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Sampling and preservation artifacts in arsenic analysis: Implications for public health issues in developing countries

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This communication presents the probable sampling and preservation artifacts in arsenic analysis of natural groundwater. It has particularly scrutinized the standard method of sampling and preservation of arsenic, where the sample is to be acidified with concentrated HNO₃ until a pH of less than 2. It has also ascertained the efficacy of sample container material in preserving the original arsenic concentration. Arsenic losses due to preservation imperfections will adversely affect the

results and their interpretation from the public health point of view. The communication also discusses that proper sampling and preservation could be the most neglected part of the whole regulation and could be the cause of under-reporting of arsenic contrasted with a higher incidence of arsenic ailments in the population, particularly in the Third World.

THE analysis of toxic elements in natural water samples is a complicated task, as they are present in a low concentration and are subject to a variety of chemical modifications after sampling. The regulatory limits of most of the toxic elements in drinking water are getting gradually lowered worldwide, as our knowledge about the health effects of trace elements is increasing. Arsenic contamination has been acknowledged as a major public health issue by the World Health Organisation (WHO) based on its international prevalence; WHO has proclaimed that it requires to be dealt with on an emergency basis¹.

As most of the trace elements cannot be determined on-site due to technical limitations, it is a general practice to collect a representative sample and preserve it until analyses in a laboratory. The preservation steps aim to maintain the original concentration of the analytes and their chemical nature. A proper estimation of the concentration and speciation is important from the health point of view, where the dose and its chemical species govern the likely effects. However, many factors contribute to the water chemistry results obtained from groundwater samples. Laboratory analytical methods for most analytes and sample types are well established and carefully documented. Errors associated with the collection and handling of a sample generally exceed those associated with the analysis².

Preservation of groundwater samples aims to retard the biodegradation reactions, hydrolysis reactions, precipitation and co-precipitation reactions, sorption reactions and any other physico-chemical reactions, which may occur in a natural sample. Sample preservation usually involves reducing or increasing pH by adding an acid or base preservative and the samples to be analysed for organics are generally preserved by cooling them to 4°C.

The total concentration of the distribution of inorganic arsenic species must be preserved in the field to eliminate changes caused by metal oxyhydroxide precipitation, photochemical oxidation, and redox reactions. Arsenic species sorbs to iron and manganese oxyhydroxide precipitates, and arsenite can be oxidized to arsenate by photolytically produced free radicals in many sample matrices³. Several preservatives were evaluated to minimize metal oxyhydroxide precipitation, such as inorganic acids and ethylenediaminetetraacetic acid (EDTA). Aqueous nitric, perchloric, hydrochloric, and acetic acids have been used to help stabilize As (III) and As (V) species, and stabilization was improved by storing samples at temperatures below 15°C. Different storage temperatures and the addition of hydro-

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chloric acid or ascorbic acid also have been investigated as possible preservation techniques; in addition, quick-freezing the sample with liquid nitrogen was recommended to preserve the As (III) and As (V) speciation in surface-water samples. Storing samples at 5°C preserved As (III) and As (V) speciation in water samples for about 30 days, while using 0.1% nitric or hydrochloric acid altered the arsenic species distribution. Some of these stabilization practices are not practical for field applications, are not amenable to analytical methodology nor have they been tested on a large number of samples with different matrix compositions³.

The standard practice for the preservation of metals, except mercury and hexavalent chromium, is the addition of HNO₃ until a pH less than 2 is obtained and the sample holding time in this state is 180 days. The recommended-sample container is a plastic bottle that is typically polyethylene, polypropylene or polyvinyl chloride⁴⁻⁷.

An unresolved issue in arsenic analysis is the on-site filtration of samples prior to analysis. The literature available on filtering is inconclusive regarding when, where and under what specific circumstances filtering should or should not be required. The decisions appear to be specific to each situation, depending primarily on: (i) the contaminants or constituents being collected and their susceptibility to alteration during filtration; (ii) the hydro-geologic environment; (iii) groundwater chemistry, and (iv) the ultimate use and purpose of filtered versus unfiltered analytical results. Filtering may remove colloids, particulates and sorbed-contaminants that are mobile under natural flow conditions. Therefore, filtering may cause an underestimation of the amount of contamination that is naturally mobile in the groundwater^{7,8}.

