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Bacterial Bioassay for Rapid and Accurate Analysis of Arsenic in Highly Variable Groundwater Samples

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In this study, we report the first ever large-scale environmental validation of a microbial reporter-based test to measure arsenic concentrations in natural water resources. A bioluminescence-producing arsenic-inducible bacterium based on *Escherichia coli* was used as the reporter organism. Specific protocols were developed with the goal to avoid the negative influence of iron in groundwater on arsenic availability to the bioreporter cells. A total of 194 groundwater samples were collected in the Red River and Mekong River Delta regions of Vietnam and were analyzed both by atomic absorption spectroscopy (AAS) and by the arsenic bioreporter protocol. The bacterial cells performed well at and above arsenic concentrations in groundwater of 7 μg/L, with an almost linearly proportional increase of the bioluminescence signal between 10 and 100 μg As/L ($r^2 = 0.997$). Comparisons between AAS and arsenic bioreporter determinations gave an overall average of 8.0% false negative and 2.4% false positive identifications for the bioreporter prediction at the WHO recommended acceptable arsenic concentration of 10 μg/L, which is far better than the performance of chemical field test kits. Because of the ease of the measurement protocol and the low application cost, the microbiological arsenic test has a great potential in large screening campaigns in Asia and in other areas suffering from arsenic pollution in groundwater resources.

Introduction

Arsenic is a worldwide recurring pollutant of natural origin with serious health effects upon prolonged intake of even low concentrations. Current estimates are that 35−50 million people in the West Bengal and Bangladesh area, over 10 million in Vietnam, and over 2 million in China are exposed to unacceptable arsenic intake through potable water consumption (*1*−*3*). Arsenicosis and visible skin lesions have been diagnosed in hundreds of thousands of persons in West Bengal, Bangladesh, and China (*2*, *4*). A similar situation may be occurring in Vietnam, where arsenic is contaminating tube wells of around 13.5% of the Vietnamese population, some 11 million persons (*1*). Although a coarse picture on arsenic distribution in groundwater in the affected areas exists, millions of family based groundwater tube wells remain to be measured and might potentially be safe for drinking water purposes (*2*, *5*, *6*). Unfortunately, arsenic is spatially very heterogeneously distributed and the arsenic contents in two nearby wells within 100 m distance can be 30-fold different (*1*, *3*). Hence, effective arsenic mitigation campaigns should screen every individual tube well (i.e., blanket screening) to determine whether the quality of the potable water complies with current arsenic guideline values (for WHO: 10 μg As/L, for Bangladesh currently 50 μg As/L).

Considering the poor technical facilities in the most exposed countries, testing a large number of wells for arsenic contamination poses an extreme challenge. So far, mostly chemistry based commercial field test kits (e.g., Merck, Hach, Arsenator, ANN, or local imitations) have been applied in Bangladesh, India, Vietnam, and other countries. The principle of these kits is the formation of volatile arsine gas (AsH_3) to separate arsenic from the aqueous matrix and subsequent colorimetric detection on a paper strip (*6*). Current chemical field kits have low precision, reproducibility, and accuracy at arsenic concentrations between 10 μg/L and 100 μg/L. Probably, one of the most important reasons for the lack of precision is the individual variability in determining the arsenic concentration from visual inspection of colored spots (*6*−*8*). Results of previous field campaigns to identify the safety of potable water in tube wells have been seriously questioned because of discrepancies between results obtained with chemical test kits and independently performed laboratory measurements. For example, among 290 wells tested both by field kits and flow injection hydride generation atomic absorption spectroscopy (FI−HG-AAS), as much as 68% of the samples in the range of 50−100 μg As/L scored false negative in the field test and 35% false positive (*7*).

Microbial reporter technologies (bacterial biosensors) have been proposed as an alternative, rapid, and cost-effective method to measure chemical species in aquatic samples. Such bioreporter microorganisms consist of genetically modified bacteria that produce a reporter protein (such as bacterial luciferase) in response to the presence of a target chemical (*9*, *10*). Luminescent bacterial biosensors reacting to arsenite and arsenate have been developed as well (*11*−*14*). So far, bacterial bioreporters have mostly only been used in laboratory applications.

