Influence of several factors during collection and preservation prior to analysis of arsenic in groundwater: A case study from West Bengal, India

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Abstract: To avoid the discrepancy in analytical results, sets of experiments were studied on mode of sampling, sample storage and time interval study and its effect were noted on groundwater arsenic concentration from West Bengal, India. Arsenic concentration decreased gradually with time and higher iron concentration, higher arsenic loss (due to co-precipitation with iron) was observed for non-acidified samples. Somewhat lesser loss of arsenic was observed under refrigerated condition, compared to room temperature and about 99% of iron was lost within a day under non-acidified condition. About 91-98% and 96-100% of arsenic were recovered within first three days for acidified samples, stored in room temperature and refrigerated condition, respectively. A considerable amount of arsenic was lost with time due to the adsorption on plastic container surface/precipitation with other elements/volatilization, especially for acidified water samples. Linear regressions showed very good correlations between initial iron concentration and loss of arsenic for non-acidified samples. Particulates (colloidal iron hydroxide) present in pumped groundwater contributed a considerable amount of arsenic and with time, less to lesser particles was coming out with pour water. Average 166% and 23.5% (up to five and fifteen months from installation, respectively) higher results of arsenic were observed for the unfiltered waters compared to the filtered (Millipore membrane filter, 0.45 μm), collected from the newly installed tubewells, 1 month and 11 months old, respectively. Whereas, an average 12% higher value of arsenic was observed in unfiltered waters compared to the filtered, for the older tubewells, 2-9 years old. Very good correlations were observed between water arsenic concentrations in filtered and unfiltered samples for older tubewells. An average ±14% variation of both arsenic and iron concentrations in groundwater (n=23) was observed round the year.

Key words: Groundwater; collection; preservation; time interval; analysis of arsenic

Introduction

Considerable concern exists globally about arsenic in potable/drinking waters extracted from contaminated aquifers (Smedley & Kinniburgh, 2002). Exposure to arsenic can cause a variety of adverse health effects, including dermal changes, respiratory, cardiovascular, gastrointestinal, genotoxic, mutagenic and carcinogenic effects as well as with disorders of the circulatory and nervous system (Mandal & Suzuki, 2002; Chen & Ahsan, 2004).

Arsenic occurs in the natural environment in four oxidation states: As(V), As(III), As(0) and As(-III). The toxicity and bio-availability of arsenic depend on its chemical form (Cullen & Reimer, 1989; National Research Council, 1999). Inorganic arsenic species are believed to be more toxic than methylated species (Cullen & Reimer, 1989) and have been established as human carcinogens (USEPA, 1988; Hopenhayn-Rich *et al*., 1996; National Research Council, 1999). The behaviour of arsenic species will change depending on the biotic and abiotic conditions in water. Arsenic is predominantly present as As(III) and As(V) in groundwater

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(Chatterjee *et al*., 1995; Kim *et al*., 2002; Rasul *et al*., 2002), with a minor amount of methyl and dimethyl arsenic compounds being detected (Shraim *et al*., 2002).

Sampling, sample storage and time interval between collection and analysis of total arsenic from aqueous solutions

It should be realized, that sampling, sample pre-treatment, transportation and storage, are an integrated part of the analysis. The sample handling procedures have not received much attention in terms of quality assurance (QA) and good measuring practice (GMP). As such they should be considered carefully in the development of any monitoring strategy.

In contrast to most metal ions which do not change their valence easily and are not transformed into organic compounds in aqueous solution, arsenic possesses a complicated chemistry characterized by equilibrium, steady state conditions or situations approaching such conditions between trivalent and pentavalent inorganic arsenic compounds and various derivatives containing arsenic-carbon bonds. The arsenic species conversion in aqueous samples can be caused by redox reactions, precipitation, adsorption, microbial activities and the extent of diffusion of O_2 from the atmosphere (Brett & Brett, 2000; Daus *et al*., 2002). In strongly reducing aquifers, As(III) is the dominant species based on the thermodynamic considerations, whereas As(V) is the more stable oxidation state under oxic conditions or in oxygenated waters. The analysis for arsenic compounds in water samples will correctly give the composition of the sample at the time of collection only if no arsenic compounds were lost, inter-conversions of arsenic compounds did not occur and no new arsenic compounds were formed during the time between collection and analysis. The chemical reaction,
$H_3AsO_3 + H_2O \leftrightarrow H_2AsO_4^- + 3H^+ + 2e^-$ can take place between sampling and analysis and has to be avoided. The standard redox potential of this reaction is +0.56 V (Brett & Brett, 2000). Oxygen alone is not able to oxidize the arsenic. Other redox couples like,
$Fe^{3+} + e^- \leftrightarrow Fe^{2+} + 0.77$ V together with $O_2 + 4H^+ + 4e^- \leftrightarrow 2H_2O + 1.23$ V are essential (Brett & Brett, 2000). The hypothesis is that, ferrous ions are oxidized by dissolved oxygen and the ferric ion can oxidize the arsenide. Other redox partners like Mn^{2+} seem to be minor importance (Daus *et al*., 2000).

Water samples are not usually analyzed for trace elements immediately after collection. Several hours or often several days elapse between collection and analysis. During this time, the chemical nature of arsenic or other trace elements can change, can be lost by volatilization, can be adsorbed on container walls and can be precipitated. Another important question of sample storage is the temperature. So preservation conditions are essential for maintaining integrity and stability of environmental water samples that are a prerequisite for successful execution of analytical determination.

Various analytical techniques are now available for determination of inorganic arsenic in water (Samanta *et al*., 1999; Hung *et al*., 2004). Limited studies had been focused on stability and preservation of arsenic species in water samples prior to analysis (Benoliel, 1994; Kramer, 1994; Daus *et al*., 2002). Acidification followed by refrigeration at 4 ºC is well known for water arsenic analysis (Benoliel, 1994). But literature is not available yet from severely arsenic-affected Bengal delta (biggest arsenic calamity in the world) on sample treatments prior to analysis to get the actual arsenic concentration in the collected groundwater. In this literature, extensive studies were done on the stability of total arsenic concentration in groundwater with and without on site filtration/ acidification during collection of water samples, maintained at room temperature/ refrigerated condition throughout the experiments and finally each day variation of arsenic concentration (loss of arsenic) for a continued 30 days period, started from the day of water collection. The question of filtering water samples on site or not is more interesting. However, this depends strongly on the purpose of the analysis. Using the groundwater as drinking water (without any water cleaning procedure), the unfiltered samples

might be more interesting to get an intake dose of arsenic. This is true to know the amount of arsenic present with particles in water and arsenic present in dissolved form in water. This may help geologists but to know the health effects of the sufferers from arsenic toxicity, on site filtration is of no use.

Importance of sampling and sample storage with respect to West Bengal's arsenic calamity

Natural groundwater arsenic-contamination, and the sufferings of people as a result, has become a crucial water quality problem in many parts of the world, particularly in Bengal Delta, Bangladesh and West Bengal, India (Chowdhury *et al.*, 2000; Smith *et al.*, 2000; Chakraborti *et al.*, 2002). Groundwater arsenic contamination in the Bengal delta has been termed the largest chemical poisoning in history (Smith *et al.*, 2000). A large part of the Ganga-Meghna-Brahmaputra plain (GMB) with an area 569,749 km^2 and population over 500 million is at risk (Chakraborti *et al.*, 2004). The situation is at its worst in the Bengal delta, with over 100 million people (Chowdhury *et al.*, 2000; Chakraborti *et al.*, 2002) living in zones with arsenic above 0.05 mg L^{-1}, the Indian drinking water standard value. Fifty districts in Bangladesh (out of a total of 64 districts) and nine districts in West Bengal (out of a total of 18 districts) are arsenic-affected. Eighty-five blocks (police stations) and approximately 3500 villages in West Bengal have been identified where groundwater contains arsenic above 0.05 mg L^{-1} (Rahman et al., 2005). The area and population of the arsenic-affected districts in West Bengal are 38,865 km^2 and 50 million, respectively. About 50 and 25% of the water samples analyzed (n=129,000) from arsenic-affected districts of West Bengal contain arsenic above 0.01 and 0.05 mg L^{-1}, respectively (Chakraborti *et al.*, 2004). About 78% of the biological samples (urine, hair, nails, skin-scales, n=28,000) contain arsenic above the normal value and about 9.7% of the population (n=92,000) has been identified with clinical manifestations (Chakraborti *et al.*, 2004). Although, entire mass of the subject areas is not yet affected, but running at risk. National and International Funding Agencies have come forward to combat the situation and large sum of money are spent for the analysis of arsenic in groundwater. During the long involvement for arsenic analysis in West Bengal and Bangladesh, it was realized that mode of collection, preservation and time interval between collection and analysis are important factors for quality assurance. Due to improper sampling and storage, results were not identical to various groups working on this problem although they used the same analytical technique for analysis. The same water samples collected and analyzed by two laboratories showed different results. Ultimately it was realized that one of the laboratories collected the water samples without addition of preservative and as a result, soluble iron present in the tubewell water was precipitated as Fe(OH)$_3$ in contact with air, that co-precipitated arsenic. So when the supernatant liquid was analyzed, obviously most of the arsenic was in the residue. On the other hand, the unfiltered water sample showed higher concentration of arsenic, compared to the filtered water sample on site. The obvious reason was that the small invisible arsenic-bearing particles in unfiltered water sample were dissolved in nitric acid (used as preservative) producing high result, while after filtering through Millipore membrane (0.45 μm), those particles were arrested. So, automatically the result became low.

