

# Simultaneous Removal of Iron and Hexavalent Chromium Via Slow Sand Filter Inoculated With *Thiobacillus ferrooxidans* and Waste management

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## Research Article

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# Abstract

Many places have common physical, biological, and chemical hazards and heavy metals contamination in drinking water is very important to study especially near industrial areas and mining sources. Application of microbes along with other physicochemical techniques can prove to be the suitable remediation technology for the removal of metals from water. This study aimed to remove iron and hexavalent chromium from water using slow sand filters inoculated with iron-oxidizing bacteria *Acidithiobacillus ferrooxidans*. An experiment was performed for 37 days under controlled conditions, a water sample was prepared synthetically and another sample was collected from water filtration plant from Abbottabad city in KP Province- Pakistan and influents and effluents were tested for different parameters like pH, turbidity, hardness, total plate count, total dissolved solids, electrical conductivity and analysis of iron and hexavalent chromium in water. *Acidithiobacillus ferrooxidans* culture was isolated from soil using the differential iron media i.e. 9K medium and was inoculated into sand filters. At the end of the experiment, the removal efficiency of ferrous iron was about 52% and that of hexavalent chromium was 60.2%. In simultaneous treatment, it was observed that removal efficiency of iron was about 68% while that of hexavalent chromium was about 93% suggesting that slow sand filters were more effective for the removal of hexavalent chromium than iron. Hence, the designed slow sand filters were able to reduce the concentration of hexavalent chromium more efficiently than ferrous iron. And it was observed that slow sand filters can effectively remove ferrous iron and hexavalent chromium from water if they are inoculated with respective bacteria.

**Key,words,.** Hexavalent Chromium, Iron Oxidizing, Sand Filters, *Acidithiobacillus ferrooxidans*, waste management.

## 1 Introduction

Availability of clean water for the community is a challenge for scientists and local authorities to provide low cost solution for safe drinking water production especially for developing countries (Abrar et al., 2020). In some regions, groundwater is no safer enough to drink directly. Many places have common physical, biological and chemical hazards and heavy metals contamination in drinking water is very important to study especially near industrial areas and mining source. Public health department has some routine analysis of metals before supplying to the community but it is in limited areas only (Qayyum et al., 2020). These heavy metals store in the body of human beings e.g., in lipids and the gastrointestinal system and can provoke health effects like cancer (Chowdhury et al., 2016). Many heavy metals e.g. copper, iron, zinc, cadmium, lead, chromium, mercury, nickel, cobalt, silver, manganese etc. have been reported in drinking water (Chowdhury et al., 2016) especially in those areas where mining practice is ongoing. Contamination of heavy metals in drinking water has many origins like rusting of pipes and water tanks made up of metal, industrial effluents, discarding of wastes of industries (Gowd and Govil, 2008), paints, battery and leather processing plants. Heavy metals migrate from surface water to groundwater from these industries (Krishna et al., 2009). One of the most important metals on earth's surface is iron that can be present in water in both soluble form (ferrous iron) and complex form (ferric

iron). The presence of iron in surface water may also have an industrial sources e.g. mining etc. (Chaturvedi and Dave, 2012). Iron is found not only in surface water but is also present in groundwater. Leaching from iron containing rocks is the main cause behind the contamination of iron in surface water (Tekerekopoulou et al., 2013). The acceptable iron concentration for drinking-water is 0.3 mg/L (Khatri et al., 2016). At initial stages, concentration of iron above this permissible level in drinking-water cannot be dangerous for health. On the other hand, non-stop intake of iron contaminated water can cause the impairment of haematopoiesis and may results in hemochromatosis, which damages different organs (spleen, liver, brain) of the body, if left untreated (Khatri et al., 2017). Other health issues like eye disorders (e.g. retinitis, conjunctivitis and choroiditis), cancer and cardiac disorders are also faced because of elevated iron concentration in the water (Alimohammadi et al., 2017). Chromium is found in environment in ores. It exists in two stable oxidation states, trivalent (III) and hexavalent chromium (VI). Cr (III) is one of the important trace elements i.e. essential for the metabolism of amino acids, glucose and lipids. From studies it is evidenced that metabolites of Cr (III) somewhat oxidize to cancer causing Cr (VI) and Cr (V) (Headlam and Lay, 2016). Cr (VI) is one of the major chromium species i.e. involved in different processes of industries. The permissible limit for total chromium comprising Cr (III), Cr (VI) and other forms and hexavalent chromium are 2 mg/L and 0.05 mg/L respectively according to drinking water guidelines by World Health organization (Gupta and Rastogi, 2009). Cr (III) is less toxic and mobile, whereas Cr (VI) is easily soluble and more toxic than trivalent. So, hexavalent chromium should be reduced to trivalent chromium to reduce adverse effects in body cell structures at increased concentrations. Cellular metabolism of Cr (VI) causes DNA damage. DNA single-strand breaks occur because of the metabolism of Cr (VI) that may cause cancers of body organs like liver, lungs and kidney (Pradhan et al., 3 2017). That's why hexavalent chromium is considered as carcinogenic.

Slow sand filters (SSF) are being manipulated since 200 years as a water purifying system (Haig et al., 2011). Slow sand filters (SSFs) because of their simple design and operation are reliable, flexible, and have relatively inexpensive installation and operation and are being successfully used for drinking water production from centuries (Calvo-Bado et al., 2003). It is dependent on the formation of microbial community on sand surface that consists of algae, bacteria, fungi, actinomycetes and protozoa etc. (Stewart-Wade, 2011). This layer is called as "Schmutzudecke layer" where pathogens and certain organic chemicals like pesticides are entrapped & degraded by microbes (Paranychianakis et al., 2006). SSFs require low flow rates and become more effective when Schmutzudecke matures or adjusts according to the particular concentration of particular pathogenic microbes or chemical to be broken down (Lee and Oki, 2013). The metal removal by inoculated sand filter has evidence that concentration of different metals like nickel, cadmium, zinc, copper, lead, etc. can be decreased via slow sand filtration (Diels et al., 2003). *Thiobacillus ferrooxidans* is a gram negative, acidophilic, chemoautotrophic bacterium that can oxidize ferrous iron. In different studies it has been discussed that *Thiobacillus ferrooxidans* has an ability to reduce toxic form of chromium i.e. hexavalent chromium to non-toxic trivalent chromium (Donati et al., 2003). In two different studies, *Thiobacillus* was used to treat iron and chromium separately. In current study both metals were tested together to see the removal by

*Thiobacillus ferrooxidans* inoculated sand filter. These filter were constructed on lab scale and effective removal of iron and chromium were analyzed through these sand filters.

## 2 Methodology

### 2.1 Design of slow sand filter column

Five different sand filters were prepared. Sand filter one was used as a control, second filter was for the treatment of iron, and third filter was for the treatment of chromium containing wastewater, fourth filter was used to treat the mixed synthetic wastewater containing both iron and Cr together at different concentrations. Last column was used only for iron and chromium mining wastewater.

### 2.2 Specification

Filter specification was as followed: Sand bed height 1m, diameter 4cm, pebbles at the bottom to retain sand 6inches, Sand particle size 0.2-0.6mm. Flow rate was adjusted with the help of peristaltic pump at 200 ml/ hr. Water inflow was from top of the column and outflow from the bottom. The sand filtration media was prepared by thoroughly rinsing the sand with water and emptying the supernatant several times to remove fine particulate matter. The washed sand was then air dried and autoclaved at 121°C for 15 minutes for sterilization then sterile sand was used to fill the column.