Arsenic-responsive bacterial bioreporters display a lower detection limit of around 4 μg As(III)/L in aqueous solution with standard deviations of around ±5%, which is more than sufficient to comply with regulatory guidelines (*11*). Their precision in real groundwater samples, however, is unknown and several compounds may potentially influence the bioreporter's response, most notably ions which can complex arsenic or inhibitory substances for the bacterial cells. A few other ions may elicit a positive response from the bioreporters. Because of the nature of the exploited biological system, the arsenic bioreporters react to antimonite with a similar sensitivity as to arsenite, and they react to bismuth and cadmium with a 100- to 1000-fold lower sensitivity (*14*, *15*). In contrast to total destructive chemical analyses, bacterial bioreporters only assess dissolved and freely diffusible arsenite and arsenate. Chemical processes, such as

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sorption of arsenic to precipitating iron(hydr)oxides from anoxic groundwater samples, may significantly lower arsenic bioavailability to the cells, leading to underestimation of the total arsenic content of the sample (*12*, *16*).

Anoxic arsenic contaminated groundwater is often iron-rich with concentrations in the range of 5−30 mg Fe/L, with varying concentrations of other ions, such as ammonia, bicarbonate, nitrate, and silicate (*1*, *17*, *18*). During groundwater sampling procedures, acids or complexing agents are usually added to preserve the sample composition and to prevent coprecipitation of arsenic onto FeOOH particles, which are rapidly formed when anoxic groundwater is exposed to air (*19*−*21*).

The aim of this study was to develop a robust bioreporter protocol for rapid and reliable quantification of arsenic in natural groundwater samples exhibiting large differences in chemical composition. The presented protocol was developed in particular to eliminate potential disturbances caused by high iron concentrations in groundwater. To our knowledge, this is the first time ever that bacterial bioreporters were applied on a large scale with natural field samples, and our results provide confidence in their performance and their predictive value.

Experimental Section

Groundwater Sampling. A total of 194 groundwater samples from groundwater tube wells (family scale) were sampled in villages located in arsenic affected areas of the Red River and Mekong River deltas, Vietnam. Groundwater was collected at the tube by hand or by electrical pumping. Samples were taken after 10 min pumping, when the oxygen concentration in the water reached a stable value, which was measured online by using a dissolved oxygen electrode (PX 3000, Mettler-Toledo). Groundwater samples (50 mL) were immediately filtered through 0.45-μm filters and were transferred to acid-washed plastic bottles. Samples were acidified to a pH of about 2 by addition of 0.1 mL HNO_3 (7.5 M, Merck) to a final concentration of 0.015 M. Water bottles were transferred to the lab, stored at 4 °C, and analyzed for arsenic within 2 weeks.

Arsenic Analysis by AAS and AFS. Arsenic in the groundwater samples was measured in parallel by using an HG-AAS (hydride generation AAS-6800, Shimadzu, Japan) at CETASD's laboratory, Hanoi University, Vietnam, and an HG-AFS (hydride generation-atomic fluorescence spectroscopy, AFS Millenium Excalibur, PS Analytical Ltd, Kent, U.K.) at EAWAG, Switzerland. Calibration standards were prepared from a commercially available stock solution of 1000 mg As(III)/L (AAS grade, Fluka, Switzerland) and deionized water. Calibration curves were established with final concentrations of 0, 1, 2, 4, 8, and 10 μg As/L (0, 0.013, 0.027, 0.053, 0.107, and 0.13 μM, respectively). The data obtained by the two methods were used to verify the Vietnamese AAS method, which was subsequently used to validate the biosensor test (see Results and Discussion). Standard reference materials such as the SPS-SW2 standard (Spectra pure Standard, Norway) and the ICP Multielement standard VI (Merck) were used to ensure correct performance of the AAS and AFS methods.

