

Original Research Paper

# Industrial Waste-an Economical Approach for Adsorption of Heavy Metals from Ground Water

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**Abstract:** A by product from steel industry mainly known as blast furnace slag is a waste product which was tested for the removal of As(III), from ground water. Steel slag is a commercial waste material mainly consisting of SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub> and CaO, the former two chemicals being major components of zeolites and the latter a major component of Hydroxyapatite (HAP). Arsenic is highly toxic, mobile and predominant species present in groundwater. Batch experiment was performed to determine the feasibility of steel industry waste as an adsorbent for treatment of heavy metal present in ground water at a wide range of pH 3-12. The results suggest that steel slag is a suitable candidate for As(V) remediation and economically viable to apply in the areas where cost of a purification process is high.

**Keywords:** Heavy Metal, Industrial Waste, Arsenic, Ground Water Contamination, Kinetic Study

## Introduction

Contamination of arsenic in ground waters has attracted worldwide attention. From many countries, incidents of arsenic contamination have been reported ([http://phys4.harvard.edu/~wilson/arsenic\\_project\\_articles.html](http://phys4.harvard.edu/~wilson/arsenic_project_articles.html)). Arsenic (As) is a highly toxic contaminant exists in our environment naturally as well as anthropogenic ally. Million of people are suffering from its contamination around the world, especially in many developing countries like Bangladesh, Nepal, India, China, Mongolia, Taiwan, Vietnam, Chile, Argentina, Romania (Feeney and Kounaves, 2000) etc. Due to the lack of low cost technology, the control of Arsenic contamination in developing countries has not yet been successful, which may create one of the most devastating situations in near future. There is an urgent need to find out low-cost, highly effective and sustainable technology to remove arsenic from the groundwater. To achieve that, the feasibility study of efficient and economical adsorbents made up of industrial materials such as blast furnace slag or steel slag will be utilized. In a recent article studied the systematic analysis of slag material mostly having oxides of calcium, iron, silicon and phosphorous. The equilibrium time is shown to be 2 h and the removal capacity is 99% with adsorbent loading of 1.25 g L<sup>-1</sup> under the experimental conditions (Chakraborty *et al.*, 2013). Using Steel slag

and other effective adsorbent for removal of the same should be considered as it is cheap as well as very effective. Some recent article has also mentioned the same concept of reducing the cost of the process as steel slag is cheaply available and is a waste product (Oh *et al.*, 2012). The US Environmental Protection Agency (EPA) has recently announced the reduction of permissible values of arsenic in drinking water from 50 to 10 µg L<sup>-1</sup> in the light of recent epidemiological evidence to support the carcinogenic nature of the ingested arsenic and its connection with liver, lung and kidney diseases and other dermal effects (EPA, 2001). Many groups are working on remediation technologies that will effect the reduction of arsenic to less than 10 µg L<sup>-1</sup> levels, in a cost effective manner with ease of operation that can be adopted at community or house-hold levels (NSF, 2015). Our main aim is to study the kinetic parameters of arsenic adsorbent with zero valent iron nanoparticle. The reason behind choosing this study is due to many different socio economic factors. Many different treatment technologies/methods including precipitation-coagulation, co-precipitation, ion exchange, electro-coagulation, oxidation and adsorption has already being used for as remediation. But out of them the adsorption method has received more attention due to its high efficiency and cost-effectiveness and is considered the most suitable

technology for developing countries. Metal oxides and hydroxides of iron or alumina (Bissen and Frimmel, 2003) are the most common adsorbents studied for the removal of As from water and wastewater. The steel slag is an industrial waste product contain iron nano-particle which has high surface area. It is a waste materials and cheaply available from the steel industry. It will not only make the process greener but also lower the footprint in the environment. However, there is an urgent need to develop low-cost alternatives due to the arising serious problem of arsenic contamination in groundwater in many developing countries such as Bangladesh, India and Nepal. This is our motivation to do this study for the development of cost effective mechanism in respect to our country's socio-economic conditions.

### *Causes of Contamination*

Arsenic contamination of the groundwater is believed to be caused by the dissolution of geological deposits containing iron, which had trapped arsenic. In addition, environmental arsenic contamination from uncontrolled industrial discharges, use of arsenical pesticides/herbicides and power generation from coal or geothermal sources also contribute to the arsenic contamination (Oh *et al.*, 2012).