### 2.3 Bacterial isolation and inoculation of the sand filter

10 g of sample was mixed in mineral salts medium (MSM) as described by (Dopson and Lindstrom, 1999). Mineral salt medium (MSM) served as an essential medium for each liquid growing medium. Composition of MSM was (for 1L distilled water):  $\text{KH}_2\text{PO}_4$ (1gm)  $\text{K}_2\text{HPO}_4$ (1gm),  $(\text{NH}_4)_2\text{SO}_4$ (1gm), NaCl (15gm),  $\text{MgSO}_4$  (0.2gm),  $\text{CaCl}_2$ (0.017gm),  $\text{FeCl}_2$  (1gm). All the ingredients were dissolved in 1000 ml distilled water, sterilized at 121°C for 20 minutes. 10gm of soil sample was added in 250 ml of flask in triplicate and were kept in shaking incubator for 3 days at 30°C. After 3 days 10ml of old MSM media was added in 250 ml of new MSM media and incubated for 48 hours and then transferred to liquid 9K medium. In MSM media 1gm of  $\text{FeCl}_3$  was added as a substrate. Silverman and Lundgren 9K broth was used for the isolation of *Thiobacillus ferrooxidans* containing:  $(\text{NH}_4)_2\text{SO}_4$  (3.0g/L), KCl (0.1g/L),  $\text{K}_2\text{HPO}_4$  (0.5g/L),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.5g/L),  $\text{Ca}(\text{NO}_3)_2$  (0.01g/L),  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (44.3g/L) (Silverman and Lundgren, 1959). The pH of the medium was adjusted by using 10N  $\text{H}_2\text{SO}_4$  and 10N NaOH. The media was sterilized and then placed in incubator for about 24 hours to check contamination (Tehreem et al., 2020). Enrichment of microorganisms was carried out with the 100ml liquid 9k medium in 250ml Erlenmeyer flasks at 300°C in a shaking incubator at 180rpm for almost 1 week. For purification, solid 9K medium with 1.5% agar was used. The plates were incubated at 30°C for about 1 week. By repeating the above plating method, isolated bacteria were obtained. After purification, *Thiobacillus ferrooxidans* culture was added to the surface of sand filter.

Before addition of iron and chromium into the treatment filters, control was run. Distilled water was used as a control to initiate the experiment and treatment filter was not inoculated. After running control for 2

weeks, filters were inoculated with the isolated bacterial strains, after which synthetically prepared iron and chromium wastewaters were added into their respective treatment filters. The bacterial count added to the filters was kept changing throughout the experiment. The calculated hydraulic retention time (HRT, time taken by the influent to remain inside the reactor) was 2 days. The influent flowing into the iron treatment filter (ITF) was containing 200 mg/L ferrous iron while the influent flowing into chromium treatment filter (CTF) containing 500mg/L of hexavalent chromium (Cr6+). The effluent passing through the filters were collected on daily basis. Flow rate was adjusted at 200 ml/hr with the help of peristaltic pump.

#### 2.4 Cell Count by Neubauer Counting Chamber under microscope

Water sample was observed under microscope using Neubauer chamber. Bacterial count in the media was measured by using microscope. While using neubauer counting chamber, coverslip was placed on upper area or on blocks of chamber for keeping sample smooth. 0.1 ml Sample was poured with the help of dropper which is very minute amount. Counting chamber was observed under the microscope with 40X magnification. The vibrating or moving bacterial cell were counted inside chamber blocks. Bacterial cell was counted in per ml of the media. For the average cell count five different fields were randomly selected and observed (Strober, 2001). Numbers of cells was estimated by using formula: Total cells x 2.5x10<sup>5</sup> x dilution factor = cells/ml is shown in the Table 1.

Table 1  
Bacterial count added to filters every  
4th day

Days	Bacterial count (CFU/mL)
1	$2.4 \cdot 10^5$
5	$2.6 \cdot 10^5$
9	$3.1 \cdot 10^5$
13	$1.6 \cdot 10^7$
17	$2.5 \cdot 10^7$
21	$3.3 \cdot 10^7$
25	$5 \cdot 10^9$
29	$5.5 \cdot 10^9$
33	$5.7 \cdot 10^9$

#### 2.5 Preparation of Ferrous ammonium sulphate (FeSO<sub>4</sub>. (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. 6H<sub>2</sub>O)

Ferrous ammonium sulphate was prepared by dissolving hydrated ferrous sulphate,  $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$  (7grams) and Ammonium sulphate,  $(\text{NH}_4)_2\text{SO}_4$  (3.5grams) in water containing little quantity of Sulphuric acid,  $\text{H}_2\text{SO}_4$ , and passed resulted solution to crystallization where pale green crystals of ferrous ammonium sulphate,  $(\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O})$ , separated out.

## 2.6 Sample preparation and collection

Two types of samples were run through slow sand filter (SSF). Type 1 sample having different concentrations of iron and chromium separately were prepared in the laboratory and then mixed. Second type of sample was collected from mining wastewater discharge. A stock solution of iron, Fe was prepared by adding 20ml of conc.  $\text{H}_2\text{SO}_4$  to 50ml water and dissolving 1.404g of ferrous ammonium sulphate in it. Then 0.1M  $\text{KMnO}_4$  was added dropwise until a faint pink colour appeared and then solution was diluted to 1000ml with water and mixed. 1ml of this solution containing 200mg/L Fe. 2.5ml of the solution was measured exactly and added to the water to make 1000ml of the solution. 1ml of this solution contained 0.5mg/L of iron. Similarly, the stock standard solution of Cr was prepared by dissolving 1.4g of potassium dichromate,  $\text{K}_2\text{Cr}_2\text{O}_7$ , in 1000ml water. 1ml of this solution containing 50 $\mu\text{g}$ /L of Cr.

## 2.7 Spectrophotometric analysis

### 2.7.1 Iron analysis via 1, 10-phenanthroline method

The absorbance measurements were made with UV-Visible spectrophotometer (Federation and Association, 2005). Different reagents were used in this method like; Hydrochloric acid, HCl (containing less than 0.5ppm iron), Hydroxylamine solution: Prepared by dissolving 10g of hydroxylamine hydrogen chloride ( $\text{NH}_2\text{OH} \cdot \text{HCl}$ ) in 100ml water, Ammonium acetate buffer: Prepared by dissolving 250g of Ammonium acetate ( $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ ) in 150 ml water and 700ml conc. (glacial) acetic acid was added to it, Phenanthroline solution: Prepared by dissolving 100mg 1, 10- phenanthroline monohydrate ( $\text{C}_{12}\text{H}_8\text{N}_2 \cdot \text{H}_2\text{O}$ ) in 100ml water containing 2 drops of HCl. Potassium permanganate, 0.1M: Prepared by dissolving 0.316 Potassium permanganate ( $\text{KMnO}_4$ ) in 100ml distilled water, Stock iron solution: Prepared by dissolving 1.404g ferrous ammonium sulphate ( $\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ ) in 50ml water in which 20ml conc.  $\text{H}_2\text{SO}_4$  was also added. 0.1M  $\text{KMnO}_4$  was added dropwise till the persistence of faint pink color. Then the solution was diluted to 1000ml with water and mixed. 1ml of this solution containing 200mg/L iron.

### 2.8 Standard iron solution

Standard iron solution was prepared daily for use. 2.5ml stock solution was pipetted into 1000ml volumetric flask and was diluted to mark with distilled water. 1ml of this solution containing 0.5mg/L iron.

For analysis of total iron, sample was mixed thoroughly and 50ml was measured into a 200ml conical flask. 2ml conc. HCl and 1ml  $\text{NH}_2\text{OH} \cdot \text{HCl}$  was added. Solution was boiled until the volume reduced to 15-

20ml and was cooled and then transferred to 100ml volumetric flask. Then 10ml ammonium acetate and 4ml phenanthroline solution was added and diluted to mark with water, mixed thoroughly and allowed for about 10 minutes for maximum color development. The absorbance was checked at 510nm.