Arsenic Analysis by Bacterial Bioreporter. The arsenic bioreporter was *Escherichia coli* DH5α (pJAMA-arsR), which was used under the cultivation and storage conditions as described previously (*11*). Briefly, arsenite determination by the bacterial bioreporter is based on bioluminescence produced by the cells in response to arsenite exposure. The intensity of the bioluminescence is proportional to the arsenite concentration and can be recorded after predefined incubation periods in a luminometer. Bioreporter cells carry a plasmid with the genes for bacterial luciferase (*luxAB*) under expression control of the ArsR transcriptional repressor protein. Cellular entrance of arsenite (or antimonite) causes release of transcriptional repression and subsequent synthesis of luciferase by the cells. Arsenate is spontaneously reduced by the cells to arsenite and hence can also indirectly cause derepression and luciferase synthesis (*11*, *15*). Bioreporter assays were conducted in 4-mL sterilized glass vials. The bacteria suspension was prepared just before the assay by mixing a 1.3-mL frozen aliquot of bacterial cells (turbidity at 600 nm of 0.5) with 10 mL sterilized Luria-Broth (LB) medium. Equal amounts of aqueous sample and cell suspension (0.5 mL) were pipetted per vial, and vials were covered with a screw-cap and were incubated on a rotary shaker at 200 rpm and 30 °C. After 90 min, 50 μL of *n*-decanal solution (18 mM in 1:1 v/v ethanol−water) was added to the vials as substrate for the luciferase reaction. Light emission was recorded after 3 min in a luminometer (Junior-Berthold, Germany) and is expressed as relative light units (RLU). Each sample was measured in triplicate, from which the average light emission was calculated. The response to samples with unknown arsenic concentrations was compared to that of a standard series of arsenite concentrations, containing 0, 7.5, 15, 30, 60, and 75 μg As/L (0, 0.1, 0.2, 0.4, 0.8, and 1 μM As), and was prepared in arsenic-free groundwater from the same area but with 20 mg Fe/L of iron (0.357 mM). Arsenic concentrations in unknown samples were determined by linear interpolation of the standard curve. In case of acidified samples, 25 μL of a 200 mM sodium pyrophosphate solution ($Na_4P_2O_7 \cdot 10H_2O$, Sigma) was added per 500 μL groundwater sample to the test vial for the purpose of raising the pH and buffering the sample at pH ∼7. All analyses of groundwater samples were conducted at CETASD in Vietnam.

Experiments To Eliminate the Disturbance of Iron on the Response of the Bioreporters to Arsenic. Solutions of Fe(II) and Fe(III) were prepared in deionized water from $FeSO_4 \cdot 7H_2O$ (p.a., Fluka) and $FeCl_3 \cdot 8H_2O$ (analytical grade, Sigma) at final concentrations in the test vials of 0, 0.28, 1.4, 2.8, 14, and 28 mg Fe/L (0, 5, 25, 50, 250, and 500 μM). All iron-containing solutions were freshly prepared and were spiked with 0.5 μM As just before starting the bioreporter assay.

To eliminate the negative influence of precipitating iron potentially lowering the availability of arsenic to the bacterial cells, several acids and complexing agents were evaluated for their suitability to keep iron in solution. For this purpose, aqueous solutions containing 0.325 μM As and 0.1 mM Fe(II) were prepared in test vials shortly before conducting a series of experiments. HCl, HNO_3 (both at 0.015 mM final concentration), and H_3PO_4 (0.025 mM) were used to lower the pH to about 2, at which iron stays in solution. In a next step, complexing agents such as disodium ethylene-diaminetetraacetatedihydrate (EDTA, Fluka), tetrasodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$, Sigma), and trisodium-nitrilotriacetate-monohydrate (NTA, Fluka) were evaluated (all at 0.1 mM final concentration) to sequester iron under neutral pH conditions and, hence, to prevent iron precipitation and subsequent adsorption of arsenic. The effect of EDTA on the response of the bioreporter in solution with 0.4 μM As and 0.2 mM Fe(II) was tested with EDTA concentrations in the range of 0−0.6 mM. All experiments were carried out in triplicates.

Results and Discussion

Effect of Iron on the Light Emission Induced by Arsenic from the *E. coli* DH5α (pJAMA-arsR) Bioreporter. The effect of iron on the bioreporter response to arsenite was tested for iron concentrations in the range of 0−28 mg Fe/L (0−0.5 mM) Fe(II) or Fe(III). The light emission from *E. coli* DH5α (pJAMA-arsR) cells decreased dramatically when the iron concentration in the assay increased from 0 to 2.8 mg Fe/L (0.05 mM), and no arsenite-inducible light response was measurable at iron concentrations above 0.05 mM (Figure