The groundwater contaminants are considered to be partitioned between two phases, a mobile phase composed of dissolved (aqueous) solutes in water transported by natural groundwater flow and a normally immobile solid phase composed of the matrix materials of the water-bearing zone. Inclusion of metals associated with these normally immobile matrix particles may bias analytical determinations, leading to elevated and improbable concentrations of mobile contaminants if suspended particle concentrations are high. As a result, groundwater samples are commonly filtered in the field to remove these suspended particles. Filtration has been considered particularly necessary under turbid conditions, where high particle (sediment) loading might lead to significant analytical bias through inclusion of large quantities of matrix metals in the analysis. Alternatively, the presence of particles in samples might also bias analytical determinations through removal of metal ions from solution during shipment and storage, because of interactions with particle surfaces.

Unfortunately, indiscriminate use of field filtration ignores the presence of particles, known as colloids, in groundwater that may exist between the extremes of solutes and sediments. Potential association of metals with

colloids has important implications for the practice of field filtration because the boundary between the particulate and dissolved matter has been operationally defined at 0.45 µm. This boundary presumes that the component retained on a 0.45 µm filter represents suspended solids, while the component that passed through the filter represents dissolved metal. Collection of groundwater samples for analysis of metal concentration is required under several US environmental regulations, including CERCLA (super-fund), RCRA Subtitle C (hazardous waste), and RCRA Subtitle D (solid waste). As a result, the debate regarding groundwater metal samples has an impact on a wide range of sampling programmes and a large number of sites, suggesting the need for further research⁹.

Pandey *et al.*¹⁰ first reported major arsenic contamination at certain locations in central-east India. A subsequent paper by Pandey *et al.*¹¹ established the regional nature of arsenic contamination in a complex geo-chemical environment. However, a wide variation in the arsenic levels reported from similar locations (unpublished report, National Environmental Engineering Research Institute, Nagpur, 2000) and recently, by Acharya¹² and our own observations since the last three years led us to carry out investigations on the reasons for the variation in arsenic levels reported from the same site. It was premised that the sampling and preservation artifacts could be expected to play an important role. Hence, this communication provides an analytical insight into the problem.

Samples were collected in passive mode, i.e. without the consumption of any energy to cause minimum deviation from the natural conditions. The sampling bottles, either glass (borosilicate) or plastic (polyethylene) were previously acid and detergent washed, and then washed with copious amount of clean laboratory water and finally rinsed with the distilled water. Two types of quality-control samples were collected, i.e. duplicate samples and equipment blanks. The purpose of the duplicate samples was to ensure the precision of sampling and analysis. The duplicate samples were collected for both field screening and for laboratory analysis. The purpose of the equipment blanks was to verify that sampling devices were not contributing to the contamination of the samples. When sample filtration was carried out, a transfer vessel was used to gently pour the grab sample into a reusable manual filtering device with a disposable 0.45 µm filter membrane and filtered using a positive pressure.

An attempt was made to observe the effect of various preservatives on maintaining the total arsenic content of the samples. Speciation of arsenic was not carried out as the mere presence of inorganic arsenic is a potential public health hazard and the redox chemistry of arsenic can cause a change in the speciation rather easily. Hence, both forms of As, i.e. (V) or (III) can undergo interconversion and vice-versa. Therefore, total inorganic As alone should be primarily focused in an area survey for arsenic prevalence.

Preservatives used in the samples were as follows:

- (i) HNO_3 (concentrated) was added until a $\text{pH} < 2$, checked at the sampling site by portable pH analyser (Orion). Four main series of samples were collected, i.e. (1) F, 4°C , Filtered and kept at 4°C ; (2) F, RT, Filtered and kept at room temperature; (3) UF, 4°C , Unfiltered and kept at 4°C ; (4) UF, RT, Unfiltered and kept at room temperature $\sim 35^\circ\text{C}$.
- (ii) Concentrated solutions of HCl and H_2SO_4 were used as above for maintaining a pH below 2.
- (iii) NaOH (1 M) was added until $\text{pH} = 12$. Precipitate, if any, was filtered immediately.
- (iv) CHCl_3 , CCl_4 and HCHO were analysed for their efficacy as arsenic preservatives and were added 10 ml each per litre of water.
- (v) EDTA (0.125 M) was prepared by dissolving 46.53 g disodium-EDTA in 1 l distilled water.