For ferrous iron, 100ml sample was acidified with 2ml HCl. 50ml from this acidified sample was separated to which 20ml phenanthroline solution and 10ml ammonium acetate solution was added with vigorous stirring and diluted to 100ml and color intensity was measured within 5–10 minutes at the wavelength of 510nm. Ferric iron was calculated by subtracting ferrous iron from total iron.

The concentration of total iron and ferrous iron was calculated by using the formula below:

$Y = mx + b$  (Y = absorbance, m = slope, x = concentration, b = Y-intercept)

### **2.8.1 Chromium analysis via 1, 5- diphenylcarbazide method**

The absorbance measurements were made with UV-Visible spectrophotometer having specification, at the wavelength of 540nm (Federation and Association, 2005). Different reagents used for this purpose were; Stock chromium solution; Prepared by dissolving 141.4mg potassium dichromate,  $K_2Cr_2O_7$  in 1000ml distilled water. 1ml of this solution containing  $50\mu\text{g Cr}$ .

### **2.9 Standard Chromium solution;**

10ml of stock chromium solution was diluted to 100ml with distilled water. 1ml of this solution containing  $5\mu\text{g Cr}$ . 18N Conc. Nitric acid ( $HNO_3$ ) and 6N Sulphuric acid, conc., Sulphuric acid, 0.2N; Prepared by diluting the 17ml 6N  $H_2SO_4$  to 500ml with distilled water, Phosphoric acid,  $H_3PO_4$ , conc, Diphenylcarbazide solution; Prepared by dissolving the 250mg 1, 5-diphenylcarbazide in 50ml acetone. NaOH (1N); Prepared by dissolving 4g of NaOH in 1L distilled water.

### **2.10 Color development and measurement:**

0.25ml/5 drops phosphoric acid,  $H_3PO_4$ , were added to 95ml water sample, 0.2N sulphuric acid and pH meter was used to adjust the pH  $2 \pm 0.5$ . Solution was transferred to 100ml volumetric flask and diluted to 100ml and mixed. 2ml diphenylcarbazide solution was added and left for 5–10 minutes for full color development. An appropriate portion was transferred to 1cm absorption cell and absorbance was measured 540nm using distilled water as a reference/ blank.

Calculation  $Y = mx + b$  (Y = absorbance, m = slope, x = concentration, b = Y-intercept).

Removal efficiency of ferrous iron and hexavalent chromium was calculated by the following formula:  
 $100 \cdot \text{Percentage removal (\%)} = \frac{Co - Ce}{Co}$  (Where Co = initial concentration of  $Fe^{2+}$  or  $Cr^{6+}$ , Ce = final concentration of  $Fe^{2+}$  or  $Cr^{6+}$ ).

### **2.11 Physio-chemical characteristics**

The multi-parameter meter, Model 520, was used for the measurement of pH, temperature, EC and TDS. The meter was standardized before use for measuring parameters. The probe of multi-meter was washed with distilled water before taking readings of effluents and influents and after use. For measurement of hardness of collected sample EDTA solution and Erichrome black T solution was prepared. EDTA solution was used as a titrant while erichrome black T was used as indicator. The hardness was calculated via following formula:

$$1000 \cdot \text{Hardness (mg/L)} = \text{EDTA used/volume of sample.}$$

The turbidity of water samples, influents and effluents were measured with the help of turbidity meter. The meter was calibrated or standardized before used and washed with distilled water after use (APHA, 2005).

Total plate count (TPC) Total plate count (TPC) in the effluent and influent collected from water filtration plant, Abbottabad was checked (Qayyum et al., 2020).. For this purpose nutrient agar was prepared by dissolving 28 grams of nutrient agar in 1000 ml distilled water and autoclaved at 121°C for 15 minutes. 1 ml of water sample was used as aliquot and spread on nutrient agar with the help of spreader in sterilized conditions and incubated for 24 hours. After 24 hours of incubation at 37°C, colony forming units were counted with colony counter.

## 3. Results And Discussion

### 3.1 Slow sand filter 1. Control

First slow sand filter setup was control setup that was run at room temperature i.e.  $25 \pm 5$ . In this setup distilled water was used as an influent that passed through slow sand filter. This setup was run without the bacterial inoculation and pH of the distilled water used was 6.6. This setup was run for about 14 days (2 weeks) and it was observed that effluent collected after filtration contained iron in a concentration of about 9.7 mg/L while there was no concentration of hexavalent chromium in the effluent Fig. 2

### 3.2 Slow sand filter 2 (Iron treatment)

During start of experiment the bacterial count was 2.4 CFU/mL. As initial concentration of ferrous iron was 200mg/L at first day of the experiment it was observed that the concentration of ferrous iron ( $\text{Fe}^{2+}$ ) in the effluent was same as that of the initial concentration and there was no decrease in iron concentration in the effluent. As the treatment filters were operated at room temperature, throughout the  $25 \pm 5^\circ\text{C}$ . At this time the pH was 2, as the treatment continued it was observed that till 6th day there was slow and gradual decrease in the ferrous iron concentration. At 7th day there was a sudden decrease in the  $\text{Fe}^{2+}$  concentration and it was decreased from 200mg/L to 161 mg/L. After that there was slow and stable decrease in concentration and at day 29 the concentration of  $\text{Fe}^{2+}$  measured was 106 mg/L and the iron removal efficiency at this point was 46.5%. On last day which was 37th day, the maximum decrease in iron concentration was 95mg/L where the bacterial count was  $5.7 \cdot 10^9$ . It was observed that the removal efficiency was maximum i.e. 52%. The mechanism of slow sand filtration involve physical as

well as biological activity. A researcher Nsengiyumva in his study suggested mechanical straining, sedimentation, adsorption and chemical and biological activity are the most well known processes of slow sand filtration (Nsengiyumva, 1997). One of the reason behind decrease in concentration can be the pores between the grain of sand that trap the ions resulting in the decrease in concentration as suggested in a study that sandbed i.e. made up of 0.15 mm grains will possess pores of about 20  $\mu\text{m}$  diameter (Paramasivam et al., 1981). A study conducted on removal of iron and manganese from natural groundwater by continuous reactor using activated and natural mordenite mineral adsorption revealed that the iron removal was much better than manganese removal. The maximum value of iron removal efficiency from natural mordenite was 2.79%/minute (Zevi et al., 2018) while in this study the value of iron removal efficiency was about 52% at last day of experiment which was recorded as the maximum value shown in Fig. 3.

### 3.3 Removal efficiency of ferrous ( $\text{Fe}^{2+}$ ) iron

Throughout the experimentation, it was observed that initially the removal efficiency of ferrous iron was very low till 4th day that was less than 1%. On 5th day, removal efficiency of ferrous iron increased to about 5%. After 1 week of continuous process, the removal efficiency was increased to about 19%. Slow sand filters were operated continuously and slow and stable increase in the removal efficiency was observed till 27th day of experimentation. On 27th day, the removal efficiency was about 39% and day 28th, it was increased to about 46% showing 46% ferrous iron was removed till 27th day. After that, from day 30 to 37 removal efficiency became stable and there was no increase in removal efficiency. On last day i.e. 37th day, removal efficiency was maximum i.e. about 52% means about half of the ferrous iron was removed till last day of experiment. A research project entitled “removal of iron from ground water by sulphide precipitation” suggested that 83% removal of iron from ground water, having initial concentration of 1 mg/L, was made possible by purifying samples of groundwater by hydrogen sulphide ( $\text{H}_2\text{S}$ ) under anaerobic conditions. It was observed that removal efficiency decreased to 80% and 75% with the increase in concentration of iron from 1mg/L to 3.55 mg/L and 5.01 mg/L respectively and the levels were higher than World health organization (WHO) standards for drinking water although the contact time was more than the former test (Jusoh et al., 2011). In this research the slow and stable increase in the removal efficiency in the concentration of ferrous iron was observed without changing the initial concentration and a point reached where there was not as much increase in the removal efficiency was observed in Fig. 4 that was in the last working days.