Considering these discrepancies of the analytical results of arsenic, it was realized that the mode of water collection, storage and time interval study between collection and analysis are to be studied further to get rid of analytical error. In this study, several factors influencing water samples stability and methods of preservation for analysis of arsenic are presented.

Materials and Methods

Water collection and storage

Change of total arsenic and iron concentrations under various conditions with time interval

Water samples were collected from the shallow hand tubewells used for drinking, cooking and other household purposes by the villagers from the arsenic-affected areas of Deganga and Hasnabad blocks in 24-Parganas (North) district, one of the nine arsenic-affected districts in

West Bengal, India. The samples were collected after pumping out a few litres of water from the tubewells for several minutes. The water samples were stored in pre-washed (1:1 HNO_3) polyethylene bottles (1 litre). One ml of HNO_3/litre of water was used as a preservative, in the particular case (Chatterjee *et al.*, 1993). The mode of water sample collection and their preservation were studied under various conditions as shown below:

Set A:

i) Without acid, kept at room temperature (around 30 ℃ throughout the experiment), filtered through Millipore membrane filter (0.45 μm) before analysis.
ii) Without acid, kept at room temperature, not filtered before analysis.
iii) With acid, kept at room temperature, filtered before analysis.
iv) With acid, kept at room temperature, not filtered before analysis.

Set B:

i) Without acid, kept in cooled icebox during transportation and finally in a refrigerator (4 ℃), filtered through Millipore membrane filter (0.45 μm) before analysis.
ii) Without acid, kept in cooled icebox and refrigerator (4 ℃), not filtered before analysis.
iii) With acid, kept in cooled icebox and refrigerator (4 ℃), filtered before analysis.
iv) With acid, kept in cooled icebox and refrigerator (4 ℃), not filtered before analysis.

Set C:

i) Without filtration during collection, followed by addition of acid, kept in cooled icebox during transportation and finally in a refrigerator (4 ℃).
ii) Filtered through Millipore membrane filter (0.45 μm) during collection, followed by addition of acid, kept in cooled icebox and refrigerator (4 ℃).

The samples were measured for total arsenic and iron concentrations on each day for a continued 30 days study (Set A and Set B) or for a single time, based on the collection of the same sample on different day (nine to thirteen experiments) within a time period (2-4 months, several days interval between each collection, Set C). The time interval between collection of water sample from the field and analysis in laboratory is about 10 hours. To compare with the experimental results under various conditions, the arsenic and iron concentrations of the same water samples were also measured under ideal condition. In this case, the water samples were collected after filtration with Millipore membrane filter (0.45 μm), followed by addition of concentrated nitric acid (1 ml HNO_3/litre of water), stored in a cooled icebox (4 ℃) and analyzed immediately after bringing back to laboratory (within around 10 hours from the collection time).

Variation of total arsenic and iron concentrations with time interval

Water samples were stored in pre-washed (1:1 HNO_3) polyethylene bottles (100 ml), followed by addition of preservative (1.0 ml of HNO_3/litre of water) and measured the variation of total arsenic and iron concentrations with time under following parameters:

Type (X): Round the year study for 20 tubewell waters after every 15 days interval.
Type (Y): One month study for one tubewell water sample on each day estimation.
Type (Z): Eight hours study for two tubewell waters after every 30 minutes interval.

Chemicals and reagents

All reagents were of analytical grade. A solution of 1.25% of sodium borohydride (Merck, Schuchardt, Germany) was prepared in 0.5% sodium hydroxide (Merck, Bombay, India) for FI-HG-AAS system. A 5.0 M solution of HCl (Merck, Bombay, India) was also used. Standard stock solutions of arsenite and arsenate (1000 mg L^{-1} as arsenic) were prepared by dissolving appropriate amounts of As_2O_3 and $Na_2HAsO_4.7H_2O$ (Merck, St. Louis, USA), were stored in glass bottles and kept in refrigerator. Dilute arsenic solutions (aliquots) were prepared daily for analysis. A mixture of 1:1 arsenite and arsenate solution was used as a standard for flow injection system. Distilled de-ionized water was used throughout. Details of the chemicals,

reagents and glassware were described in earlier publications (Chatterjee *et al.*, 1995; Das *et al.*, 1995; Samanta *et al.*, 1997). For spectrometric analysis of arsenic, a filtered solution of 0.5% Ag-DDTC in chloroform with 3% hexamethylene tetramine was used. Concentrated HCl, 15% KI solution, 40% $SnCl_2$ solution (in concentrated HCl), granulated Zn were also used. Details of the reagents and glassware were described elsewhere (Chakraborti *et al.*, 1982; Roychowdhury, 1999). For spectrometric analysis of iron, hydroxylamine hydrochloride (for reduction of ferric to ferrous solution), 1:1 NH_3 solution (for adjustment of pH) and 1, 10-phenanthroline solution (for colour development) were used. The details of the methodology and reagents were available in literature (Fries *et al.*, 1977; Roychowdhury, 1999).

Analysis

Three sets of individual water sample were collected and processed for each experiment throughout and the mean concentration was considered. The total concentration of arsenic was determined both by FI-HG-AAS and spectrometric (using Ag-DDTC) methods. A Perkin-Elmer Model 3100 atomic absorption spectrometer (USA) equipped with Perkin-Elmer EDL system-2, arsenic lamp (current 400 mA) and Hewlett-Packard Vectra computer with GEM software and Varian AAS Model Spectra AA-20 with hollow-cathode lamp (current 10 mA) were used. The flow injection assembly consisted of an injector, Teflon T-piece, tigon tubing and other parts for the FI system from Omnifit UK. The peristaltic pump (VGA-76) from Varian and Minipuls 3 from Gilson were incorporated in to the FI system. Details of the instrumentation, optimization conditions and methodology of FI-HG-AAS system were described in earlier publications (Chatterjee *et al.*, 1995; Das *et al.*, 1995; Samanta *et al.*, 1997). By using modified spectrometric method, water samples containing high arsenic (above 40 μg L^{-1}) can be determined with 95% confidence, whereas the detection limit of arsenic for FI-HG-AAS system was 3 μg L^{-1} with 95% confidence. A Shimadzu double beam spectrometer, Model UV-150-02 (Japan) was used for spectrometric analysis of both arsenic and iron. Details of the instrumentation and methodology were described in earlier publications (Fries *et al.*, 1977; Chakraborti *et al.*, 1982; Roychowdhury, 1999).

Results and Discussions

Change of total arsenic and iron concentrations in water samples under various conditions with time interval

Set A and Set B:

Table 1 represents the loss of total arsenic concentrations in four water samples having different iron concentrations with time (after 7 and 30 days) under various conditions. Figure 1(a) represents the trend of loss of arsenic in the four water samples on each day measurement for Set A (i), carried on without addition of acid and kept in room temperature throughout. From the Table 1 and Figure 1(a), it appears that for all the four water samples, the arsenic concentration decreases with time and higher the iron concentration, higher the loss of arsenic. The loss of arsenic might be an effect of the high pH values in the solutions that led to a precipitation of iron hydroxides. For Set A (ii), the results are almost identical to that of Set A (i). The obvious reason is that, in the collected bottle, arsenic was co-precipitated with iron and settled at the bottom of the container. The supernatant water sample was considered for arsenic measurement without disturbing the bottom residue. As a result, filtration through Millipore membrane filter is not so significant here. Thus, under this condition results of Set A (i) and Set A (ii) are almost identical. The important finding of this study is that the loss of arsenic depends upon the initial iron present in the water sample. The arsenic concentration rapidly decreases for the first two days, and then the increasing rate of loss of arsenic becomes slow and finally almost constant after 15 days, for most of the cases. In the case of iron, 91 and 99% are lost within 10 hours and one day, respectively. Figure 2(a) shows the trend.