The biological effects of arsenic depend mainly on the chemical form in which the element is ingested, the route of entry, the dose and the duration of exposure. Inorganic As(III) and (V) are identified to be more toxic than its organic forms. The arsenic is built up through intake of food or potable water contaminated with arsenic. In water, arsenate is more prevalent in aerobic surface waters and arsenite is more likely to occur in anaerobic ground waters.

### *Treatment Technology*

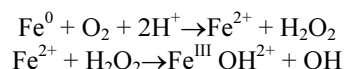
Variety of treatment technologies have been used for the removal of arsenic from water (Xu *et al.*, 2002). The common methods adopted for arsenic treatment include (i) co-precipitation (using  $\text{Fe}_2(\text{SO}_4)_3$  or  $\text{FeCl}_3$ ); (ii) coagulation (with ferric or aluminium salts such as  $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$  as coagulants); (iii) passing through activated alumina; (iv) ion exchange; (v) use of adsorption media like activated carbon, ferric oxide, titanium oxide, bone charcoal, iron oxide/ $\text{MnO}_2$  coated sands, cellulose materials (saw dust and news paper pulp), steel slag etc.; (vi) the use of zero valent iron [http://www.sciencedirect.com/science?\\_ob=ArticleURL&\\_udi=B6TGF-4378T8B-9&\\_user=1521315&\\_coverDate=06%2F29%2F2001&\\_alid=223968761&\\_rdoc=6&\\_fmt=full&\\_orig=search&\\_cdi=5253&\\_sort=d&\\_st=13&\\_docanchor=&\\_acct=C00053471&\\_version=1&\\_urlVersion=0&\\_userid=1521315&md5=62e7ac04a33ae79c1e194e35f7c353ba](http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TGF-4378T8B-9&_user=1521315&_coverDate=06%2F29%2F2001&_alid=223968761&_rdoc=6&_fmt=full&_orig=search&_cdi=5253&_sort=d&_st=13&_docanchor=&_acct=C00053471&_version=1&_urlVersion=0&_userid=1521315&md5=62e7ac04a33ae79c1e194e35f7c353ba) in the presence of sulphate; and (vii) reverse osmosis and electro dialysis.

Despite the fact that a variety of treatment methods are available, the efficiency of these methods is not completely known. Many of them have been reported to be capable of removing arsenic to levels lower than  $50 \mu\text{g L}^{-1}$ . With the impending revision of the permissible levels to  $10 \mu\text{g L}^{-1}$  or lower, it is necessary to investigate remediation approaches that would consistently provide drinking water with arsenic at less than  $10 \mu\text{g L}^{-1}$  levels (<http://phys4.harvard.edu/~wilson/arsenic-conf.html>).

This study describes the development of a remediation approach based on the using the steel slag. This approach is simple, cost effective and produces water with total arsenic concentration after some time and the value is close to  $50 \mu\text{g L}^{-1}$ . We have used artificially added arsenic with various concentrations throughout our investigations.

### **Materials and Methods**

In this study the steel slag comes from the Pohang Steel Industry. It was treated with hydrogen peroxide at  $60^\circ\text{C}$  for 24 h to oxidize the adhering organic matter before use. In our work we use  $\text{H}_2\text{O}_2$  instead of other acids such as  $\text{H}_2\text{SO}_4$  or  $\text{HNO}_3$ . If we see the reaction mechanism clearly we can see that there is a formation of  $\text{Fe}^{2+}$  and  $\text{H}_2\text{O}_2$  on the corroding  $\text{Fe}^0$  surface in turn forms OH radical (Voegelin and Hug, 2003; Joo *et al.*, 2004a):



After grounding, it was washed with distilled water to remove fine particles and was dried at  $100^\circ\text{C}$  for 12 h. It was then screened to get different geometrical sizes of 0 to 75, 75 to 180, 180 to 425, 425 to 600 and 600 to 1700  $\mu\text{m}$  for the adsorption study. The steel slag was prepared by adding hydrochloric acid onto the steel slag using the reported method (Apak *et al.*, 1998) and the material was stored in vacuum desiccators for further use. All other chemicals used in this study were of analytical grade, unless otherwise specified. Stock solution of As(III) were prepared from reagent grade  $\text{NaAsO}_2$ , Conc HCL and Potassium Iodide all from the Aldrich Chemical Corporation, Germany. All the chemicals used are of analytical reagent grade unless specified otherwise. The used De-ionised water which is made by instrument (Double Distilled DI water 18  $\text{m}\Omega$ .  $\text{NaBH}_4$  (Sigma-Aldrich Chemical, Germany) was freshly prepared in ice-cold water. After the sample were made we used the HGAAS-5100PC made by Perkin-Elmer Corporation, USA for determine the concentration of arsenic. We used polypropylene copolymer centrifuge tube made by Corning Corporation, USA. For all the materials, the leaching potential of pollutants using water was determined by a Korean standard test (Ahn *et al.*, 2003). In this test, 500 mL of deionized water (pH 5.8-6.2)

was added to 50 g of each material and mechanically shaken for 24 h. The concentrations of As and dissolved metals in the leachate were determined by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, Jobin Yvon 138 Ultrace).