### 3.4 Slow sand filter 3 (Hexavalent Chromium treatment

At the start of experiment bacterial count added was 2.4 CFU/mL. As the initial concentration of hexavalent chromium ( $\text{Cr}^{6+}$ ) was 500mg/L, on first day of experiment it was observed that the concentration of hexavalent chromium ( $\text{Cr}^{6+}$ ) in the effluent was same as that of the initial concentration and there was no decrease in chromium concentration in the effluent. As the treatment filters were operated at room temperature, throughout the experimentation the temperature was  $25\pm 5^\circ\text{C}$ . At this time the pH was 4–5, as the treatment continued it was observed that till 4th day there was slow and gradual decrease in the  $\text{Cr}^{6+}$ . On 5th and 6th day there was a sudden decrease in the  $\text{Cr}^{6+}$  concentration and it

was reduced from 500mg/L to 364 mg/L and 268 mg/L respectively. After that there was slow and stable decrease in concentration and on day 30th the concentration of  $\text{Cr}^{6+}$  measured were 202 mg/L and the  $\text{Cr}^{6+}$  removal efficiency at this point was 59.6%. On last day which was 37th day, the maximum decrease in the  $\text{Cr}^{6+}$  concentration was 198.6mg/L where the bacterial count was  $5.7 \times 10^9$ . It was observed that the removal efficiency on last day was maximum i.e. 60.2%. In another study, the researchers used a novel process for the removal of iron from ground water in which they used limestone packed column to increase efficiency of oxidation of ferrous iron ( $\text{Fe}^{2+}$ ) and conduct alkylation and aeration at the same time. While observing the effect of hydraulic retention time (HRT) on the removal of iron, it was observed that by increasing the flow rate of water, the iron removal yield decreased as the contact time between the column and iron decreased (Sim et al., 2001). As shown in the Fig. 5 HRT was calculated as about 48 hours and flow rate was adjusted at 200 ml/hour in order to increase the contact time between the sample and sand granules 33 and added biofilm and to increase the removal efficiency of ferrous iron from synthetic wastewater.

### **3.5 Removal efficiency of hexavalent chromium ( $\text{Cr}^{6+}$ )**

Initially it was observed that removal efficiency of hexavalent chromium ( $\text{Cr}^{6+}$ ) was very low till 3rd day that was about 1%. On 4th day, a slight increase in removal efficiency was seen i.e. about 4% and on 5th day, removal efficiency for  $\text{Cr}^{6+}$  suddenly increased to about 27%. After 1 week of continuous process, at 7th day the removal efficiency was increased to about 50%. Slow sand filters were operated continuously and slow and stable increase in the removal efficiency was observed till 27th day of experimentation. On 17th day, the removal efficiency was about 56% and on day 28, it was increased to about 59.3% means 59% hexavalent chromium was removed till 27th day. After that, the removal efficiency was increasing stably and slowly. The maximum removal efficiency was observed on day 31 i.e. about 61% and at last day of experiment that was 37th day the removal efficiency was about 60%. In present research an iron oxidizing bacteria was used as hexavalent chromium reducer. The basic mechanism behind this is that these organisms obtain energy for their growth from oxidation of different inorganic sulphur compounds (Donati et al., 2003). Oxidation of such compounds results in production of reducing agents that are present on colloidal sulphur and result in reduction of hexavalent chromium ( $\text{Cr}^{6+}$ ) to trivalent chromium ( $\text{Cr}^{3+}$ ) that is more stable and non-toxic form of chromium than  $\text{Cr}^{6+}$  (Quilntana et al., 2001). Reduction ability of colloidal sulphur increases in case of low pH (Quilntana et al., 2001). So a clear decrease in the concentration of hexavalent chromium was observed in present study. A study on speciation and removal of chromium from aqueous solution by white, yellow and red sand revealed that at  $\text{pH} < 4$ , the adsorption of hexavalent chromium ( $\text{Cr}^{6+}$ ) was very low i.e. 10% while Cr (III) was totally adsorbed. In this study removal of trivalent chromium ( $\text{Cr}^{3+}$ ) and hexavalent chromium ( $\text{Cr}^{6+}$ ) from aqueous solution by different types of sand as low-cost abundant absorbent was investigated (Khamis *et al.*, 2009). In another study, the adsorption ability of hexavalent chromium on dolochar was studied which is a less expensive naturally occurring adsorbent. Dolochar was found to be an efficient adsorbent for chromium removal from synthetic waste water. Batch adsorption experiment have been conducted by varying adsorbent dose, adsorbate concentration, pH, particle size, time on removal of chromium of these metal ions (Panda

*et al.*, 2011). It was found that more than 94% of the removal was achieved under optimal condition while in the present research 60% removal was achieved where normal sand was used as adsorbent and bacterial culture was also used to see the combine effects on the removal of hexavalent chromium from sythetic wastewater. In another study researchers used another strategy for the removal of hexavalent chromium from aqueous solution in which they used *Thiobacillus thioparus* for the indirect reduction of hexavalent chromium. Their results in the Fig. 6 showed the values for reduction of hexavalent chromium very close to 100% at different pH (Donati *et al.*, 2003).

### 3.6 Comparison of Removal efficiency

In this research, iron and hexavalent chromium was removed through slow sand filtration technique. The figure showing comparison between removal efficiency of iron and hexavalent chromium ( $\text{Cr}^{6+}$ ) via slow sand filters (SSF). At start of experiment a very low increase in the removal efficiency of  $\text{Fe}^{2+}$  and  $\text{Cr}^{6+}$  was observed. On 5th day, it was observed that the removal efficiency of ferrous iron was 5 % while that of the chromium was 27.2 %. On 30th day it was observed that removal efficiency of hexavalent chromium was 59.6 % as compared to ferrous iron whose removal efficiency was 51.3 %. After that slow and stable increase in removal efficiency was observed. On last day that was 37th day it was seen that the removal efficiency of hexavalent chromium ( $\text{Cr}^{6+}$ ) was 60.2 % while the removal efficiency of ferrous iron ( $\text{Fe}^{2+}$ ) was 52.3 %. The Fig. 7 overall suggests that the designed slow sand filters were more efficient in the removal of hexavalent chromium as compared to iron.