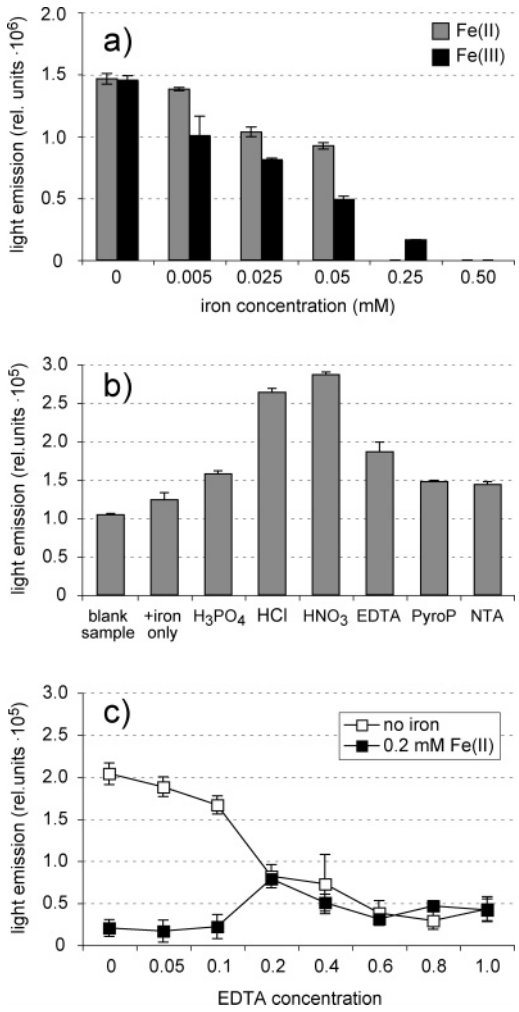

FIGURE 1. Effects of iron and iron-hydroxide solubilizing agents on the bioreporter response. (a) Light emission from the bacterial cells after 90 min incubation with 0.5 μM arsenite and different iron concentrations, as indicated. (b) Light emission from the bioreporter cells with 0.3 μM arsenite and 0.1 mM Fe(II) in the absence or presence of 0.015 mM HCl or HNO_3, 0.025 mM H_3PO_4, 0.1 mM EDTA, tetrasodium pyrophosphate, or NTA. Blank sample: no iron, arsenite, or agents added. (c) Effect of EDTA at different concentrations on the light emission induced by 0.4 μM arsenite in the presence or absence of 0.2 mM Fe(II).

1a). Already, 5 μM Fe(III) was sufficient to diminish the response from the bioreporter cells to 0.5 μM arsenite. Since iron itself is not toxic for the bacterial cells, this suggests that the availability of arsenite for the cells diminished in the presence of colloidal iron hydroxides by adsorption. Fe-oxyhydroxides (any mixture of iron oxides and iron hydroxydes) are formed rapidly in aqueous solution at neutral pH and oxic conditions and can adsorb 80−90% of soluble arsenite or arsenate within minutes (*22*, *23*). Therefore, we concluded that the bacterial cells are not capable of sensing arsenic adsorbed to colloidal iron-hydroxide particles. We envisioned that complexing reagents or acidification of the sample to a pH lower than 2.5 could prevent iron oxyhydroxide formation and retain the level of luciferase induction expected from the same arsenite concentration in iron-free media.

Evaluation of Agents for Acidification and Chelation. For this purpose, HCl, HNO_3, and H_3PO_4 at pH 2 or complexing agents were evaluated (see Figure 1b). In all cases, an aqueous solution containing freshly prepared 0.3 μM As and 0.1 mM Fe(II) was used as the basis. The acids HCl and HNO_3 (both at 0.015 mM final concentration) and H_3PO_4