As(III) adsorption studies: Stock solutions of 1000 mg L<sup>-1</sup> As(III) and As(V) were prepared by dissolving NaAsO<sub>2</sub> and Na<sub>2</sub>HAsO<sub>4</sub>·7H<sub>2</sub>O, respectively, in Deionized (DI) water. Batch adsorption of As(III) was studied in 50 mL polypropylene copolymer centrifuge tubes containing 200 mg steel slag (unless otherwise specified) in 20 mL of As(III) reaction solution as described previously (Manning *et al.*, 2002). The pH of the solution was not controlled from the beginning and monitored during the experiments. Equilibrium experiments were conducted for 72 h at 25°C (unless otherwise specified) in a shaken water bath (185 rpm) kept in the dark by covering with aluminum foil. The 72 h reaction time was shown to be adequate to attain equilibrium. After 72 h, the supernatant solution was filtered through a 0.45 µm membrane filter (Millipore), with a disposable syringe and analyzed by Hydride Generation Atomic Absorption Spectrophotometry (HGAAS; Perkin-Elmer 5100 PC) with a detection limit of 1 µg L<sup>-1</sup> As. As(III) was the initial form of As in all experiments (unless otherwise specified) and total As (AsT) was measured after adsorption with steel slag. As(III) and As T concentrations were also measured in certain experiments using an anion exchange cartridge method and competing anionic experiments and speciation of As(III) studies were performed as described in our previous report (Manning *et al.*, 2002). All the experiments has been performed at least 3 times in order to make it reproducible and standard error has been calculated which is within the range of 5-7%.

### Sample Preparation and Instrumentation

First we take known amount of steel slag. After adding Distilled (DI) water and known amount of arsenic we put the sample in the mechanical shaker (Universal Shaker US-RRA) in room temperature at 185 RPM for shaking all the solutions. After a frequent time interval we take the sample and filtered it through 0.45 µ pore size membrane filter and take the filtered sample in an another tube. With the required amount of sample in the tube and adding required amount of potassium Iodide (KI) solution and concentrated Hydrochloric Acid (HCL) and kept the solution for more than 1hr and 20 min to react. After that the sample was made and turns yellowish we took it to the Hydride Generator Atomic Adsorption Photo spectrometer (HGAAS-5100 PC) and the concentration of the As was measured.

### Instrumentation

The chemical composition of the steel slag was determined by the Philips PW 2400 X-Ray Fluorescence

(X-RF) analyzer. The steel slag material was characterized by powder X-Ray Diffraction (XRD) using a Rigaku diffractometer and monochromatized CuKα radiation (generator tension = 40 kV, current = 40 mA). Diffractograms were recorded from 5 to 85° (2θ) with a step size of 0.02° and a count time of 5 s per step. Morphological analysis of the samples was performed by Field Emission Scanning Electron Microscopy (FE-SEM) using a Hitachi S-4700 microscope (at 15 kV).

### Analysis

Arsenic (III) standard was prepared by dissolving arsenic powder at a room temperature. In this test, required amount of deionized water (DI-water) (pH 5.8-6.2) was added to a certain amount of each sample and mechanically shaken for 0, 2, 4, 6, 8, 10, 12 h respectively. The concentrations of As in the leachate were determined by HG AAS-5100 PC. For this HG AAS-5100 PC we need to prepare the Sodium Boro Hydride solution as a diductant and dilute hydrochloric acid as a Carrier by using the light acattering method we determine the concentration of Arsenic. The pH was not controlled during the test. At prescribed intervals (0,2,4, 6, 8,10,12) those 7 tubes (the 6 materials and one blank) were sampled and then filtered through 0.1 µm membrane filters and analyzed for residual As by HG AAS-5100. The pH was determined of all the solutions with a HORIBA D-24 pH meter. We take the value of the pH of the solutions after and before shaking and compare it with the other parameters of the test like percentage of adsorbent, rate constant, as well as the dose of the arsenic and the steel slag to determine the cost effective adsorbent. It seems that oxidation of As(III) will occur with time so we also study the time effect of the arsenic removal.