The hydride generation atomic absorption spectrophotometer with background correction facility (HG-AAS, Chemito-201) was used for arsenic analysis by following the standard methods^{4,5}. Merck-certified standard solutions and chemicals were used for quality control. For speciation, As (III) was determined directly by hydride generation at pH 5–7 and then As (V) was measured at $\text{pH} < 1$ using a pre-reductant, viz. potassium iodide–ascorbic acid.

Occasionally, scanning UV-visible spectrophotometer (Chemito-UV 2100) was also utilized using silver diethylthiocarbamate method for arsenic analysis.

A proper quality assurance and quality control protocol was followed, consisting of the following steps: First, measurement of certified calibration standards as samples, maximum allowed difference was 3%. Second, measurement of calibration check standards with known analyte concentration but of different origin than the calibration standards, maximum allowed difference was 5%. Third, measurement of sample blanks and laboratory blanks to control the instrument contamination and contamination arising out of the sample preparation respectively. Fourth, laboratory check samples were prepared from unexposed containers with standard addition of elements; maximum deviation allowed was 5%. And fifth, a standard reference material (SRM) of urban dust from National Institute of Standards and Technology, SRM-1648 was used for ensuring quality control.

Elemental standards used were prepared by dilution of the stock-certified single-element solution for AAS supplied by Merck, Germany. The dilution water used was deionized, double-distilled water. All other reagents used were arsenic-free, analytical grade chemicals obtained from Merck, India/Germany. Background checks were also made and the intrinsic trace element content thus deduced was appropriately integrated in the result. The detection limits were calculated as three times the standard deviation of nine measurements of blanks for every wavelength.

Analytically, the results of the study show major artifacts in the standard method of arsenic sampling and analysis when applied on groundwater of the studied location. Results of a large number of analyses wherein the effect of non-preservation, effect of preservation at lower temperatures, preservation behaviour of nitric acid, efficacy of other chemicals as preservatives and the effect of various types of containers, etc. are presented.

Acidification of the collected samples with nitric acid until a pH below 2 is the standard practice in trace element analysis, as discussed above. Figure 1 shows that even after acidification with HNO_3 , the filtered samples have registered a continuous loss of arsenic from the groundwater. The rate of loss of arsenic in the filtered samples kept at room temperature was astoundingly high (about 17% within 24 h). Similarly, the filtered samples kept at 4°C also registered sizeable losses of arsenic (about 6% within 24 h). However, the unfiltered samples registered an increase in arsenic concentration initially. This appears to be a direct effect of dissolution of arsenic sorbed on the colloidal iron present in the samples. Yet, after initial increase due to the dissolution as described above, both sets of samples kept at different temperature regimes registered regular loss of arsenic which was more pronounced in the samples kept at room temperature compared to similar unfiltered samples kept at 4°C . Overall, the rate of loss of arsenic was high in the unfiltered samples compared to the filtered samples at all temperature regimes.

On the other hand, the arsenic samples prepared artificially by spiking of distilled water or regular municipal, treated drinking water did not show a significant pattern of losses when treated in a similar fashion as above. This corroborates the theoretical efficacy of addition of HNO_3 until a $\text{pH} < 2$, as a preservative.

Concisely, the results show that nitric acid preservation is not free from artifacts and continuous loss of total

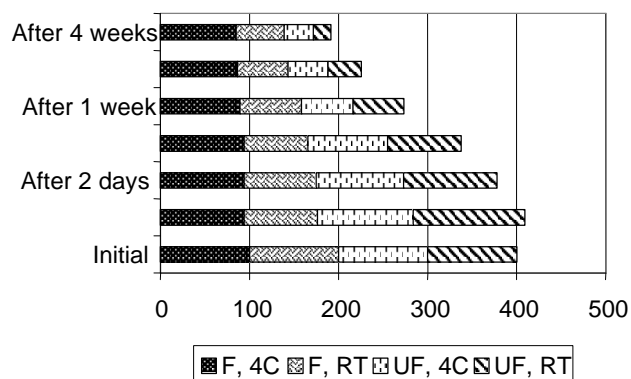


Figure 1. Arsenic loss pattern of four series of groundwater samples ($n = 57$) preserved with HNO_3 at $\text{pH} < 2$, under differing temperature and filtration conditions. F, 4C, Filtered and kept at 4°C ; F, RT, Filtered and kept at room temperature; UF, 4C, Unfiltered and kept at 4°C ; UF, RT, Unfiltered and kept at room temperature.