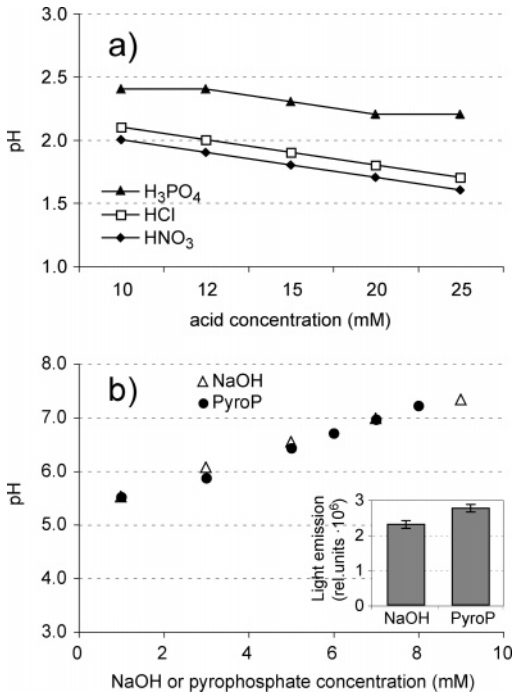

FIGURE 2. Amount of acid or base needed to acidify and thereafter neutralize groundwater samples. (a) Acidification and resulting pH of groundwater with HCl, HNO_3, or H_3PO_4. (b) Neutralization of HNO_3 acidified (pH 2) groundwater samples with NaOH or pyrophosphate and the resulting pH at the indicated concentrations. Inset shows the light emission after the procedure with a groundwater containing 62 μg/L (0.83 μM) total arsenic at the 5 mM concentration of neutralizing reagent.

(0.025 mM) were added to lower the pH to about 2 and to keep iron in solution. When the sample was subsequently mixed in a 1:1 v/v ratio with the suspended bioreporter cells, the pH of the assay mixture rose to 5.5. The bacterial cells were active under acidified conditions (pH 5.5), resulting in a partially restored arsenic-inducible response in the presence of iron (Figure 1b), with HNO_3-acidified samples producing the highest light intensity.

Direct application of complexing agents, such as EDTA, pyrophosphate, and NTA, all at 0.1 mM final concentration without pH adjustment, resulted in a lower response than for HCl and HNO_3 (Figure 1b). We tested whether the effect of EDTA could be optimized by using different EDTA concentrations in a range between 0 and 0.6 mM (Figure 1c) on the arsenic-inducible bioreporter response with 0.4 μM As and 0.2 mM Fe(II). The optimum for EDTA addition occurred at 0.2 mM EDTA with restoration of 30% of the bioreporter response in comparison to assays without iron (Figure 1c). However, at higher EDTA concentrations, the arsenite-induced light emission decreased. Also in iron-free solutions with 0.4 μM As(III), the light emission declined strongly at EDTA concentrations of 0.2 mM and higher. EDTA therefore seems to inhibit the activity of the bacterial cells, which might be attributed to chelation of essential cations in the cell membrane (*24*). Although a positive influence of EDTA has been reported at both lower Fe and EDTA concentrations (*25*), we conclude that EDTA addition alone is not useful for bioreporter detection of arsenite in iron-rich groundwater.

Further optimization of the protocol was then conducted by first acidification and subsequent neutralization. Different acids such as HNO_3, HCl, and H_3PO_4 in concentrations between 0.01 and 0.025 mM (all p.a. grade, Merck) were tested to generate a pH of about 2 in the groundwater sample (Figure 2a). Acidified samples were then neutralized before adding

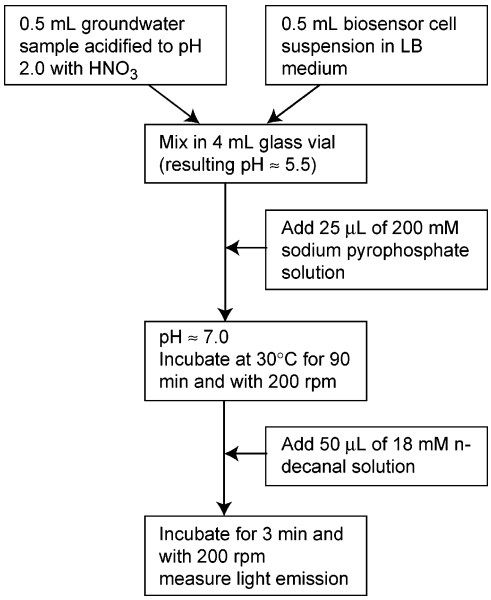

FIGURE 3. Schematic outline of the optimized procedure for arsenic bioreporter measurements in a broad variety of groundwater compositions, including iron concentrations of up to 50 mg/L.

the bioreporter cells by using either 200 mM NaOH or 200 mM $Na_4P_2O_7$ aqueous solutions in final concentrations of 2, 4, 6, and 8 mM. Subsequent pH change and bioreporter responses in a groundwater sample with 0.83 μM As were measured (Figure 2b). From this, we concluded that the combination of HNO_3 and $Na_4P_2O_7$ at a final concentration of 5 mM was optimal to dissolve any iron hydroxide complexes, neutralize the pH, and maintain arsenite available in solution for the bioreporter cells (inset in Figure 2b).