### 3.7 Slow sand filter 4 (Simultaneous treatment)

This setup was run for almost 6 days at room temperature ( $25\pm 5$ ) in which simultaneous removal of iron and hexavalent chromium was observed. Bacterial count that was inoculated in the filter was approximately  $5.7 \cdot 10^9$  CFU/mL and iron and chromium wastewaters were added to the same filter as influent while effluent collected was the mixture of both solutions. The figure showing the removal efficiency of both ferrous iron and hexavalent chromium when they were treated within the same SSF. It was observed that on first day removal efficiency of hexavalent chromium ( $\text{Cr}^{6+}$ ) was 64.13% whereas removal efficiency of ferrous iron was 43.47%. As the experimentation continued, it was observed that removal efficiency was increasing stably. At the end of the experiment i.e. 6th day, removal efficiency of hexavalent chromium was about 93% while the removal efficiency of ferrous iron was 68.4%. Hexavalent chromium ( $\text{Cr}^{6+}$ ) can be reduced abiotically by aqueous  $\text{Fe}^{2+}$  (Bishop *et al.*, 2014). So this property of ferrous iron can be reason for increased removal efficiency of hexavalent chromium in the present study. A study was conducted on synergistic effect of iron (III) minerals and dissimilatory metal reducing bacteria on the reduction of hexavalent chromium revealed that Iron (III) minerals and anthraquinone-2,6-disulfonate (AQDS) synergistically enhance bio-reduction of hexavalent chromium (Meng *et al.*, 2018). Oxidation of such compounds results in the production of reducing agents that are present on colloidal sulphur and causes the reduction of hexavalent chromium ( $\text{Cr}^{6+}$ ) to trivalent chromium ( $\text{Cr}^{3+}$ ) that is more stable and non-toxic form of chromium than  $\text{Cr}^{6+}$  (Quilntana *et al.*, 2001). Reduction ability of colloidal sulphur increases in case of low pH (Quilntana *et al.*, 2001) as shown in the Fig. 8.

### 3.8 Field Application

Water sample was collected from Water filtration plant, Abbottabad that was tested for different parameters and then was compared to the results of treated water of currently studied slow sand filter. Different parameters like pH, turbidity, hardness were checked before and after treatment. It was observed that pH of the water before and after passing through slow sand filter (SSF) remained same. A very slight change in turbidity of tested water was observed i.e. before treatment turbidity of water was  $1.27 \pm 0.48$  and after treatment it was  $0.96 \pm 0.05$ . It was also observed that hardness of water was also being reduced very slightly after treatment of water through SSF Regarding microbiological analysis like total plate count (TPC) was checked. A decrease in Total plate count (TPC) was observed on nutrient agar i.e.  $22 \pm 4.12$  CFU in average (after treatment) as compared to  $40.5 \pm 6.36$  (before treatment). As shown in the Table 2

Table 2  
Analysis of water before and after treatment through lab scale SSF

S. No.	Parameters	Before treatment	After treatment
1	pH	$8.6 \pm 0$	$8.6 \pm 0$
2	Turbidity, NTU	$1.27 \pm 0.48$	$0.96 \pm 0.05$
3	TPC (cfu/ml)	$40.5 \pm 6.36$	$22 \pm 4.12$
4	Hardness, (mg/L)	$169.33 \pm 16.772$	$120 \pm 20$

Water samples collected from water filtration plant were also checked for different parameters as shown in the table below. It was observed that there was a very slight change in the pH, temperature, total dissolved solids, and electrical conductivity table (3) However, it was observed that turbidity was decreased to permissible limit. Similarly, value of hardness reduced to half till the end of the process or collection of effluent. According to the test report provided by the higher authority of the filtration plant, no *E.coli* colony was found in the water.

Table 3  
Analysis of effluent and influent collected from Water filtration plant Abbottabad

S.No	Parameters	Raw water	Slow sand filtration effluent
1	pH	8.12±0.05	7.94±0.005
2	Temperature (°C)	29±0.1	29.9±0.15
3	Turbidity (NTU)	6.34±0.2	0.29±0.2
4	Hardness (mg/L)	32±1	16 ± 0
5	TDS (ppm)	60.33±2.08	61.3±1.5
6	Electrical conductivity ( $\mu^s$ )	125.66±0.57	124±1

Iron and hexavalent chromium was also checked in the treated water after slow sand filtration. It was observed that total iron in the influent was about 22 mg/L while in effluent it was 8.13mg/L approximately and slow sand filters were able to reduce iron upto 37% approximately. But it was observed that the concentration of ferrous iron in the effluent (treated water) was more than the concentration in the influent. Concentration of hexavalent chromium ( $Cr^{6+}$ ) was also analysed in the effluents and influents. It was seen that concentration of hexavalent chromium was reduced from 40mg/L to 28mg/L approximately.

Schmutzdecke layer from the slow sand filter was also collected to check the presence or absence of iron oxidizing *Acidothiobacillus ferrooxidans* bacteria. It was observed that after 7 days of incubation, only single colony of iron oxidizing bacteria was grown on the media. In this research instead of native schmutzdecke layer, the synthetic layer was applied that was composed of isolated culture of *Acidothiobacillus ferrooxidans* i.e. the iron oxidizing bacteria. So the bacterial strains were also performing their functions and oxidizing the ferrous iron into ferric iron, hence reducing the concentration of ferrous iron in the effluent. In a study it was revealed that these organisms have ability to drive energy for their growth via oxidation of ferrous ( $Fe^{2+}$ ) iron by oxygen ( $O_2$ ) resulting in the formation of ferric iron ( $Fe^{3+}$ ) and water ( $H_2O$ ) and fixation of  $CO_2$  from environment (Campodonico et al., 2016).

## 4. Conclusion

In this research, slow sand filters were designed that were inoculated with iron oxidizing bacteria *Acidothiobacillus ferrooxidans* and were allowed to remove ferrous iron and hexavalent chromium from water samples via oxidation of ferrous iron to ferric iron and reduction of hexavalent chromium to trivalent chromium,  $Cr^{3+}$ . At the end of the experimentation the removal efficiency for ferrous iron was about 52.3% means decreased the concentration from 200 to 106 mg/L. The removal efficiency of hexavalent chromium was about 61% (decreased from 500 to 198.6 mg/L). So, in conclusion, the

designed slow sand filters were able to reduce the concentration of hexavalent chromium more efficiently than ferrous iron. And it was clearly observed that slow sand filters can effectively remove ferrous iron and hexavalent chromium from water if they are inoculated with respective bacteria.

## Declarations

**Ethics approval and consent to participate:** This is non-human subject research, and waived the need for informed consent.

**Declaration of interests :**The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Funding:** No funding

**Consent to Participate/Publish:** Authors contributed to the study conception and design. The authors read and approved the final manuscript.