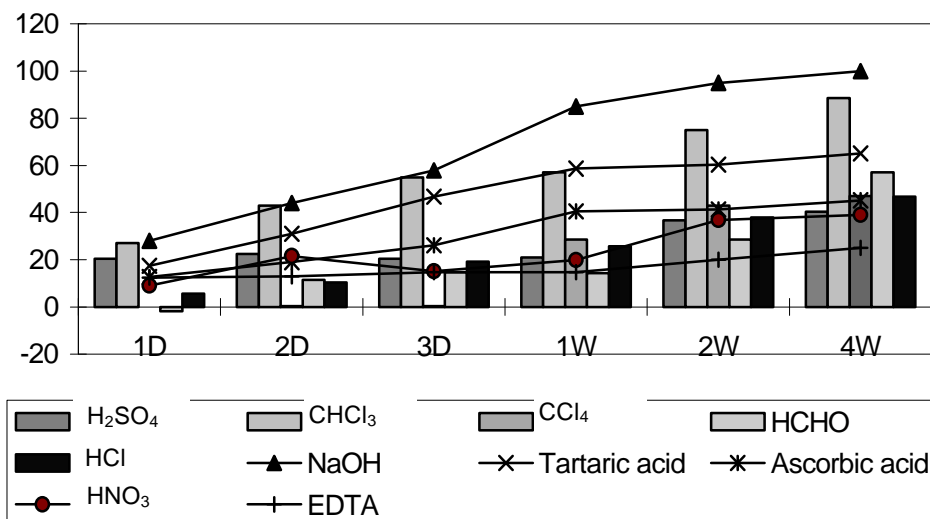


Figure 2. Performance (mean) of various chemicals in preserving total arsenic concentration in unfiltered samples kept at room temperature (showing % As loss).

arsenic takes place in the preserved samples. Based on this it is obvious that arsenic speciation cannot be maintained. As the phenomenon is pronounced in natural samples obtained from central-east Indian locations, the samples for arsenic measurement should be filtered, acidified and stored at 4°C until analysis and the samples should be analysed within a day of collection.

Recently EDTA has been reported as an effective preservative for maintaining arsenic speciation and hence (obviously) the total arsenic content of water samples obtained from groundwater and acid mine drainage samples. Bednar *et al.*³ also reported that storing EDTA-preserved samples in opaque polyethylene bottles eliminated the effects of photochemical reactions.

The second set of studies has verified the efficacy of organic liquids, i.e. CHCl₃, HCHO, and CCl₄ and a base, NaOH apart from the acids (HCl, H₂SO₄, HNO₃), the effect of temperature regimes and effect of container material on arsenic preservation using EDTA. In the experiments, the samples were filtered with a 0.45 µm filter (if required) and kept at room temperature (30 ± 5°C).

The mean results of a series of experiments involving at least three samples for every chemical studied are presented in Figure 2. The results obtained show that overall, in a period of 4 weeks storage time, EDTA is the best preservative with mean loss of arsenic at about 16%, closely followed by CCl₄ with 19% loss.

However, if the three-day period is concerned reasonably sufficient prior to analysis, then CCl₄ preservation efficacy surpasses what EDTA or any other acid offers. With total loss of about 0.3% of the initial arsenic concentration, CCl₄ is clearly the best preservative. At the moment, the reasons for this excellent preservative action are unclear. As formaldehyde has also shown a reasonably good performance during this period, it appears that

the anti-microbial action of organic solvents could be the reason for preservative action. Further research is needed in assessing the efficacy of organic solvents as arsenic preservatives and their effect on the hydroxide precipitation and photolytic reactions.