## Results and Discussion

### Characterization of the Adsorbent

The chemical composition of steel slag by XRF analysis is listed in Table 1. Steel slag is a complex heterogeneous material mainly composed of total iron (43.14%), CaO (35.43%), SiO<sub>2</sub> (10.08%), Al<sub>2</sub>O<sub>3</sub> (3.24%), MnO (2.52%) and MgO (2.06%). The XRD study also confirmed that the main components of slag were oxides of iron and calcium (Fig. 1). The Brunauer, Emmett and Teller (BET) results showed that the specific surface area and pore size of steel slag is SBET = 12.56 m<sup>2</sup>/g and 794 nm, respectively. The isoelectric point of steel slag in aqueous solution was measured by a light scattering instrument, which was found to be 3.2. Whereas (Lopez-Delgado *et al.*, 1998) also reported the isoelectric point of blast furnace sludge was 3.23. The morphological appearance of the steel slag using SEM is shown in Fig. 2A and B. In both

samples, a hexagonal shaped structure was clearly seen, which was due to the presence of iron oxide. Finally TEM-EDX analysis was performed on As-adsorbed steel slag (Fig. 3B) and its control (Fig. 3A) to further confirm the As adsorption on steel slag. The spectrum showed that there was no As on the control sample (Fig. 3A), however, the As-treated steel slag contained different elements such as As, O, Fe, Ca, Al, Si, S, K, Cu and C (Fig. 3B), where the appearance of a C and Cu peak arose from the TEM grid (carbon coated copper grid). FTIR was used to find out the adsorption of As(III) on steel slag (Fig. 4). It was found that as the concentration of As(III) increased from 0 to 1000 mg L<sup>-1</sup>, the peaks at 426, 474 and 510 cm<sup>-1</sup> were shifted to 428, 477 and 516 cm<sup>-1</sup>, respectively, whereas the peaks at 2359 and 2520 cm<sup>-1</sup> were shifted to 2330 and 2359 cm<sup>-1</sup>, respectively.

Table 1. Chemical composition of blast furnace slag

Chemical composition of blast furnace slag (STEEL SLAG) by XRF analysis

| Contents                       | %weight |
|--------------------------------|---------|
| Fe <sub>2</sub> O <sub>3</sub> | 43.14   |
| SiO <sub>2</sub>               | 10.08   |
| Al <sub>2</sub> O <sub>3</sub> | 3.24    |
| TiO <sub>2</sub>               | 0.48    |
| ZnO                            | 0.21    |
| SO <sub>3</sub>                | 1.35    |
| MnO                            | 2.52    |
| MgO                            | 2.06    |
| CaO                            | 35.43   |
| V <sub>2</sub> O <sub>5</sub>  | 0.26    |
| K <sub>2</sub> O               | 0.09    |
| SrO                            | 0.04    |
| P <sub>2</sub> O <sub>5</sub>  | 0.91    |