Further investigations were studied under refrigerated condition. Figure 1(b) represents the trend of loss of arsenic in the water samples on each day measurement for Set B (i), carried on without addition of acid and kept in refrigerated condition throughout. It has the same trend as of Set A (i), but with somewhat lesser loss of arsenic. Figure 2(b) shows the trend of iron. About 87 and 99% iron are lost within 10 hours and one day, respectively. In this case, co-precipitation of arsenic also occurs with iron, but not so rapidly like Set A (i) and A (ii), due to maintaining the refrigerated condition. Set B (ii) is almost identical with Set B (i).

Table 1. Analytical results of arsenic and iron concentrations in the studied water samples under various conditions

Tubewell water	Experiment number	With/without acid	Room temp./refrigerated	Filtered/not filtered (F/NF)	No. of days observed	Conc. under ideal condition		Final concentration		Loss of As (%)	
						As (mg L^{-1})	Fe (mg L^{-1})	As (mg L^{-1})	Fe (mg L^{-1})	After 7 days	After 30 days
W-1	Set A (i), A (ii)	Without acid	Room temp.	F/NF	30	0.57	1.17	0.27	0.10	39.1	53
Do	Set B (i), B (ii)	Without acid	Refrigerated	Do	30	0.57	1.17	0.40	0.10	20.9	30.4
Do	Set A (iii), A (iv)	With acid	Room temp.	Do	30	0.61	1.15	0.43	1.15	13	28.7
Do	Set B (iii), B (iv)	With acid	Refrigerated	Do	30	0.54	1.20	0.46	1.20	2.83	15.6
W-2	Set A (i), A (ii)	Without acid	Room temp.	Do	30	0.25	3.52	0.11	0.10	40.7	55.7
Do	Set B (i), B (ii)	Without acid	Refrigerated	Do	30	0.25	3.52	0.15	0.10	32.8	38.7
Do	Set A (iii), A (iv)	With acid	Room temp.	Do	30	0.25	3.45	0.18	3.45	13.5	28.9
Do	Set B (iii), B (iv)	With acid	Refrigerated	Do	30	0.24	3.60	0.19	3.50	5.14	18
W-3	Set A (i), A (ii)	Without acid	Room temp.	Do	30	0.62	8.0	0.15	0.10	64.8	75
Do	Set B (i), B (ii)	Without acid	Refrigerated	Do	30	0.62	8.0	0.27	0.10	41.9	56
Do	Set A (iii), A (iv)	With acid	Room temp.	Do	30	0.49	8.25	0.35	8.10	14.1	28.3
Do	Set B (iii), B (iv)	With acid	Refrigerated	Do	30	0.55	9.50	0.45	9.50	6.3	18.9
W-4	Set A (i), A (ii)	Without acid	Room temp.	Do	30	0.38	10.8	0.025	0.10	87	93.5
Do	Set B (i), B (ii)	Without acid	Refrigerated	Do	30	0.38	10.8	0.08	0.10	70.1	79.2
Do	Set A (iii), A (iv)	With acid	Room temp.	Do	30	0.41	10.5	0.29	10.5	14.6	29.3
Do	Set B (iii), B (iv)	With acid	Refrigerated	Do	30	0.41	11	0.33	10.9	7.0	19.5

These results clearly show that most of the arsenic in water is lost due to co-precipitation with iron (almost 100% loss of iron) within 0-2 days and rate of co-precipitation of arsenic is directly proportional to the initial iron concentration. A small amount of arsenic is also lost due to the adsorption on plastic container surface/ precipitation with some other elements/ volatilization from the medium *etc*. Thus, arsenic concentration in groundwater should not be analyzed without the addition of preservative and dissolved iron in water plays an important role which is directly associated with arsenic. In general, iron is a common element in arsenic-contaminated waters. Mine waters often have high iron concentrations in addition to arsenic contamination (Williams, 2001). Most of the arsenic in the groundwater of United States has their source in Fe-oxides (Welch *et al*., 2000). A positive correlation between the concentrations of arsenic and iron was also found in the groundwater and borehole sediments of Bengal delta (Roychowdhury *et al*., 1999; Chakraborti *et al*., 2001; Meng *et al*., 2001).
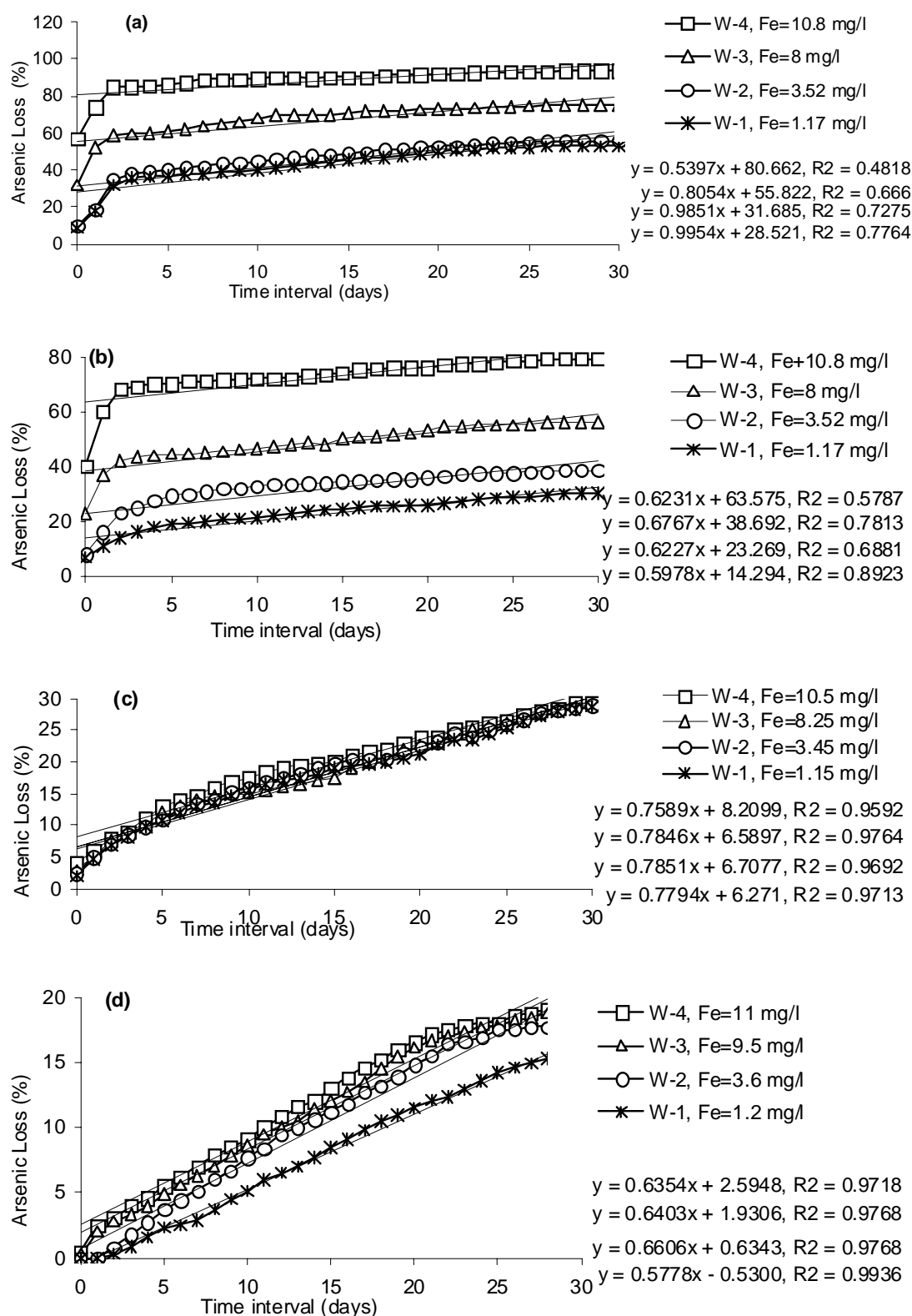
Figure1. Loss of arsenic in waters having different Fe concentrations with time, under various conditions (a) without acid, room temperature, FM/NFM; (b) without acid, refrigerated, FM/NFM; (c) with acid, room temperature, FM/NFM; (d) with acid, refrigerated, FM/NFM.