**Authors Contributions:** “Not applicable”

**Availability of data and materials:** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## Figures

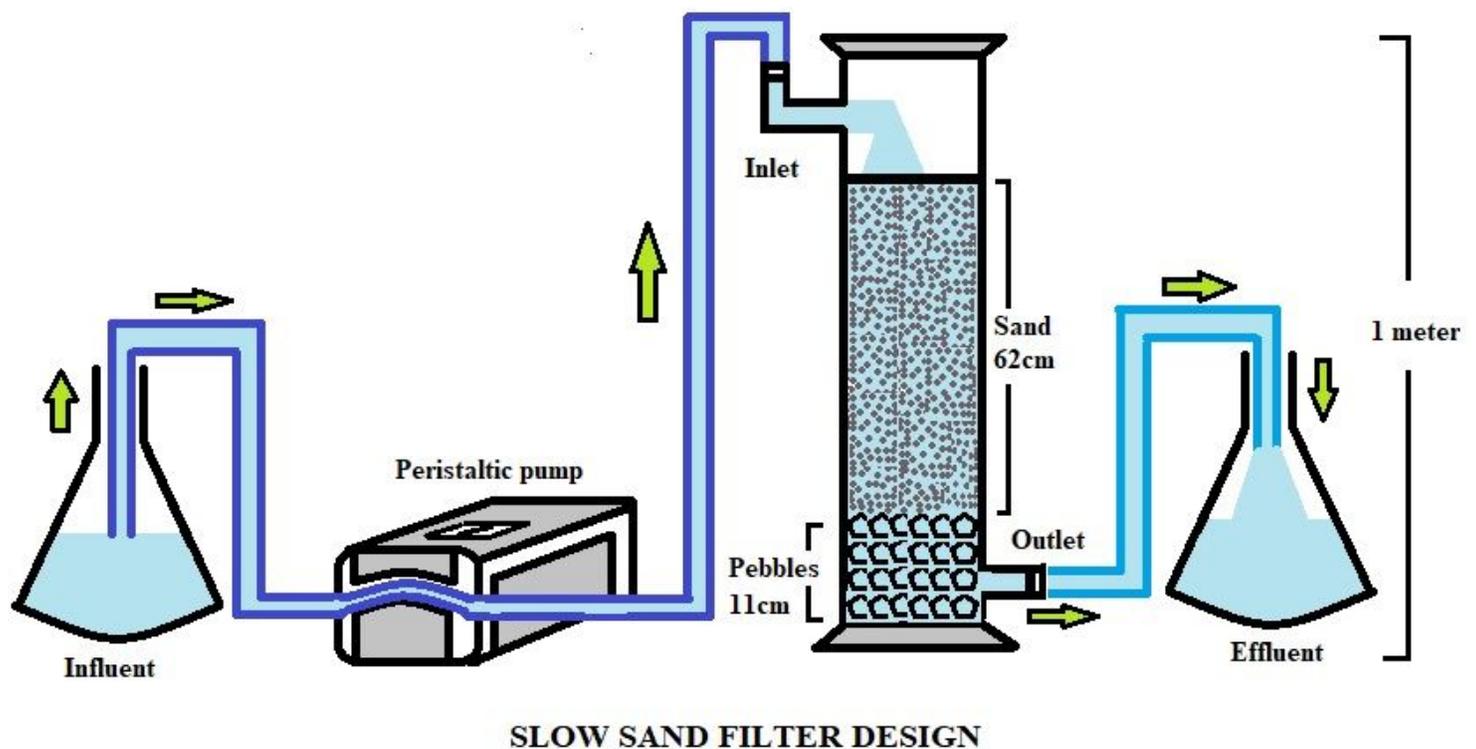
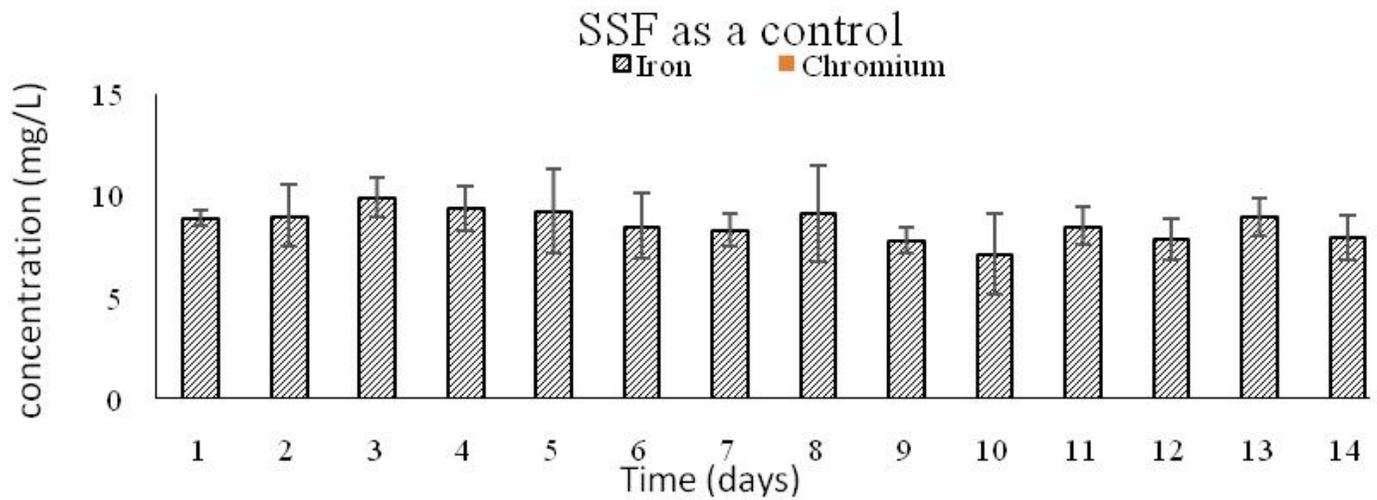