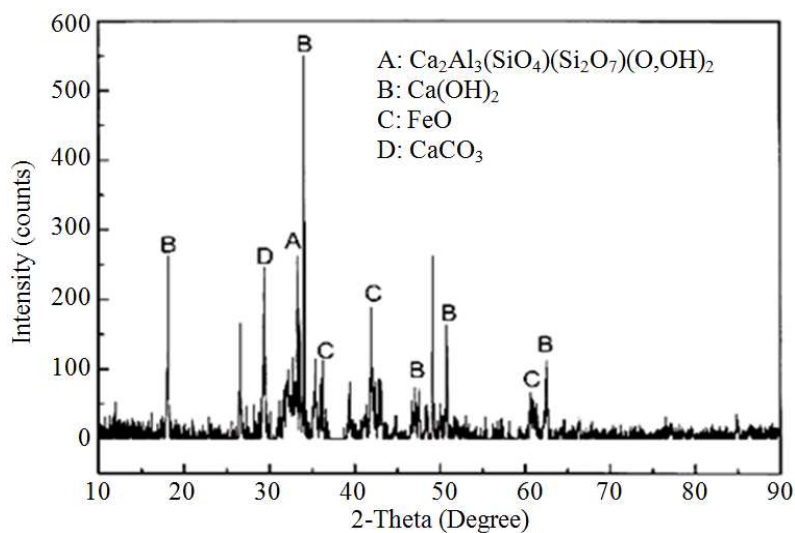


Fig. 1. Main components of the waste steel slag

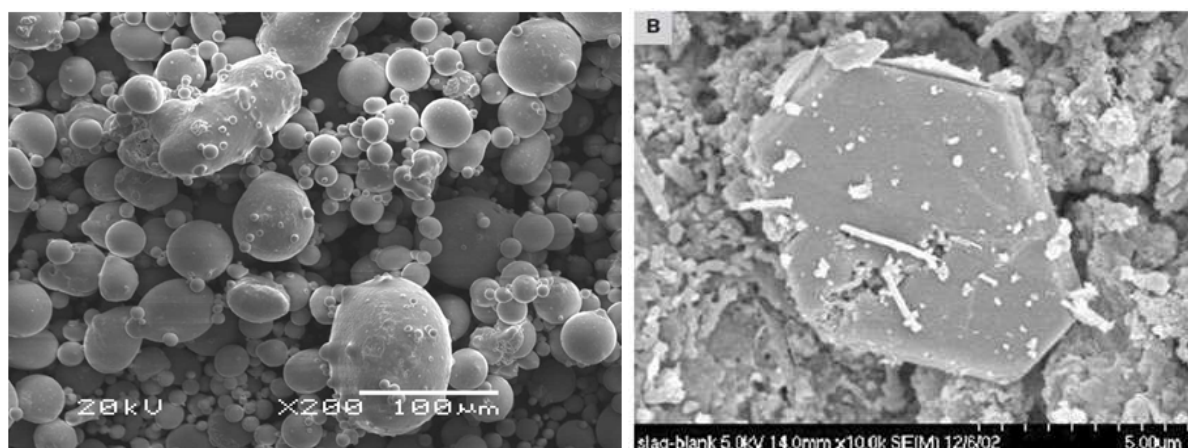


Fig. 2. SEM images of the steel slag

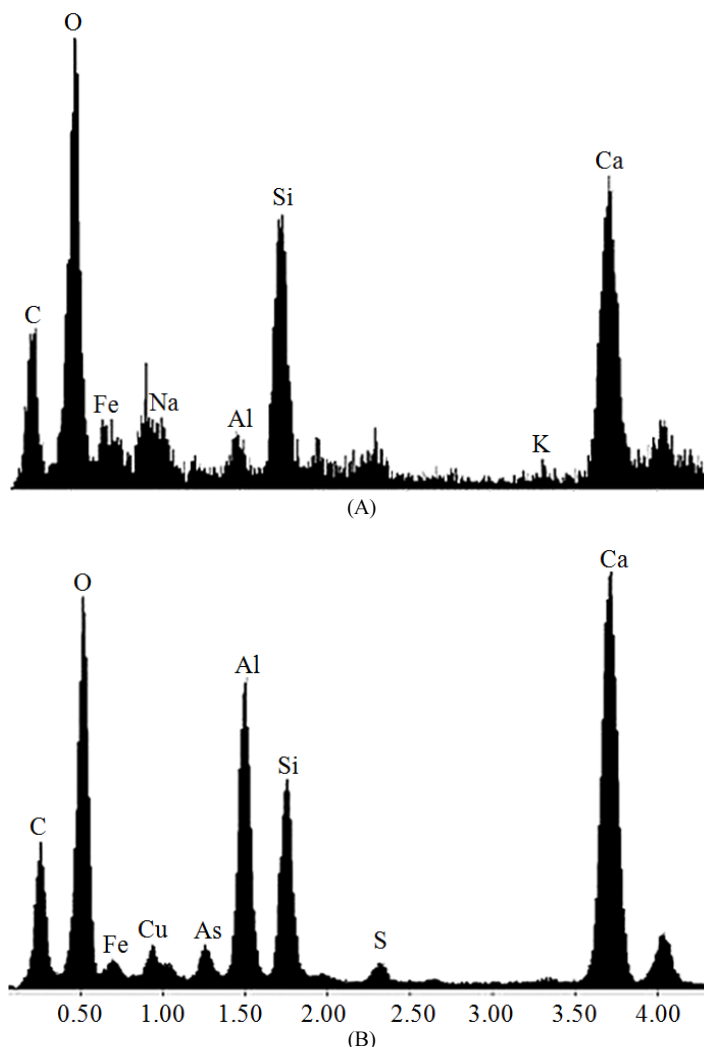


Fig. 3. (A, B): TEM-EDX analysis of adsorbed steel slag

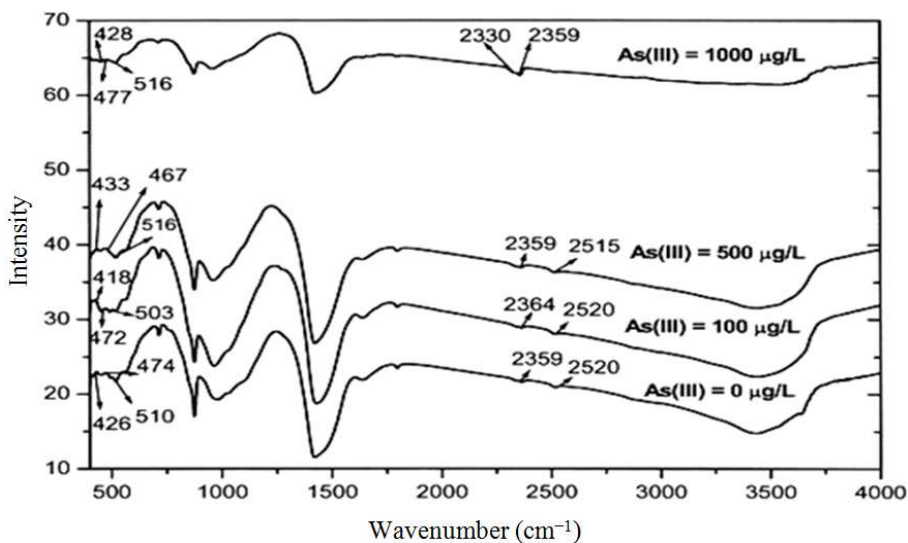


Fig. 4. FTIR image of the steel slag after As adsorption

### Dose Effect

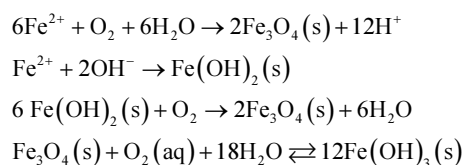
The data was taken from a sample with the adsorption capacity of arsenic and the percentage adsorption (Table 2) which describe about the dose of the arsenic and the adsorption capacity by steel slag and we put the values in the Fig. 5 which describe that adsorption is increases with the increases of dose of Steel slag and this is due to the increases of surface area of the Steel slag i.e., adsorbent. The As(III) adsorption is enhanced with increasing the amount of Steel slag and it is dependent on the effective surface area of the bound slag (Ahna *et al.*, 2003). The significant decrease of effective specific surface area for steel slag is probably due to the clogging of the microspores by the excess deposit of the bound aluminum species.