Finally, the most successful protocol for iron-rich groundwater samples consisted of acidification to pH 1.8−2.0 by the addition of HNO_3 to a concentration of 0.015 mM and then mixing the acidified groundwater sample with LB (Luria Broth) solution containing the bacteria suspension in a 1:1 volumetric ratio, after which pyrophosphate solution (at 5 mM final concentration) was added to readjust the pH to about neutral. This protocol depicted in Figure 3 was applied for all field samples.

Chemical Variability of Groundwater Samples and Validation of Reference Method. To establish a comparison between arsenic bioreporter and atomic absorption spectrophotometric (AAS) measurements, we first validated the AAS reference method for total arsenic determination at the CETASD institute in Vietnam by comparison with the AFS method performed at the EAWAG in Switzerland on a set of 111 groundwater samples collected in Vietnam. As shown in Figure 4a, the AAS and AFS measurements of total arsenic concentrations on the same sample set were perfectly in agreement (r^2 = 0.992 by linear regression), hence giving confidence that the AAS applied in Vietnam would give a proper calibration for comparisons to the bioreporter-obtained values afterward. The chemical compositions of the groundwater samples were additionally determined and were highly variable with respect to arsenic, iron, bicarbonate, phosphate, ammonium, or chloride. The concentrations of these species as well as oxygen values measured during sampling are summarized in Table 1.

Calibration Curves. Since the arsenic bioreporters' absolute light response is not only related to the arsenic concentration but is dependent on incubation time and amount of cells, the arsenic concentration in unknown samples must be inferred from a calibration curve with known arsenite concentrations analyzed simultaneously. Calibration of the bioreporter response with the new protocol (Figure 3) was therefore carried out in an arsenic-free (<1 μg/L) but iron-containing (0.36 mM Fe, 20 mg/L) groundwater sample to which known As(III) concentrations between 0 and 225 μg/L (3 μM) were spiked. The light response of the bioreporter cells was linearly proportional to the arsenite concentration in the range between 0 and 75 μg/L (0−1 μM) with r^2-values equal to 0.997 (Figure 4b). At higher As(III) concentrations, the bacteria response became saturated. These results were in agreement with previous calibration data in tap water (*11*). The detection limit in the protocol (as the value of the blank plus 3 times the standard deviation measured in the blank) was thus at 7.5 μg As(III)/L (0.1 μM). Consequently, the sensitivity of the bioreporter was sufficiently adequate to identify arsenite in groundwater as low as 10 μg/L. Theoretically, a similar concentration of antimonite may elicit an equally large response from the bioreporter cells (*15*). Therefore, a priori, without further knowledge on the types of water, the bioreporter response may be caused by either arsenite or antimonite or both. Antimonite concentrations in the Vietnam groundwater were mostly between 1 and 4 μg Sb/L, with one exception of 13 μg/L (unpublished data) and, thus, have not contributed largely to the observed bioreporter responses (see below).

Rapid Screening of Field Samples with the Bacterial Bioreporter. AAS and the bioreporter assay were then used simultaneously at CETASD to measure arsenic concentrations in 194 groundwater samples collected in July 2004 from the Red River and Mekong delta regions. A comparative plot of all values generated by AAS and by the bioreporter method showed a good correlation between both methods (Figure 4c and d), especially in the low concentration range from 7 to 75 μg/L (0.1−1 μM, r^2 = 0.882). For practical reasons, water samples were used directly in the bioreporter test, leading to a 2-fold dilution, which is the reason for the cellular response being linear up to 150 μg/L (Figure 4d). If dilution factors >2 are applied, the accuracy of determining arsenic concentrations >150 μg/L with the bioreporter cells becomes better. At the other end of the concentration scale (5−100 μg As/L), the cells measured rather accurately, thus giving the bioreporter assay an important advantage over most other field kits at present.