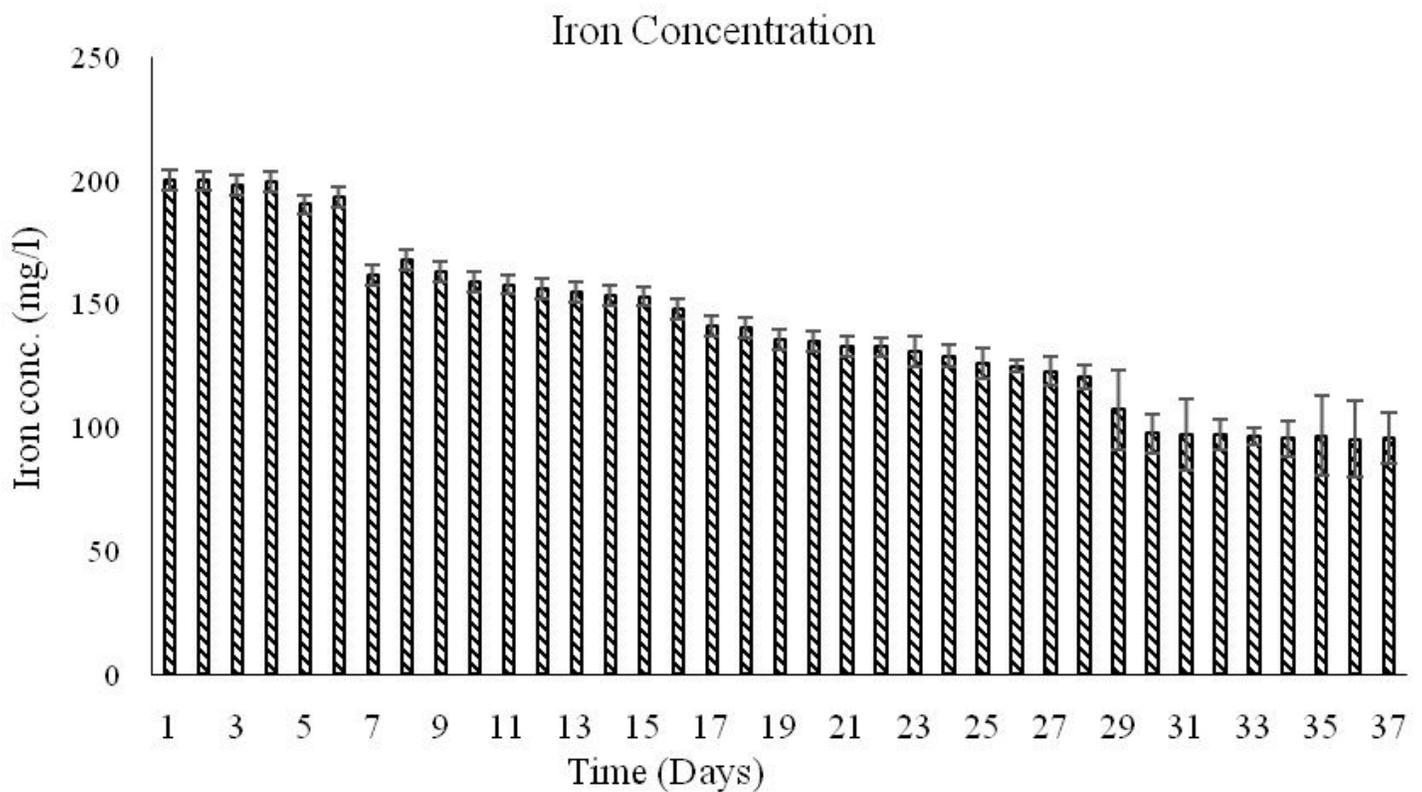
Figure 1

Slow sand filtration setup design



**Figure 2**

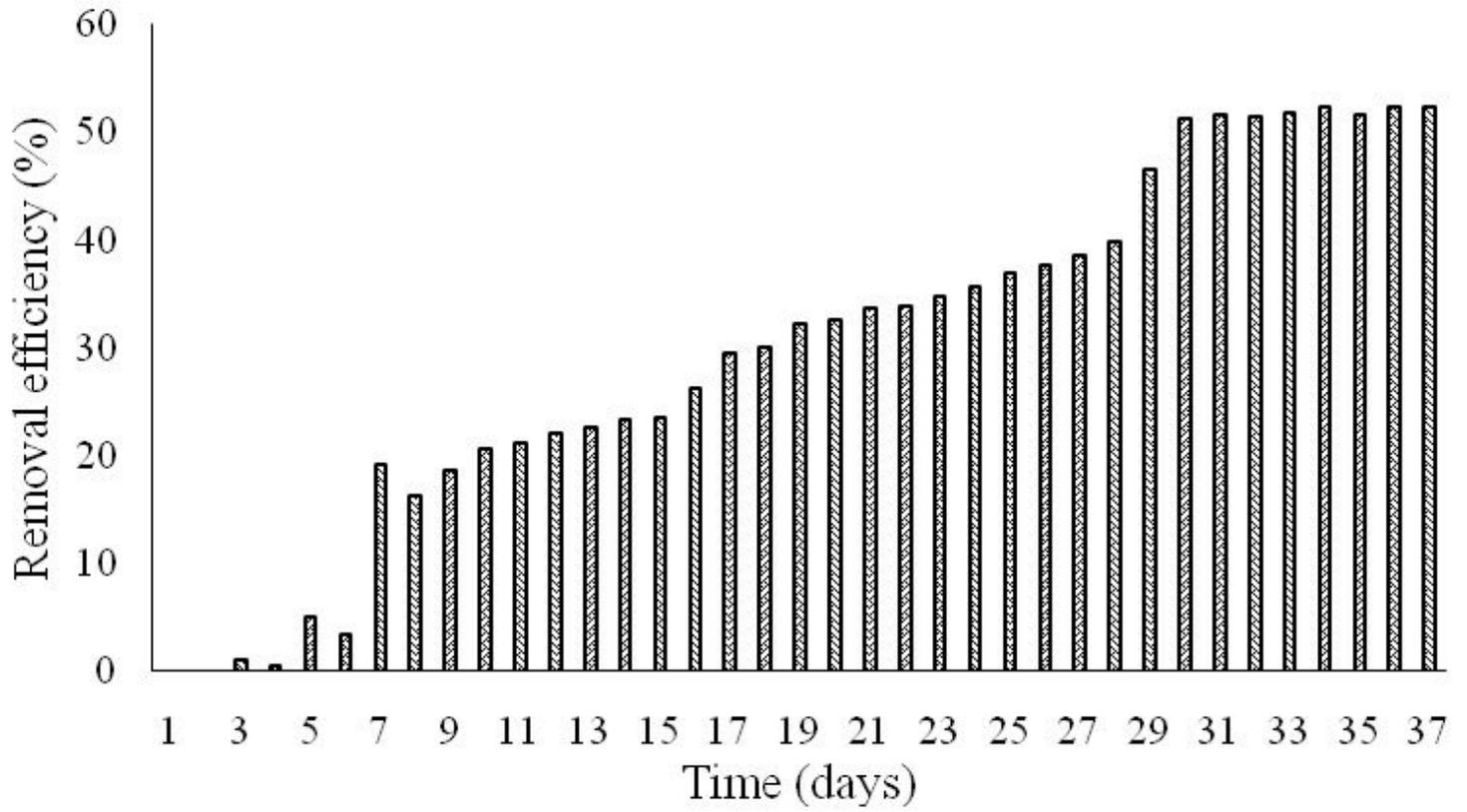
Concentration of Ferrous iron and Hexavalent chromium in the effluents of control setup



**Figure 3**

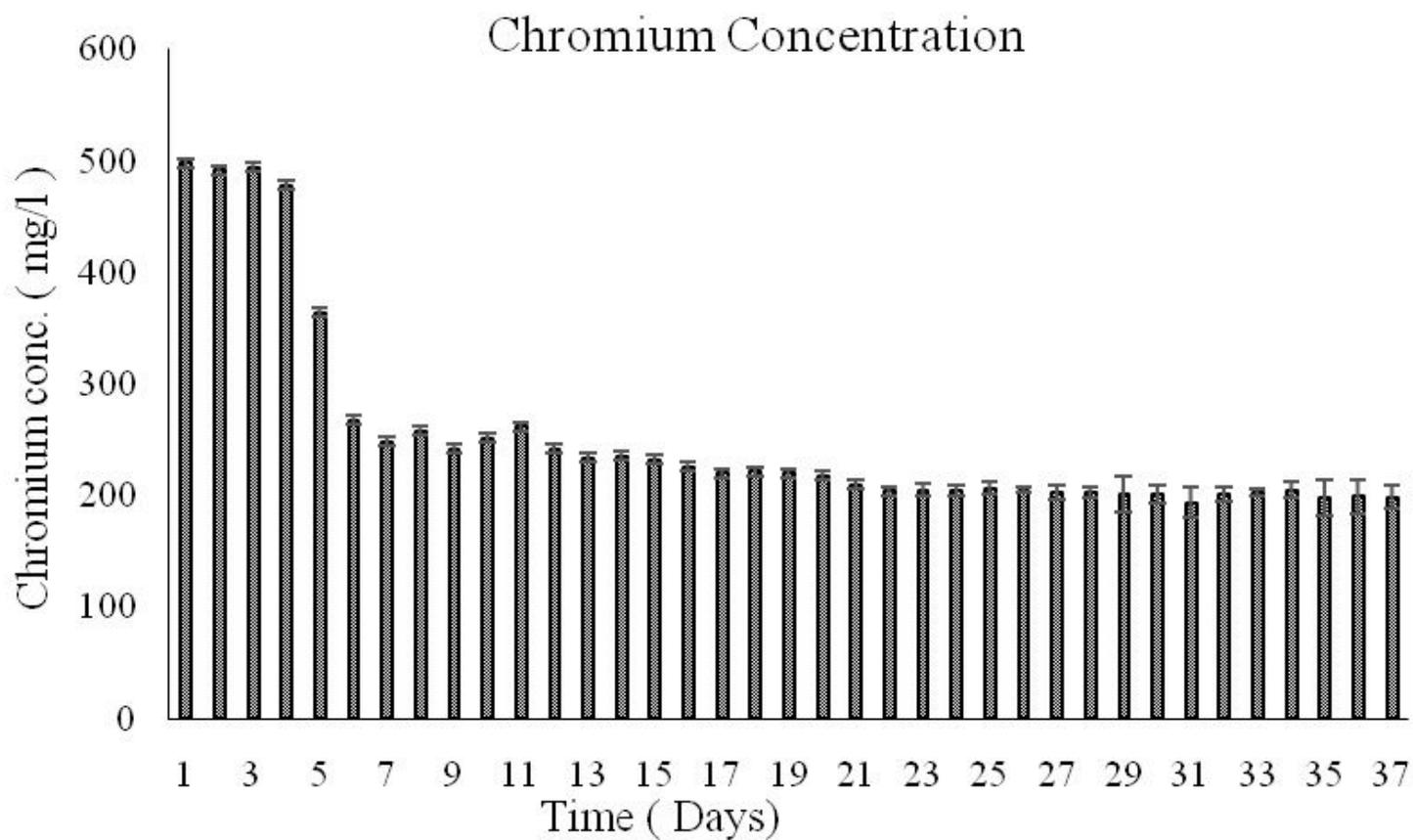
Concentration of ferrous iron in the effluents after treatment