### Effect of pH on As(III) Adsorption

The dependence of removal percentage for As(III) upon pH of the aqueous solution is shown in Fig. 6 by using two dosages of Steel slag. The adsorption by Steel slag is found slightly dependent on the initial pH in the range 3-10 (Ahna *et al.*, 2003; López-González *et al.*, 1994; Joo *et al.*, 2004b).

The final pH of the sample was taken and plotted on the Fig. 6 for various samples and we compare it with the adsorbent capacity to determine the effect of pH in this experiment.

The As(III) removal mechanism is mainly due to spontaneous adsorption and co-precipitation of As(III) with iron(II) and iron(III) oxides/hydroxides, which form in-situ during ZVI oxidation (corrosion) (Manning *et al.*, 2002; Farrell *et al.*, 2001; Charlet and Manceau, 1993). The oxidation of ZVI by water and oxygen produces ferrous iron (Ponder *et al.*, 2000):



Fe(II) further reacts to give magnetite ( $\text{Fe}_3\text{O}_4$ ), ferrous hydroxide ( $\text{Fe}(\text{OH})_2$ ) and ferric hydroxide ( $\text{Fe}(\text{OH})_3$ ) depending upon redox conditions and pH: Heterogeneous reactions at the corroding ZVI surface are complex and result in a variety of potential adsorption surfaces for As(III) and As(V).