The question as to whether the groundwater samples should be filtered or not, becomes much more complicated in light of this study. According to Heidlauf and Bartlett¹³ and Puls and Barcelona¹⁴ there are diverse views on the filtration of groundwater before sample preparation and analysis for metals. Results from filtered samples may best represent the concentration of metals being transported by groundwater flow. Results from unfiltered samples may sometimes be a function of well installation and development and on the other hand, may provide a true picture of groundwater contamination. The option selected should be based on the objectives of the specific project, use of analytical data, comparability with previous site results, and the position of the regulators.

We have observed that arsenic concentration in unfiltered samples from the maximum arsenic contaminated sites, such as Hand Pump no. 6 located at the Bazaar Square of Kaurikasa village, Rajnadaon district was slightly higher than the filtered samples indicating the presence of sorbed arsenic. This was confirmed by the analysis of the filtration residue, which showed the presence of iron hydroxide and arsenic. This is chemically plausible, given the strong adsorptive power of the oxy-hydroxides of iron. However, the subsequent faster rate of arsenic losses in unfiltered samples compounds the issue, as we did not witness an increase in the arsenic content of the filtration residues collected subsequently. This means that arsenic loss cannot be wholly attributed to the presence of iron in the system, though it may play a significant role in controlling the redox reactions. Particularly, the dissolved

ferrous iron (Fe^{2+}) reacts immediately to an increase in the dissolved oxygen (DO) content of the sample. The DO content of groundwater is always prone to increase from the moment it is drawn up. The more the application of energy for lifting of the water (i.e. the use of pumps, etc.), greater will be the sample perturbation and hence increase in the DO content. The DO accepts an electron from Fe^{2+} , thus oxidizing the iron and changing it to ferric iron (Fe^{3+}). Ferric iron then precipitates out of solution as oxyhydroxide. To avoid such a possibility, we had used the method of passive sampling in which no energy was used for sample collection. Further, iron (if present) was in Fe^{3+} state rather than Fe^{2+} oxidation state, thus plummeting the possibility of redox reactions subsequent to sampling at the study locations.

On the contrary, locations that were far away from the high-arsenic zone contained arsenic mainly in adsorbed state in fine clay and/or iron oxide. Filtration of these samples resulted into low concentration of arsenic. Generally, the groundwater obtained in most parts of the world is consumed directly without any filtration. Therefore, from the public health point of view, filtration of samples prior to analysis is undesirable. This fact is attested by the development of palmer and planter keratosis of the first to second stage in people living in villages supposedly free or with less danger from arsenic risks¹¹.

Geologically, most of the affected locations in central-east India, where this work was carried out, fall in the geologic anomalous zone where there exists a volcanic metamorphism and wide-ranging shearing and fractured bedrock. Such geology is bound to favour higher mobility of a variety of colloids and contaminants. At such locations, filtration of samples will certainly underestimate the actual risk. Yet, the higher rate of arsenic losses in the unfiltered samples kept at room temperature presents a bewildering situation.

Another important analytical issue dealt within this work, was the type of sample container. Generally, two types of sample containers, i.e. glass (borosilicate) or plastic (polyethylene or its equivalent) are used. The standard methods for general metal analysis (including arsenic) prescribe the use of either type of container^{4,5}.

Bednar *et al.*³ have reported that storing the samples in opaque polyethylene bottles eliminates the effects of photochemical reactions, but they have not presented a comparison with opaque borosilicate glasses. The results produced by them showing the preservation of arsenic species distribution over a three-month period for a groundwater sample (Colorado well) and an acid mine drainage sample (Koehler Tunnel), show fluctuations. Hence, an effort was made to test the efficacy of the two sample containers with nitric acid and EDTA preservation.

To assess the container efficacy, four series of natural samples were taken wherein the arsenic concentration of natural groundwater samples was 0.7, 0.9, 1.2 and 1.5 ppm. These samples were preserved with HNO_3 (until a pH = 2

with continuous pH monitoring) and EDTA (300 μl of 0.125 M EDTA) to the 30-ml sample obtained from Kaurikasa village (Figure 3).

Results confirm 0% loss of total arsenic in glass bottles preserved with EDTA and HNO_3 up to 336 h. However, when kept up to 672 h, a loss of about 6 and 14% was observed with EDTA and HNO_3 respectively.