Figure 1(c) represents the trend of loss of arsenic in the water samples on each day measurement for Set A (iii), carried on with addition of acid and kept in room temperature throughout. The loss of arsenic is 13-15%, 17-20% and 28-29% after 7, 15 and 30 days respectively, whereas the loss is 2-9% within first three days for all the water samples from the day of collection (Figure 1c and Table 1). Thus, 91-98% arsenic is recovered if analyzed within 3 days. Further, filtration through Millipore membrane filter is not so significant here. Because the water samples treated with filtration and without filtration before analysis do not show any appreciable change of both arsenic and iron concentrations. The obvious reason is that hydrolysis does not occur in this case due to the presence of iron in acid media throughout. Figure 2(c) shows the trend of iron. The iron concentration remains almost same during the whole period. Thus, Set A (iii) is almost identical with Set A (iv). But the loss of 28-29% of arsenic after 30 days is due to the adsorption on plastic container surface/ precipitation with some other elements/ volatilization from the medium etc.

Figure 1(d) represents the trend of loss of arsenic in the water samples on each day measurement for Set B (iii), carried on with addition of acid and kept in refrigerated condition throughout. It has the same trend as of Set A (iii), but with somewhat lesser loss of arsenic. The loss of arsenic is 3-7%, 8-13% and 15-19% after 7, 15 and 30 days respectively, whereas the loss is 0-4% within first three days for all the water samples from the day of collection (Figure 1d and Table 1). Thus, 96-100% arsenic is recovered if analyzed within first 3 days. Figure 2(d) shows that iron concentration remains almost same during the whole period. Set B (iv) is almost identical with Set B (iii). The loss of arsenic due to adsorption on plastic container surface/ precipitation with some other elements/ volatilization is lesser in this case, compared to Set A (iii) and Set A (iv), due to maintaining the refrigerated condition.

Regression analyses have been studied between loss of arsenic (%) and time interval study for the water samples (Figure 1). The linear regressions show good correlations for all the cases. But very good correlations are observed for the acidified samples (Figure 1c and 1d), compared to the non-acidified samples (Figure 1a and 1b). This is due to the fact that most of the arsenic was co-precipitated with iron within first two days for non-acidified water samples. About 73.2% (range: 60-91%) and 66.6% (range: 46-86%) of arsenic were lost within first two days at room temperature and refrigerated condition respectively, with respect to the total loss of arsenic after 30 days. Finally the increasing rate of loss of arsenic became slow for the rest of the period. That means there was a fluctuation of loss of arsenic at a certain stage in the solution. But in the case of acidified water samples, the loss of arsenic increased slowly throughout the whole period, maintaining a steady rate. About 25.3% (range: 24-27%) and 9.26% (range: 2-16%) arsenic were lost within first two days at room temperature and refrigerated condition respectively, with respect to the total loss of arsenic after 30 days. Thus, it is expected that some other factors were also responsible for loss of arsenic in acidified waters, as co-precipitation of arsenic with iron does not occur in this case.

Loss of arsenic and its average deviation on different days (0, 1, 2-10, 11-20, 21-30) in the four water samples having different iron concentrations (1.17, 3.52, 8 and 10.8 mg L^{-1}) under various conditions is shown in Table 2. Regression analyses have been carried out between the iron concentrations and loss of arsenic (%) in the water samples with time (Figure 3). The linear regressions show very good correlation in the case of non-acidified water samples, both under room temperature (Figure 3a) and refrigerated condition (Fig. 3b). The result clearly indicates that the loss of arsenic in non-acidified water is dependent upon the presence of initial iron concentration and it is directly proportional to the iron concentration. On the other hand, satisfactory correlations are not observed for acidified water samples, both under room temperature (Fig. 3c) and refrigerated condition (Figure 3d), compared to non-acidified samples. The mean concentrations of the average deviations of loss of arsenic in non-acidified samples having different iron concentrations (with a wide range of variation) on different days (under room temperature and refrigerated conditions) are 17.4 and 15.9%, respectively (Table 2).
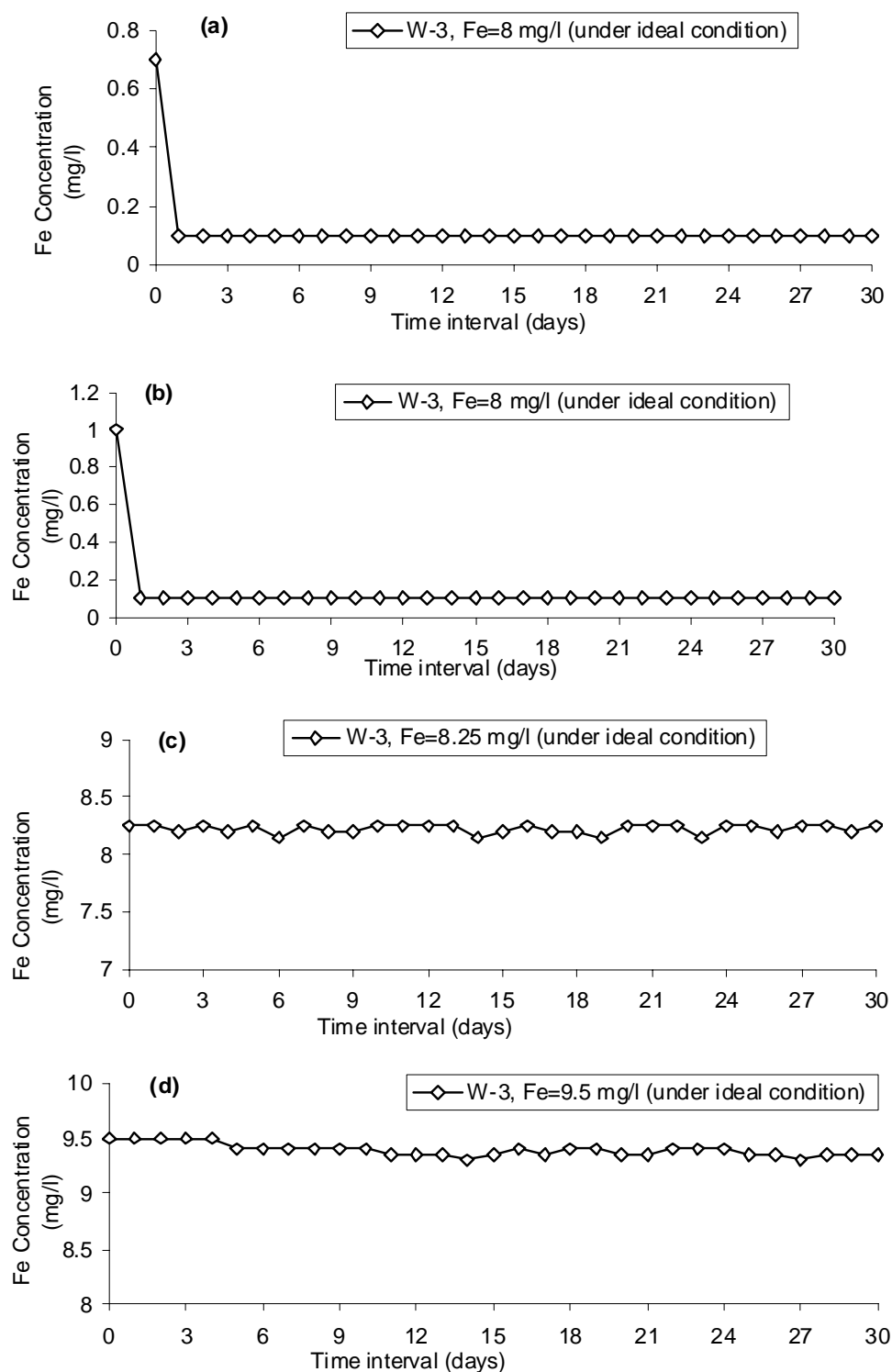
Figure 2. Variation of iron concentrations in water samples with time, under various conditions (a) without acid, room temperature, FM/NFM; (b) without acid, refrigerated, FM/NFM; (c) with acid, room temperature, FM/NFM; (d) with acid, refrigerated, FM/NFM.