Robustness of the Bioreporter Assay: Performance Indicators and Outlook. Assuming that the data obtained by AAS had a higher probability for being true, we calculated the percentage of false positive and false negative results obtained by the bioreporter assay for arsenic concentrations in the range of smaller than 10, from 10 to 100, and higher than 100 μg As/L (Table 2). The bioreporter measurement was considered false negative when the As-determined concentration was lower than the concentration for that category, whereas the concentration determination by AAS showed it was above. At the other way around, bioreporter measurements were considered false positive. Both of these predictions are important, because a false negative will identify a groundwater well being safe (lower than the risk category) whereas it might not be safe with potential negative consequences for human health. False positives will identify a groundwater well as being not safe despite that the arsenic level is below the guideline values of 10 μg/L (*7*).

Among the 194 tested samples, 112 samples (58%) were determined to be safe for potable water (arsenic concentration lower than 10 μg/L). For 38 samples (19%), arsenic concentrations ranged between 10 and 100 μg/L, and 44 samples (23%) contained more than 100 μg/L arsenic (see Table 2). In the range lower than 10 μg As/L, nine samples were to be considered false negatively determined by the bioreporter (8.0%). However, arsenic concentrations of those nine samples determined by AAS ranged between 10 and 19 μg/L, indicating that they were not extremely off and would