Plastic containers in case of both preservatives, have registered loss in arsenic concentration within 24 h after preservation (Figure 3). The loss percentage is small (0.5 and 0.2% for EDTA and HNO_3 respectively). Yet it could be considered significant in trace elemental analysis. These losses approach to as high as 25% with both preservatives up to 672 h.

This result questions the efficacy of plastic containers (at least for arsenic) compared to glass, where the loss of trace levels of metal from the glass container has been reported by the sorption mechanism¹⁵. This study also shows that the glass container is better-suited for arsenic sampling. To account for arsenic loss, it is hypothesized that there could be a conversion of arsenic in the natural samples from this location to the volatile phase, which the higher temperature aggravates and the diffusion of the gaseous arsenic species is facilitated by the apparent porosity of the plastic container. The role of microorganisms needs to be investigated, which may act as catalysts to speed up the otherwise slow redox reactions.

The results obtained above bring the whole gamut of trace and toxic elements analysis into sharp focus in a developing country. A serious public health implication of this phenomenon is evident in Kaurikasa village. We had reported as early as in 1998, through local media, about the presence of arsenic in groundwater and through a detailed scientific paper¹⁰, about the level of arsenic and cases of arsenicosis in the affected population (Table 1). Yet, the different government agencies reported the arsenic level far below those measured by us and in most cases, they did not even detect the presence of arsenic (pers. commun.).

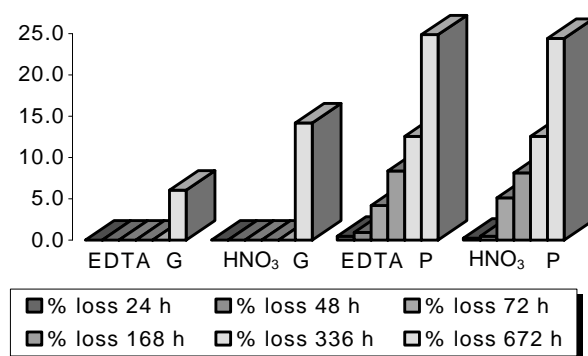


Figure 3. Percentage loss of arsenic in two types of sample containers at 4°C. G, Opaque borosilicate glass; P, Opaque polyethylene.

Table 1. Comparison of reported arsenic levels from the same locations in Kourikasa village

Identifying number	Location	Mean arsenic ($\mu\text{g/l}$) 2000 (this study)	Mean arsenic ($\mu\text{g/l}$) 1999 (this study)	Total as ($\mu\text{g/l}$) (NEERI, 2000)
HP 1	Girls Primary School	350	200	199
HP 2	Thakurtola	510	140	153
HP 3	Shiva temple	450	250	217
HP 4	Durga chowk	800	500	19
HP 5	Gram panchayat	1700	1510	1660
HP 6	Kunjam house	3050	1965	1890
HP 7	Sonjhariapara	550	400	374
HP 8	Bhagirath house	50	10	3
HP 9	Anganwadi	1120	960	826
HP 10	Margiyaram house	60	50	BDL
HP 11	Girls ashram	190	170	135
HP 12	Old boys hostel	1265	300	245

NEERI, 2000: Study of arsenic contamination in the groundwaters of Block Chowki, District Rajnandgaon (MP), National Environment Engineering Research Institute, Nagpur, 2000.

The major reasons for this incongruity have been identified as follows:

- (i) Non-filtration of samples at the sampling site.
- (ii) Keeping the samples at ambient temperature.
- (iii) Long-distance transport, including road transport to laboratories of the regulatory agencies located at distant places like Nagpur, Bhopal, Kolkata, New Delhi etc.
- (iv) Long holding time before arsenic analysis is actually performed.
- (v) Inadequate preservation or non-preservation of samples.

We have reported that there are about 25,000 people with substantial risks of arsenic contamination in Rajnandgaon district, Chhattisgarh; but the regulatory agencies have underplayed the danger based on their own results which are at times based on unscientific sampling and analysis or genuine artifacts in the whole procedure. This can be ascertained from the fact that the Indian standard method for the sampling and testing of arsenic in water and wastewater¹⁶ does not make any specific mention of the suggested sampling and preservation procedure. This puts a big question mark on the entire regulatory procedure in a country of 100 million people and which encompasses the world's greatest arsenic-contaminated region, i.e. Bengal Delta Plain.