The values for acidified water samples are 0.52 and 1.06%, respectively. Such kind of small values of average deviation of loss of arsenic for acidified water samples having a wide range of iron concentrations is only possible when the loss of arsenic is independent of iron

concentration. Several other factors like adsorption, precipitation with other elements, volatilization might be responsible for loss of arsenic in this case.

Set C:

The experiment was carried out to know the amount of arsenic coming out in dissolved form or a portion in particulate form with groundwater. The experiments were carried out with two different aged tubewells.

Model A: newly installed tubewells (within one year)
Model B: tubewells installed more than one year.

For both Model A and Model B, water samples were collected with and without filtration, followed by addition of concentrated nitric acid and refrigeration. The same water samples were collected and analyzed for arsenic on nine different days within a period of four months from two tubewells (Model A) and on thirteen different days within a period of two months from three tubewells (Model B).

Model A:

The experiment was carried out for two tubewells, 1 month (≈ 50 m depth) and 11 months old (≈150 m depth). Figure 4(a) and 4(b) show the variation of arsenic concentrations in water, with and without using Millipore membrane filter during collection from the tubewells, 1 month and 11 months old, respectively. About 166% (up to five months from the installation) and 23.5% (up to fifteen months from the installation) average higher results of arsenic were observed for the water samples that were not filtered through Millipore membrane filter compared to the filtered, collected from the two tubewells, 1 month and 11 months old, respectively. Much higher result of arsenic (about 223% on an average) were observed for the initial period (up to three and a half months from the installation) for the water samples, collected from the one month old tubewell and finally the value gradually decreased to 51% for the next one and a half months period. A good amount of arsenic was coming out through a large number of small particles (invisible through naked eyes) that increased the arsenic level of unfiltered water samples, especially for the newly installed tubewells. These arsenic-bearing particles were arrested in the case of filtered water samples. It was reported that the particulates presence in pumped groundwater of South-western United States contributed much of the arsenic, based on a comparison of arsenic concentration in filtered and unfiltered samples (Welch *et al.*, 1998). Similar findings were observed in other cases also (Daus *et al.*, 2002). With time, less and less particles were coming out with pour water. To get a more clear idea, Model B experiment was carried out.

Model B

The experiments were carried out for three tubewells, 2, 6 and 9 years old, respectively. The depths of these tubewells were ≈ 50 m. Figure 5(a), 5(b) and 5(c) show the variation of arsenic concentrations in water samples, with and without using Millipore membrane filter during collection from the tubewells, 2, 6 and 9 years old, respectively. About 11.7, 12.4 and 12.4% of average higher results of arsenic were observed for the water samples which were not filtered through Millipore membrane filter compared to the filtered from the three tubewells, respectively. Thus, it appears that for older tubewells, arsenic concentration does not change appreciably before and after filtration. With time, the number of particles coming out with water was decreased and it was within a certain limit. That is why the results were quite similar for the three different aged tubewells.

Regression analyses have been carried out between arsenic concentrations in unfiltered and filtered water samples from the newly installed tubewells as well as from older tubewells (Figure 6). No correlation is observed in the case of 1 month old tubewell (Figure 6a). Whereas, some kind of correlation is found in the case of 11 months old tubewell (Figure 6b), but very

good correlation is found for older tubewells (Figure 6c). Thus, it appears that arsenic-bearing particles were coming out randomly with pumped water for an initial period after installation of the tubewell, which was responsible for higher concentration of arsenic. But with time (within 1-2 years), its appearance becomes controlled in pumped water.
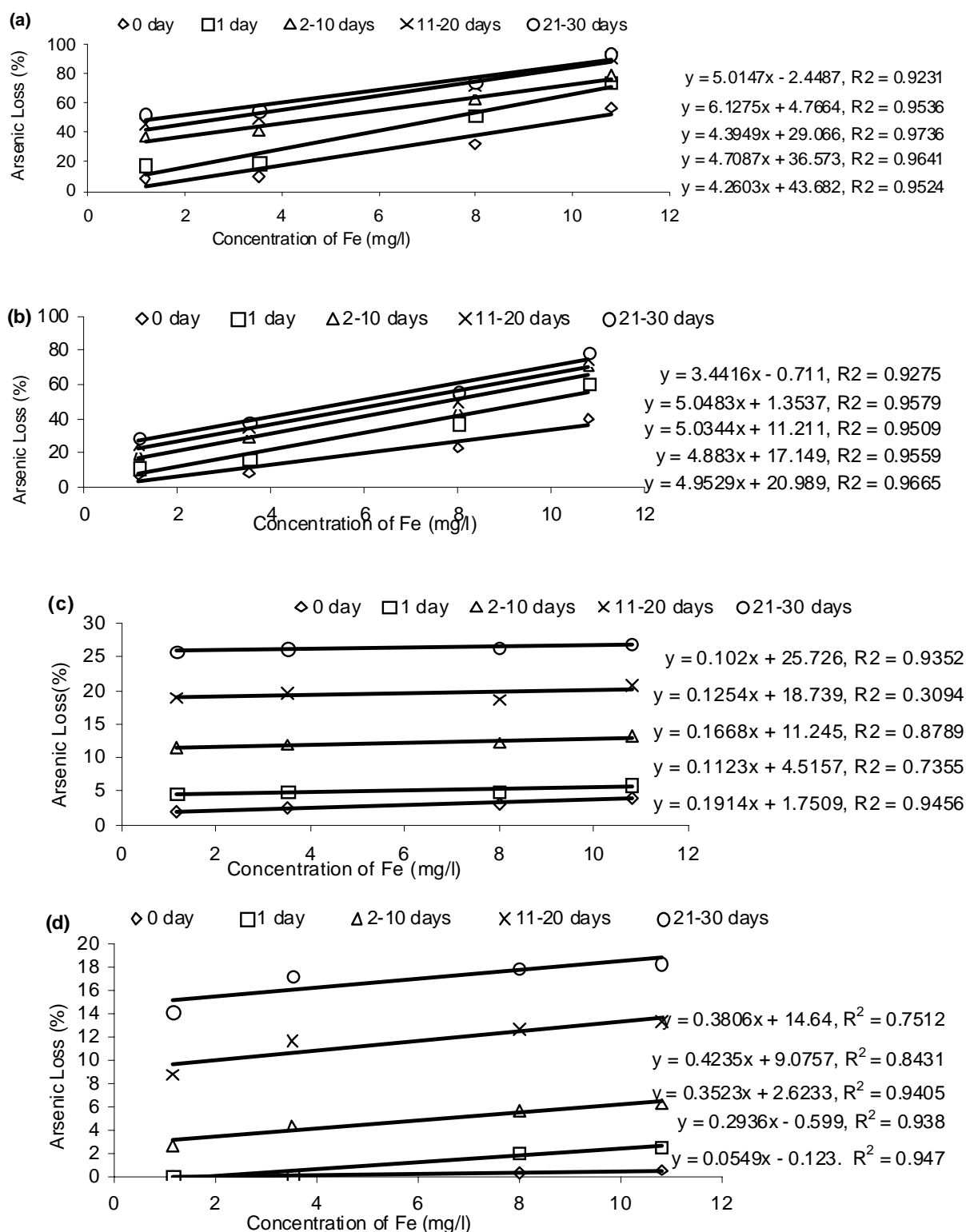
Table 2. Loss of arsenic on different days in the water samples having different iron concentrations under various conditions

Experiment	Type	Fe (mgL^{-1})	Loss of arsenic (%)								
			0 day	Av. Dev.	1 day	Av. Dev.	2-10 days	Av. Dev.	11-20 days	Av. Dev.	21-30 days	Av. Dev.
(a)	Without acid, room temperature	1.17	9	17.5	18	22.2	37.4	15.7	45.8	16.6	52.5	15
		3.52	10		19		40.9		49.4		54.9	
		8	32		52		62.7		71.2		74.4	
		10.8	57		74		78.5		90.5		93	
(b)	Without acid, refrigerated	1.17	7	12	11	17.5	18.9	16.7	24.5	16.3	28.8	16.8
		3.52	8		16		29.2		34.6		37.8	
		8	23		37		44.6		49.9		55.3	
		10.8	40		60		70.4		74.3		78.4	
(c)	With acid, room temperature	1.17	2	0.62	4.7	0.41	11.5	0.54	18.9	0.72	25.8	0.32
		3.52	2.5		5		11.9		19.6		26.2	
		8	3		5		12.2		18.6		26.4	
		10.8	4		6		13.3		20.8		26.9	
(d)	With acid, refrigerated	1.17	0	0.2	0	1.12	2.64	1.21	8.75	1.4	14.1	1.39
		3.52	0		0		4.33		11.6		17.2	
		8	0.3		2		5.59		12.6		17.9	
		10.8	0.5		2.5		6.21		13.3		18.3	

From all these study results, filtration (through Millipore membrane filter) followed by acidification and finally refrigeration is strongly recommended for analysis of arsenic and iron in groundwater for quality assurance. But for collection of a large number of water samples per day in the field, filtration followed by acidification and finally refrigeration needs a long time and also a cooling system. So for routine analysis, without filtration (except the newly installed tubewells) followed by acidification and without refrigeration, about 2-9% and 13-15% loss of arsenic (due to not maintaining the refrigerated condition) are considered if measured within first 3 days and after 7 days from the day of collection of water samples (considering also an average of 12% higher result of arsenic for unfiltered water samples).