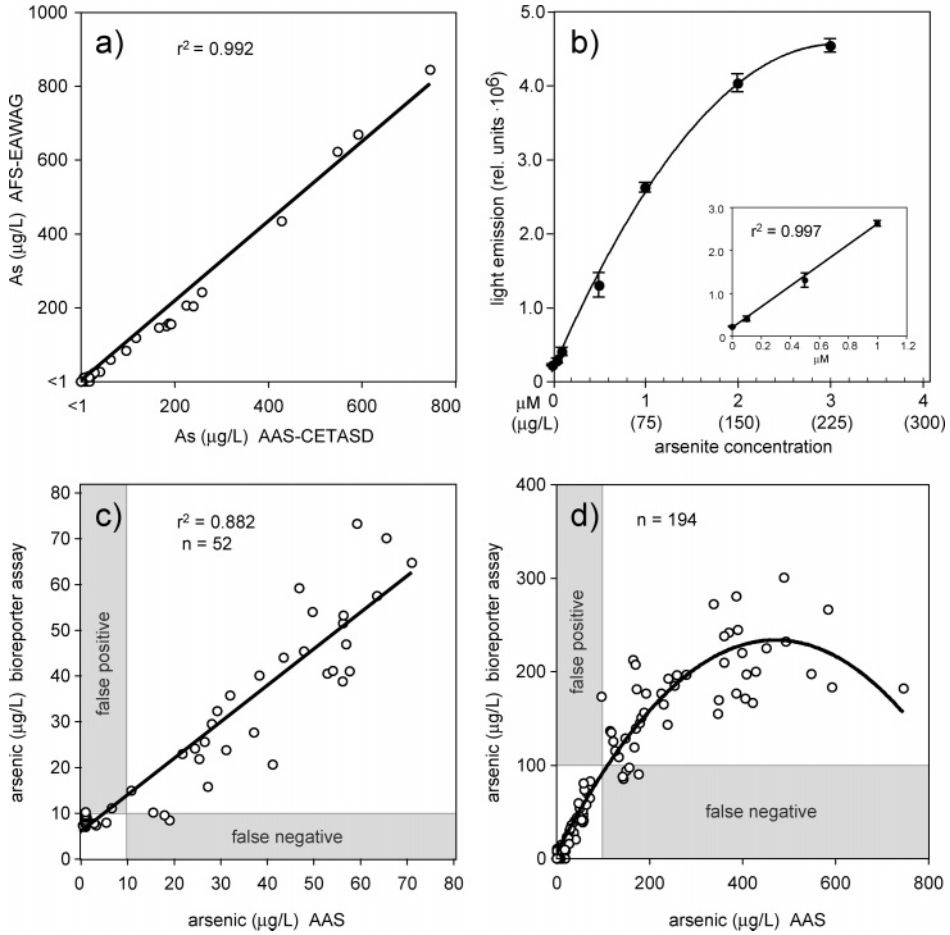

FIGURE 4. Methodological calibrations and cross-analysis of 194 groundwater samples from Vietnam by the arsenic bioreporter protocol and by atomic absorption spectroscopy (AAS). (a) Comparative calibration of the AAS-method at the CETASD institute in Vietnam with the AFS-method at the EAWAG, Switzerland, on 111 groundwater samples from Vietnam, in a concentration range of between 0 and 800 μg As/L. (b) Light emission from the arsenic bioreporter *Escherichia coli* DH5α (pJAMA8-arsR) as a function of arsenite concentration measured after 90 min incubation at 30 °C. The line represents the hyperbolic fit of the calibration. Aqueous matrix for preparing the calibration curve was arsenic-free but iron-containing groundwater from well TD26 (20 mg Fe/L). The inset shows a linear fit of the concentration range between 0 and 1 μM arsenite. (c) and (d) Cross-analysis of 194 groundwater samples by AAS and the bioreporter protocol. Arsenic concentrations in unknown samples were interpolated from the linear (0−2.8·10^6 light units) or hyperbolic fits (>2.8·10^6 light units) of the calibration curve in panel b. Panel c is an enlargement of the region between 7 and 70 μg As/L of panel d. A large proportion of samples was below 10 μg As/L in both methods (Table 2) and did not contribute to the calculation of the r^2-value (linear fit) in panel c.

TABLE 1. Chemical Composition of Groundwater Samples Analyzed in This Study

chemical parameter		Red River Delta (n = 83)			Mekong River Delta (n = 111)		
		min	max	average	min	max	average
pH		7.0	7.7	7.2	6.0	7.6	7.0
As	(μg/L)	1.3	460	140	<1	850	39
Fe	(mg/L)	0.1	26	7.4	0.05	56	2.6
Mn	(mg/L)	<0.01	2.8	0.8	<0.01	34	3.4
Ca	(mg/L)	17	160	103	1.0	620	77
NH_4^+	(mg/L)	<0.1	24	8.6	1.5	25	7.5
HCO_3^-	(mg/L)	310	730	582	96	900	318
Cl^-	(mg/L)	<0.05	37	11.6	2.1	8600	690
O_2	(mg/L)	<0.05	1.4	0.20	<0.05	3.9	0.28

TABLE 2. Comparison of AAS with Bioreporter-Determined Arsenic Concentrations Categorized for the Vietnam Groundwater Samples (n = 194)

	arsenic concentration range			
	<10 μg/L	>10 μg/L	10−100 μg/L	>100 μg/L
number of samples in category	112	82	38	44
percentage (%)	58	42	19	23
number of false negatives	9 (8.0%)		5 (13%)	
number of false positives		2 (2.4%)	2 (5.3%)	1 (2.3%)

still be below the safety level of 50 μg As/L. Among the 38 samples identified in the 10−100 μg As/L range, five samples (13%) were recorded as false negative by the bioreporter assay, with AAS-determined arsenic concentrations being in the range of 142−176 μg/L, whereas two samples (5.3%) were false positive. In 44 samples, the bioreporter-determined concentration of arsenic was higher than 100 μg/L. Among those, there were no false negative determinations, but one sample (2.3%) was false positive with an AAS-determined value of 97 μg/L. However, this is very close to 100 μg/L and can be considered as a discrepancy that can also occur between AAS tests among different laboratories (*6*). In summary, if all the wells were categorized as safe or not safe on the basis of the WHO guideline value for arsenic in drinking water (10 μg/L), 9 of 112 samples were false negative (8.0%) and 2 of 82 were false positive (2.4%).

In light of the horrifying high rate of false negatives with chemical field test kits of up to 68% at arsenic concentrations in the range even of 50−100 μg/L (*7*), the performance of the bioreporter assay is very promising. Validation with a larger number of real samples from a variety of other environments as well as higher dilution ratios in the case of highly contaminated samples will improve the predictive value of the bioreporter measurements even further. However, we are confident that the assays and the protocol for using the luminescent bacterial strain *E. coli* DH5α (pJAMA-arsR) can already be an important new tool for rapid screening of arsenic in groundwater in developing countries.

The bioassays were performed directly in Vietnam. It was the first time ever that such a microbial reporter system was tested under local conditions on a large variety of environmental samples (see Table 1). The average processing time with the single vial test was about 50 samples per day. The system can easily be upgraded to multiwell-plate analyses, allowing measurements of hundreds of samples per day, even in a moderately equipped laboratory, which is much more than can be achieved by AAS or AFS. Production of the bioreporter cells can be achieved at low costs while maintaining good quality if simple rules of handling bacteria are followed. Thus, we believe that extensive screening of many wells by this microbial reporter technology has become a more realistic opportunity to counteract the arsenic crisis.

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