Keeping the above in view, the following sampling and analysis strategy should be adopted strictly by the worst-affected countries like India and Bangladesh, where proper sampling and analysis procedure is followed more in letter than in practice:

- Sampling with minimum perturbation of groundwater and preferably without any application of energy.
- Use of pre-cleaned glass (borosilicate) container instead of plastic container; especially for arsenic analysis.

- Collection of two series of groundwater from every location along with the field blanks and duplicate samples. One set should be filtered immediately with 0.45 μm membrane filter and other should be unfiltered.
- The filtration method should be by application of positive pressure rather than suction, which will create greater perturbation.
- All the bottles should be filled completely and kept at a temperature of 4°C until analysis.
- EDTA may be considered as an alternative preservative as it will effectively preserve the total arsenic content as well the speciation of arsenic. Based on our work we suggest using 5 ml of 0.250 M EDTA to 1 l of sample so as to effectively preserve all major analytes.
- Samples should be analysed preferably on-site or else as early as possible, within 24 h.
- The unfiltered sample should be analysed first and only in the event of arsenic concentration being greater than the regulatory limit should the filtered sample be analysed.
- Results may be reported on filtered and unfiltered basis along with the holding time and should be accordingly interpreted.
- For regulatory purpose, unfiltered arsenic concentration should be taken into consideration.
- The on-site analysis as recommended in this protocol has the limitation of non-availability of a good field method and the instrumental limitations, particularly in a developing country. This calls for further research in the aspect.

Thus, there could be major analytical artifacts in arsenic analysis if the proper sampling and on-site filtration is not carried out and the samples are not kept at 4°C until analysis. This communication reports that even after on-site

filtration, acidification until a $\text{pH} < 2$ by nitric acid and storage of the samples at 4°C until analysis, there could be a loss of total arsenic content. Further, glass containers are better-suited for arsenic sampling and preservation. EDTA could be considered as an alternative preservative for the analysis of total arsenic content and its speciation. However, Bednar *et al.*³ have prescribed varying molar concentrations of EDTA depending on Fe, Mn and other metal cation concentrations. Arsenic concentration in the unfiltered samples witnessed an increase during the first 24 h after the sampling and preservation.

Analytically, the nature of arsenic compounds present and other concomitant parameters in the contaminated samples in Kaurikasa village need a further study to explain the higher rates of arsenic loss compared to the synthetic samples or similar samples from different locations.

The results also show that the sampling and preservation artifacts may result into serious under-reporting of arsenic levels, particularly in developing countries. This is probably the major reason for the failure of scientists to reach a consensus on the dose–effect levels for arsenic. Secondly, the Indian regulators will do well if QA/QC controlled sampling and analysis is carried out for arsenic, which should encompass all the seasons in any arsenic contaminated region.

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Using an optical trap to fold and align single red blood cells

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We report on the trapping dynamics of single red blood cells (RBCs) in an optical trap. The optical trap was constructed using linearly polarized, infrared laser light (1064 nm). The trapped RBC shows folding behaviour due to the elastic nature of the cell membrane. On removal of the trap, the RBC regains its original shape, indicating that there is no cell damage induced by the optical field. The folding time for the RBC is less than 1 s, while the relaxation time is ~ 6 s. The folded RBC aligns itself along the direction of the electric field of the laser due to the action of polarization-induced forces; introducing a half-wave plate in the trap and forcing the trapped RBC to follow the direction of the electric field vector of the laser confirms this.

THE utility of optical tweezers to trap and manipulate microparticles is now well established¹. In recent years, the advent of optical tweezers has opened new vistas for both basic and applied research in diverse areas of life sciences, like single-cell molecular biology², laser-assisted *in vitro* fertilization³, development of cell biosensors⁴, micromanipulation of relevance to cell sorting and cellular microchips^{4,5} and studies of the mechanics of single DNA molecules⁶. Optical trapping also makes feasible single-cell testing of erythrocytes that are linked to pharmacophores for use in drug therapy⁷.

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