Variation of total arsenic and iron concentrations with time interval
Table 3 shows the mean, range, standard deviation and variation of arsenic and iron concentrations of 20 tubewell water samples, round the year study for every 15 days interval (Type X), one month study for one tubewell water sample on each day estimation (Type Y) and eight hours study for two tubewell water samples after every 30 minutes interval (Type Z)

(a)

y = 5.0147x - 2.4487, R2 = 0.9231
y = 6.1275x + 4.7664, R2 = 0.9536
y = 4.3949x + 29.066, R2 = 0.9736
y = 4.7087x + 36.573, R2 = 0.9641
y = 4.2603x + 43.682, R2 = 0.9524

(b)

y = 3.4416x - 0.711, R2 = 0.9275
y = 5.0483x + 1.3537, R2 = 0.9579
y = 5.0344x + 11.211, R2 = 0.9509
y = 4.883x + 17.149, R2 = 0.9559
y = 4.9529x + 20.989, R2 = 0.9665

(c)

y = 0.102x + 25.726, R2 = 0.9352
y = 0.1254x + 18.739, R2 = 0.3094
y = 0.1668x + 11.245, R2 = 0.8789
y = 0.1123x + 4.5157, R2 = 0.7355
y = 0.1914x + 1.7509, R2 = 0.9456

(d)

y = 0.3806x + 14.64, R^2 = 0.7512
y = 0.4235x + 9.0757, R^2 = 0.8431
y = 0.3523x + 2.6233, R^2 = 0.9405
y = 0.2936x - 0.599, R^2 = 0.938
y = 0.0549x - 0.123. R^2 = 0.947

Figure 3. Regression plots between concentration of iron and loss of arsenic in waters with time
(a) without acid, room temperature, FM/NFM; (b) without acid, refrigerated,
FM/NFM; (c) with acid, room temperature, FM/NFM; (d) with acid, refrigerated,
FM/NFM.

Type (X): The variation of arsenic and iron concentrations of a water sample (No. 19 in Table 3), out of twenty, was shown in Figure 7(a). The average variation of arsenic and iron concentrations of 20 water samples were ± 20.5% (range: 11.8-30%) and ±20.3% (range: 3.77-39.9%), respectively.

Type (Y): The variation of arsenic and iron concentrations of the water sample (No. 21 in Table 3) was shown in Figure 7(b). The average variation of arsenic and iron concentrations were ± 11.2% and ±3.81%, respectively.

Type (Z): The variation of arsenic and iron concentrations of a water sample (No. 23 in Table 3), out of two, was shown in Figure 7(c). The average variation of arsenic and iron concentrations was ± 10.7% (range: 9.42-12%) and ±16.5% (range: 15.6-17.3%), respectively.
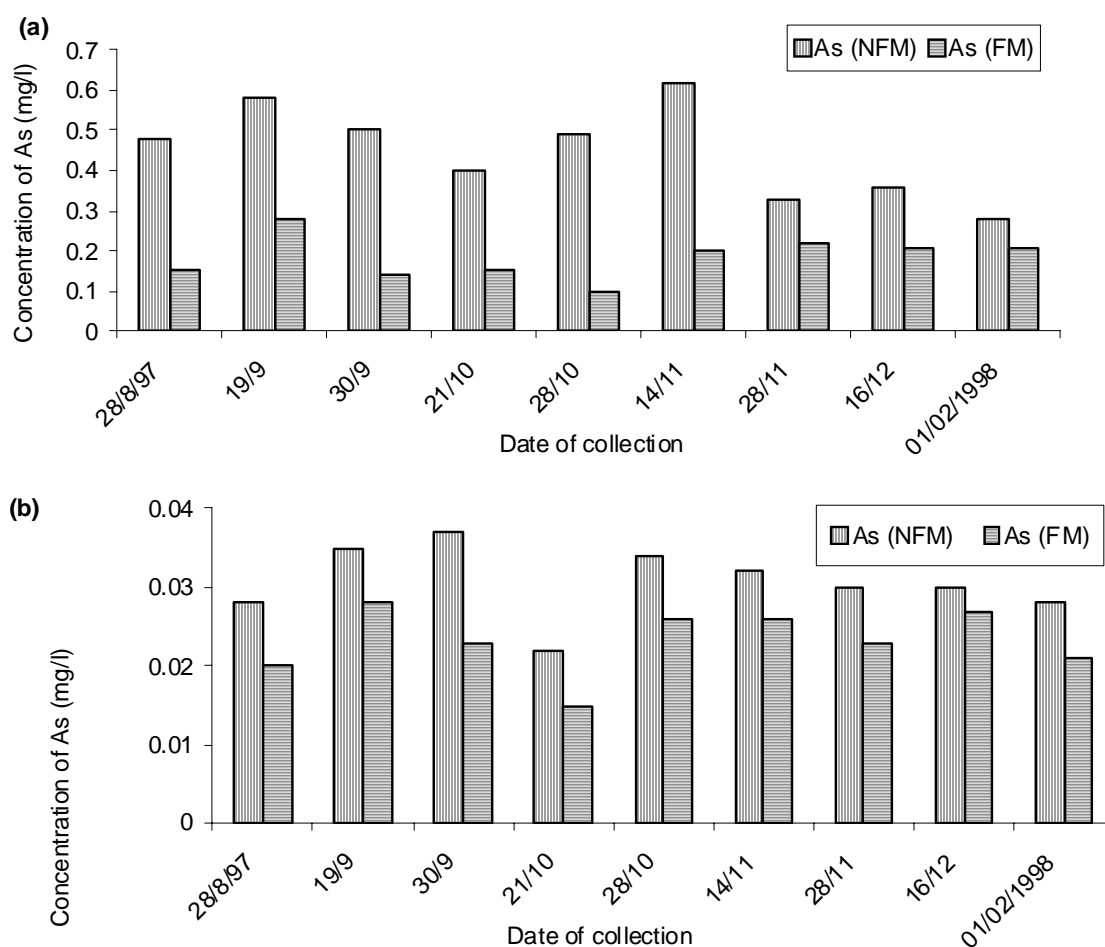
Figure 4: Variation of arsenic in water, with and without using Millipore membrane filter during collection from newly installed tubewells (a) 1 month old; (b) 11 months old.