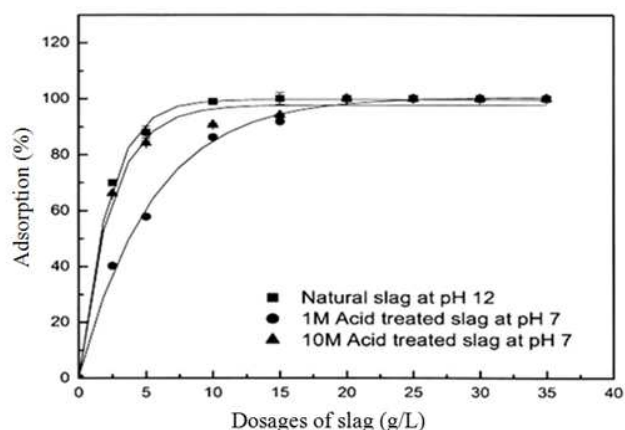
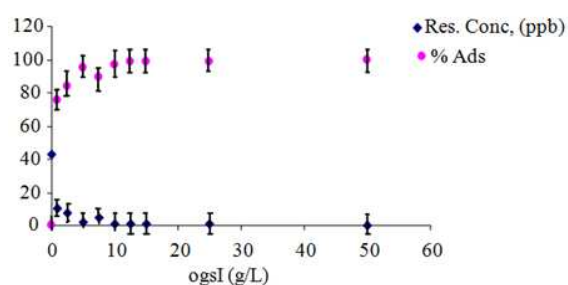


Fig. 5. Sorption of As(III) wrt doses of OGS in g/L

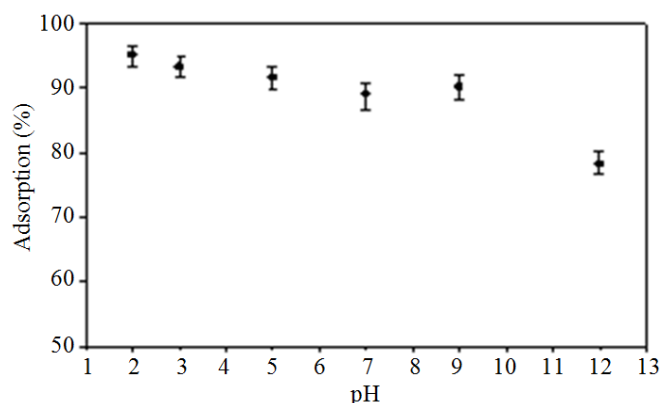


Fig. 6. pH effect of As(III) adsorption by steel slag

Table 2. Dose effect on steel slag

| Amount (g) of steel slag (20 mL) | Dose (g/L) | Res. Conc, ppb | % Ads |
|----------------------------------|------------|----------------|-------|
| 0.00                             | 0.0        | 42.4400        | 0.00  |
| 0.02                             | 1.0        | 10.5800        | 75.07 |
| 0.05                             | 2.5        | 7.0290         | 83.44 |
| 0.10                             | 5.0        | 2.0898         | 95.06 |
| 0.15                             | 7.5        | 4.5620         | 89.25 |
| 0.20                             | 10.0       | 1.3500         | 96.83 |
| 0.25                             | 12.5       | 0.5800         | 98.64 |
| 0.30                             | 15.0       | 0.6200         | 98.55 |
| 0.50                             | 25.0       | 0.5600         | 98.68 |
| 1.00                             | 50.0       | 0.2400         | 99.43 |

Despite this complexity, studies using X-ray absorption spectroscopy showed that the products after reaction of As(III) and As(V) with steel slag contain iron nano particle were inner sphere As(III) and As(V) surface complex oxides/hydroxide corrosion products (Farrell *et al.*, 2001; Charlet and Manceau, 1993).

From the Fig. 6 we can say that the pH of the solution is increasing with decreasing of the amount of As(III). This is due to (-Ve) charge gain in as well as in the Steel slag (Voegelin and Hug, 2003; Joo *et al.*, 2004a; Apak *et al.*, 1998; Ahn *et al.*, 2003; Manning *et al.*, 2002; Le and Ma, 1997; Morin *et al.*, 1991; Cantrell *et al.*, 1995; Su and Puls, 2001; Bothe Jr. and Brown, 1999). This is either due to preferential binding of arsenate with metal ions or higher diffusible characters of the interfering anions than arsenate present in water. Although a further precise mineralogical study is needed to verify the above results, iron oxides, the dominant components in steel mill waste materials (Steel slag), may become significant As adsorbents under near-neutral pH conditions.

### Kinetic Analysis

In order to analyze the adsorption kinetics of As(III) onto steel slag from aqueous solution, the first order, pseudo-first order and pseudo-second-order kinetic equations were applied.