(a)

(b)

(c)
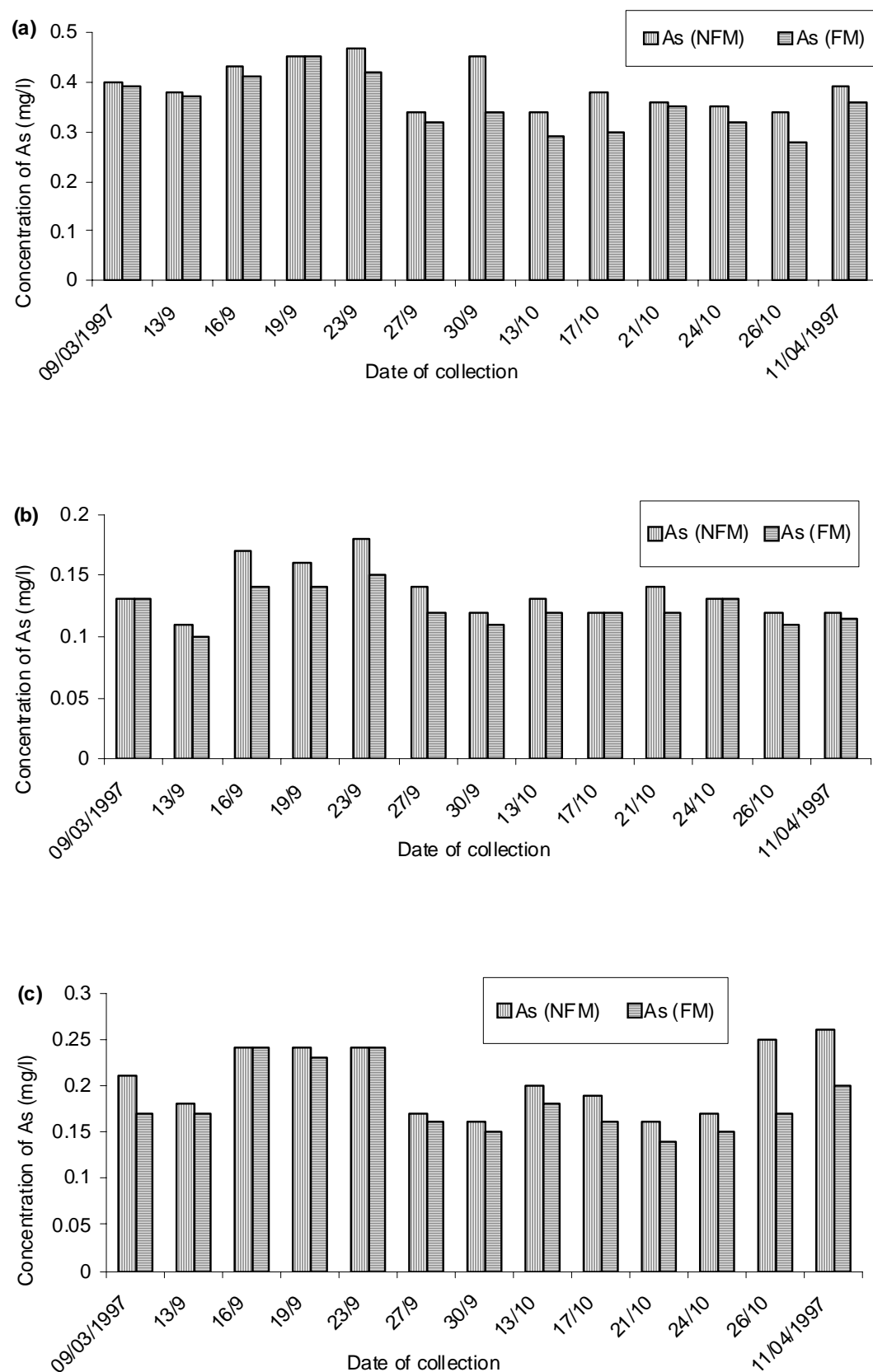
Figure 5. Variation of arsenic in water, with and without using Millipore membrane filter during water collection from the tubewells, used for long run (a) 2 years old; (b) 6 years old; (c) 9 years old.

Table 3. Parametric presentation of variation of arsenic and iron concentrations (mg L^{-1}) in water samples with time interval

Tubewell water	Depth (m)	Exp. No.	Time Interval	No. of obs.	Concentration of As				Concentration of Fe			
					Mean	Range	Std. Dev	Variation (± %)	Mean	Range	Std. Dev	Variation (± %)
No. 1	21.2	Type X	15 d	24	0.36	0.2-0.5	0.08	22.4	1.02	0.56-1.35	0.20	19.6
No. 2	21.2	Type X	15 d	24	0.31	0.21-0.48	0.07	24	3.17	2.59-4.4	0.46	14.6
No. 3	21.2	Type X	15 d	24	0.39	0.25-0.56	0.08	21.4	2.96	1.45-4.8	0.86	29
No. 4	21.2	Type X	15 d	24	0.19	0.12-0.31	0.04	23.3	1.21	0.7-1.95	0.39	32.2
No. 5	21.2	Type X	15 d	24	0.29	0.19-0.46	0.06	19.5	0.75	0.3-1.45	0.29	38.2
No. 6	21.2	Type X	15 d	24	0.17	0.11-0.25	0.03	19.4	1.45	0.95-2.35	0.41	28.3
No. 7	21.2	Type X	15 d	24	0.12	0.07-0.23	0.03	26.4	0.61	0.33-1.2	0.24	39.9
No. 8	30.3	Type X	15 d	24	0.20	0.13-0.31	0.04	22.1	1.99	1.25-3.3	0.49	24.6
No. 9	21.2	Type X	15 d	24	0.19	0.11-0.27	0.04	23.5	1.51	0.9-2.85	0.55	36.4
No. 10	21.2	Type X	15 d	24	0.33	0.24-0.47	0.05	16.2	5.03	3.85-6.6	0.63	12.6
No. 11	21.2	Type X	15 d	24	0.03	0.02-0.06	0.01	30	4.94	3.95-6.1	0.49	9.92
No. 12	21.8	Type X	15 d	24	0.47	0.31-0.67	0.07	15.2	0.95	0.35-1.3	0.25	26.1
No. 13	22.7	Type X	15 d	24	0.16	0.09-0.23	0.04	22.6	6.88	5.15-8.0	0.64	9.26
No. 14	21.2	Type X	15 d	24	0.21	0.13-0.32	0.04	20.7	3.32	2.55-4.0	0.35	10.7
No. 15	21.2	Type X	15 d	24	0.26	0.16-0.32	0.04	16.5	2.28	1.78-3.06	0.26	11.5
No. 16	25.4	Type X	15 d	24	0.14	0.10-0.23	0.03	24.1	3.95	2.35-5.9	0.80	20.2
No. 17	21.2	Type X	15 d	24	0.27	0.21-0.38	0.05	17.9	2.73	2.1-3.5	0.42	15.6
No. 18	21.2	Type X	15 d	24	0.18	0.14-0.23	0.02	14.1	5.50	4.3-6.15	0.58	10.5
No. 19	21.2	Type X	15 d	24	0.28	0.21-0.34	0.03	11.8	4.82	4.45-5.2	0.18	3.77
No. 20	30.3	Type X	15 d	24	0.32	0.22-0.43	0.06	18.1	3.87	3.05-4.8	0.49	12.6
No. 21	30.3	Type Y	1 d	30	0.56	0.45-0.65	0.06	11.2	8.22	7.25-9.25	0.31	3.81
No. 22	30.3	Type Z	30 min.	16	0.57	0.46-0.68	0.05	9.42	8.05	5-9.25	1.26	15.6
No. 23	22.7	Type Z	30 min.	16	0.14	0.12-0.17	0.02	12	7.74	7.1-12.3	1.34	17.3

From all the three experiments, an average ± 14% variation of both arsenic and iron concentrations in groundwater was observed whether studied for year or hour or minute. The variation included also experimental and personal error.
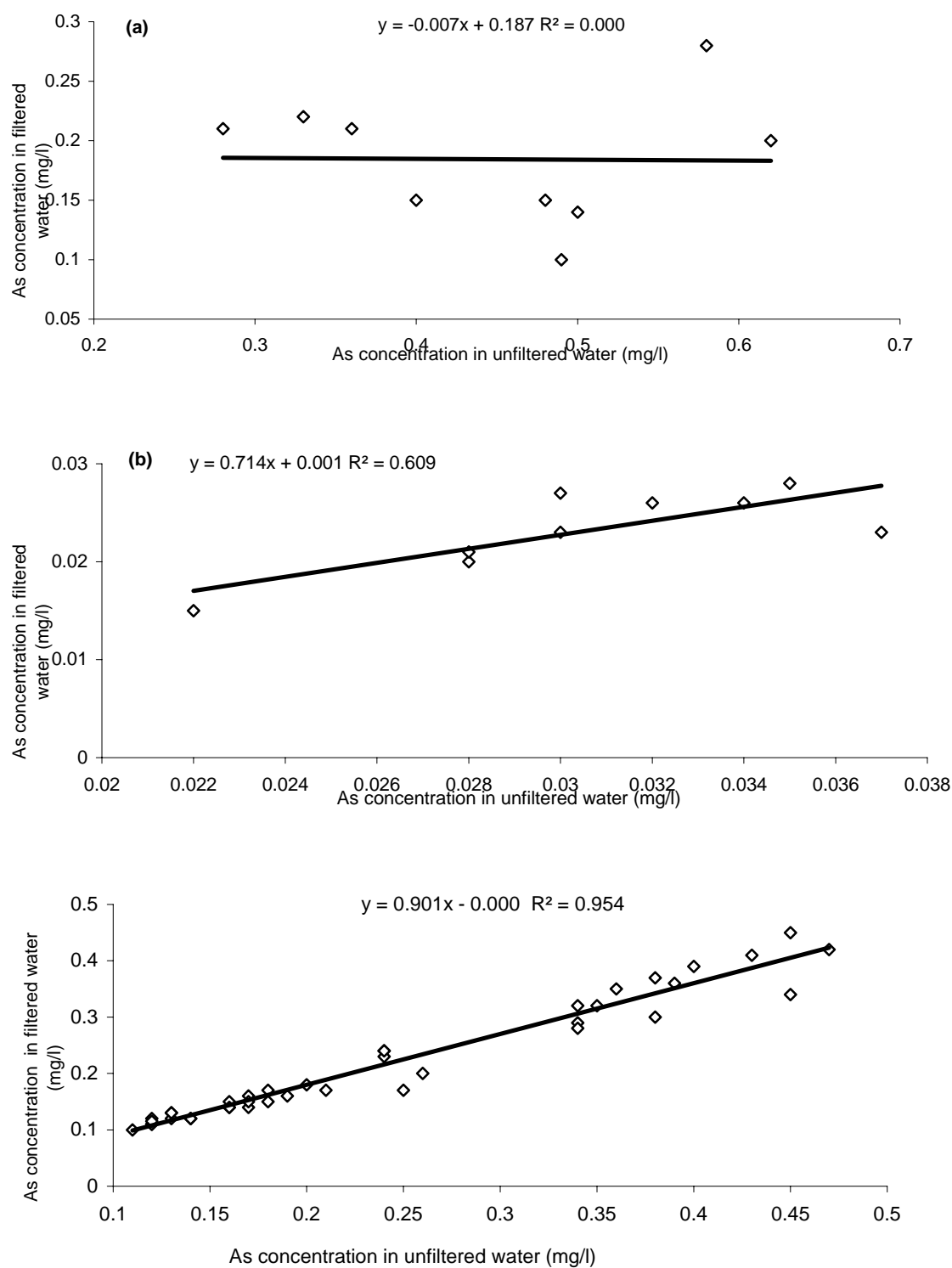
Figure 6. Regression plots between concentrations of arsenic in unfiltered and filtered waters from newly installed and older tubewells (a) 1 month old; (b) 11 months old; (c) older tubewells (2, 6 and 9 years old).
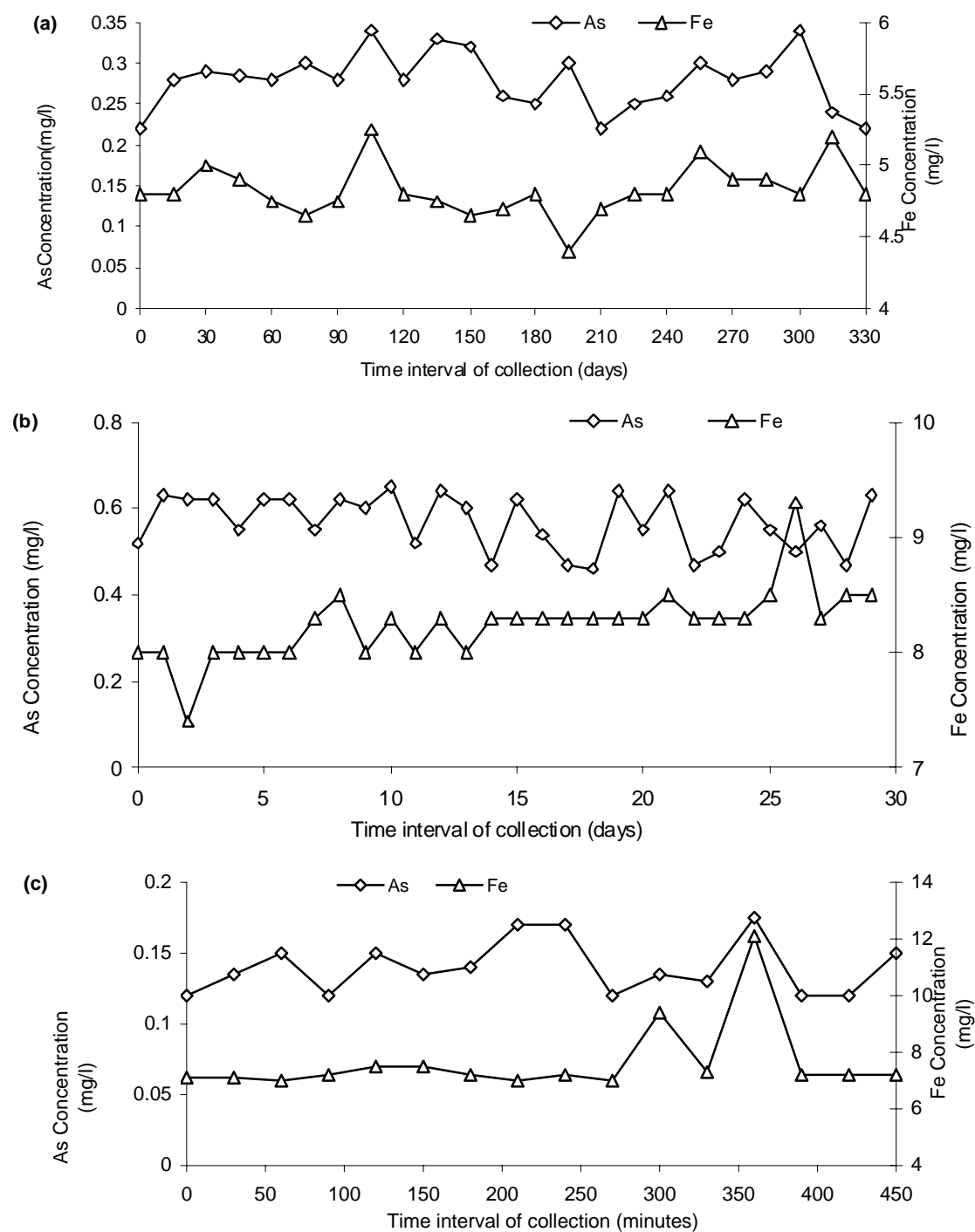
Figure 7. Variation of As & Fe concentrations in tubewell waters with time (a) 15 days interval, No. 19 in Table 3; (b) each day interval, No. 21; (3) 30 minutes interval, No. 23.

Conclusion

From all these experimental results, the following conclusions can be made:

- Loss of arsenic in non-acidified water is due to co-precipitation with iron. Soluble iron in water precipitates as $Fe(OH)_3$ in contact with air, that co-precipitates arsenic. About 99%

iron is lost from water under non-acidified condition within a day. Rate of co-precipitation of arsenic is directly proportional to the initial iron concentration present in the water.

- Loss of arsenic in acidified water is not dependent on iron concentration. Several other factors like adsorption, precipitation with other elements, volatilization might be responsible for loss of arsenic in this case.
- Refrigeration is prescribed throughout for water samples after addition of concentrated nitric acid (1 ml of nitric acid/ litre of water) for the analysis of both arsenic and iron. In this case, 96-100% of arsenic is recovered within first three days and the recovery is up to 93-97% even after 7 days.
- At room temperature, loss of 2-9% and 13-15% of arsenic were observed within first three days and after 7 days from the day of collection of acidified water samples, respectively.
- Just before analysis, filtration through Millipore membrane filter is not so significant for both acidified and non-acidified water samples.
- A good amount of arsenic was coming out through a large number of small particles, containing mainly colloidal iron hydroxides (invisible through naked eyes) from newly installed tubewells that increased the arsenic level of unfiltered water samples.
- For old tubewells (more than a year), if not filtered through Millipore filter, an average of 12% higher value of arsenic is expected due to the presence of arsenic-bearing particles in water. With time (within 1-2 years), its appearance becomes controlled in pumped water.
- For precise analysis of arsenic, on site filtration through millipore membrane filter (0.45 μm) followed by acidification and finally refrigeration at 4º C (during transportation as well as inside the laboratory) is recommended. But to know the health effects of the sufferers from arsenic toxicity, on site filtration is of no use.
- An average ± 14% variation of both arsenic and iron concentrations in groundwater was observed round the year.

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