The first order kinetic equation is as follows:

$$\log\left(\frac{C_t}{C_0}\right) = -kt + b \quad (1)$$

where,  $C_0$  and  $C_t$  are the As(III) ions concentration at initial and time,  $t$  respectively.  $t$  the time ( h) and  $k$  the rate constant. The values for  $k$ , calculated from the slope of the respective linear plot of  $\log(C_t/C_0)$  versus  $t$  are 6.4, 3.4, 2.3  $h^{-1}$ , respectively.

From the first principle, the pseudo-first order rate expression is followed by:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (2)$$

where,  $q_t$  represents amount of dye adsorbed at any time  $t$  ( $g \cdot g^{-1}$ ) by steel slag and  $k_1$  is the pseudo-first-order rate constant ( $h^{-1}$ ). The integral linear form of Equation 2 is:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} \times t \quad (3)$$

Pseudo-first-order rate constant,  $k_1$  is evaluated by plotting  $(q_e - q_t)$  versus  $t$ . Expression for the pseudo-second order rate expression is followed by:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (4)$$

where,  $k_2$  was the pseudo-second-order rate constant ( $g \cdot g^{-1} \cdot h^{-1}$ ). The integral linear form of Equation 4 is:

$$\frac{t}{q_t} = \frac{1}{k_2 \times q_e^2} - \frac{t}{q_e} \quad (5)$$

Pseudo-second-order rate constant,  $k_2$  is evaluated by plotting  $(t/q)$  versus  $t$ . From the analysis of experimental data, it may clearly say that the adsorption is follow first order kinetics. The values of  $k$  and  $b$  have found to be 0.1  $h^{-1}$  and 0.25. It is observed that adsorption of As(III) onto steel slag was found time dependent as seen in Fig. 7. It was revealed that the adsorption was rapid in the first 30 min and then slowed considerably as the reaction approached equilibrium. The adsorption rate was found to accelerate with an increase in the amount of steel slag. The adsorption rate was described by a first-order equation, as demonstrated by the results in Fig. 7, plotted in a figure. The rate equation can be expressed for As(III) ions as follows (Bissen and Frimmel, 2003):

$$\text{Log}\left(\frac{C_t}{C_0}\right) = -kt + b$$

where,  $C_0$  and  $C_t$  are initial As(III) ions concentration and concentration at time  $t$ , respectively,  $t$  the time (h) and  $k$  the rate constant. The values for  $K$ , calculated from the slope of the respective linear plot of  $\log(C_t/C_0)$  versus  $t$  are 6.4, 3.4, 2.3  $h^{-1}$ , respectively. This rapid kinetics has significant practical importance as it will facilitate smaller reactor volumes ensuring efficiency and economy.

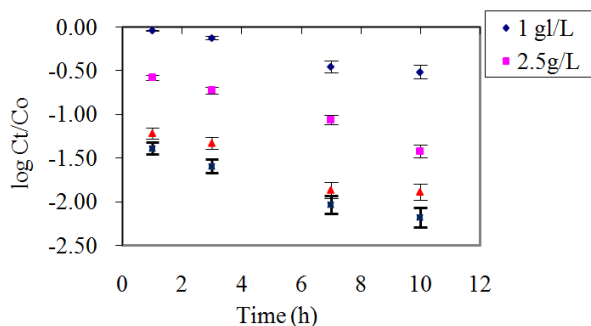


Fig. 7. Kinetic of adsorption

## Conclusion

We have presented evidence that As(III) can be removed efficiently without any pretreatment by adsorption on low-cost and widely available BFS. Hardened paste of steel slag has been shown to be an effective adsorbent for arsenic removal. In view of the study reported here it appears possible to remove arsenic almost quantitatively (>99%) from drinking water. Many kind of adsorbent were used before but after doing this experiment we find that the steel slag is another cost effective material that has a great capability to remove arsenic from ground water as well from other water sources. We have also presented evidence that As(III) can be removed by adsorption/precipitation on steel slag (at neutral pH) in a relatively short time of only several minutes. As(III) strongly adsorbs on NZVI over a wide pH range, through the co precipitation of various iron oxide corrosion products. Finally, the study results presented here have confirmed the potential of steel slag as an efficient material for the treatment of As(III) and one that may be used as a possible solution for both in situ and ex situ groundwater arsenic contamination removal remediation. Promising results, indicating that it has great potential to be used as an effective adsorbent for groundwater treatment in developing countries.

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## Author's Contributions

All authors equally contributed in this work.

## Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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