## Fe<sup>2+</sup> Removal efficiency



**Figure 4**

Removal efficiency of ferrous (Fe<sup>2+</sup>) iron



**Figure 5**

Concentration of Cr<sup>6+</sup> in the effluents after treatment

### Removal efficiency of Cr<sup>6+</sup>

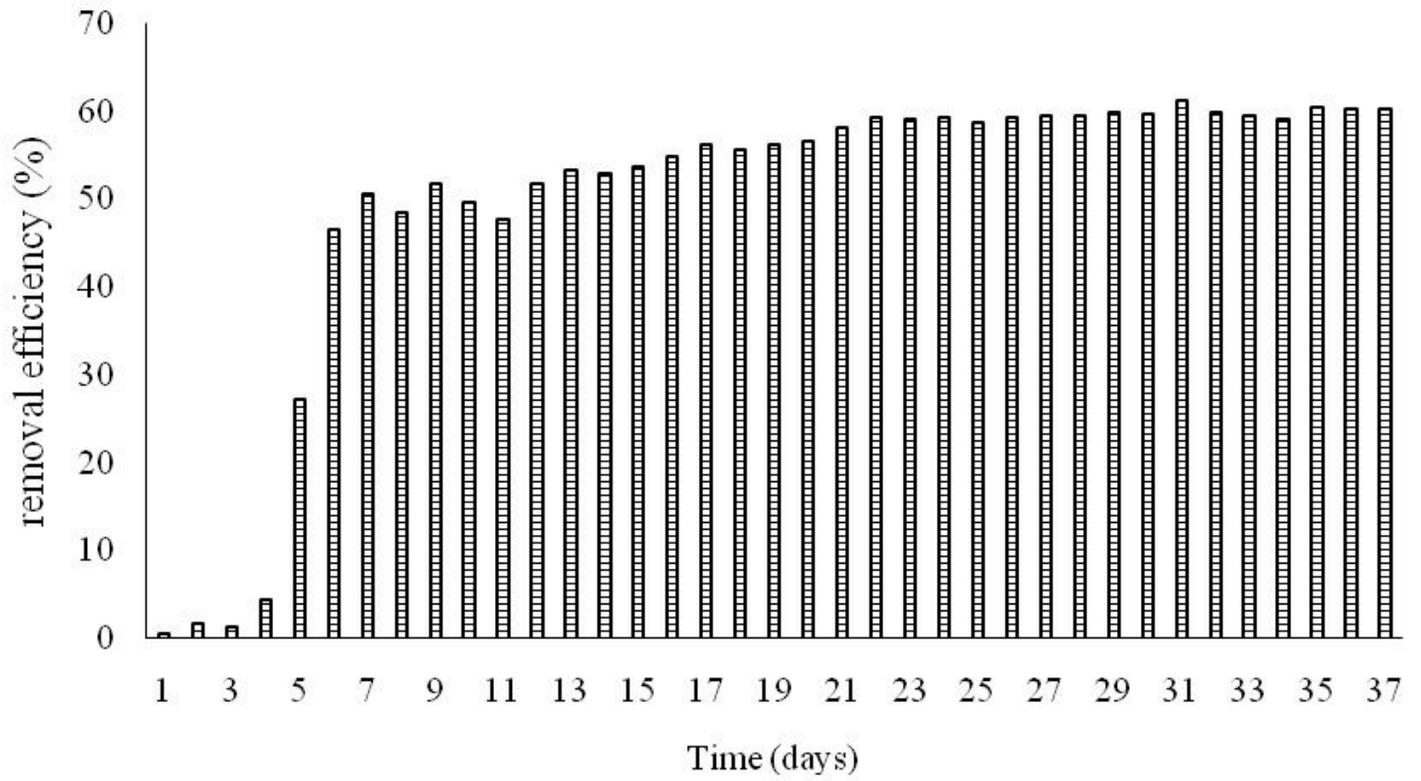


Figure 6

Removal efficiency of hexavalent chromium (Cr<sup>6+</sup>)(Qayyum et al., 2020)

### Comparison of Removal Efficiency

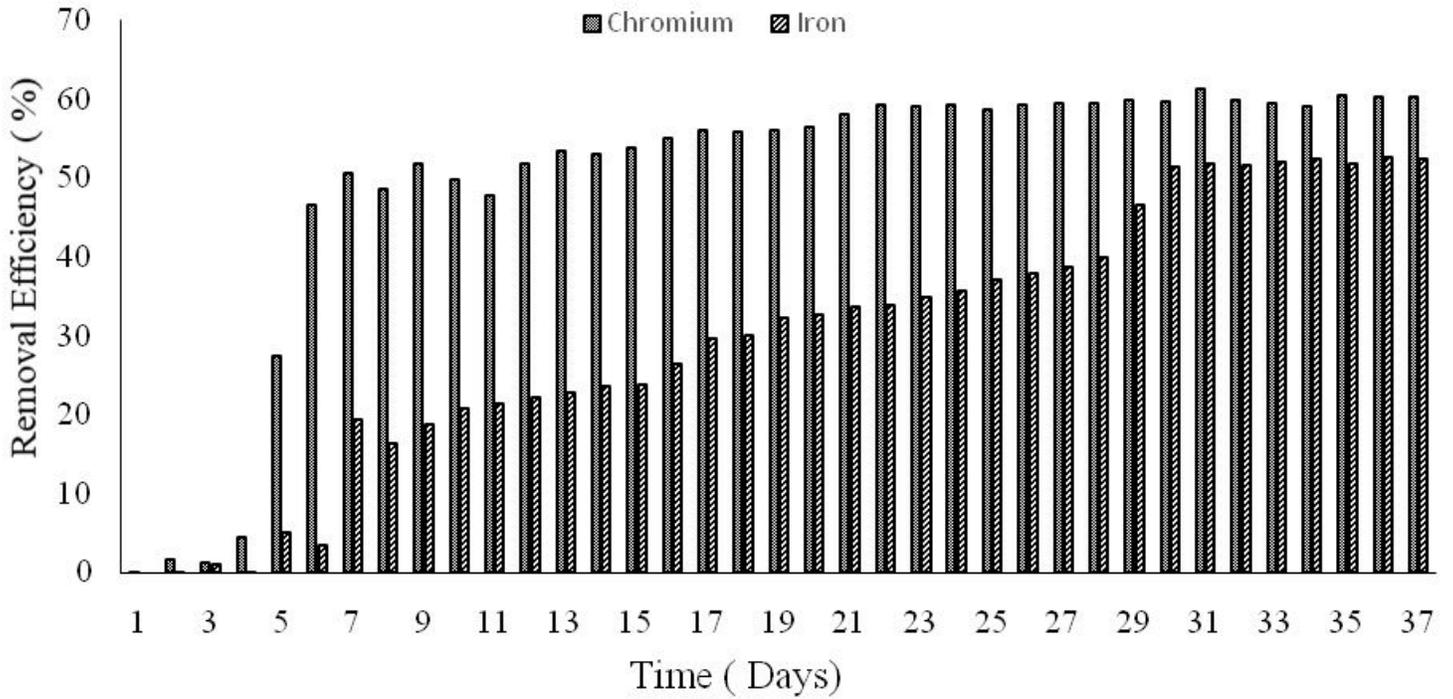
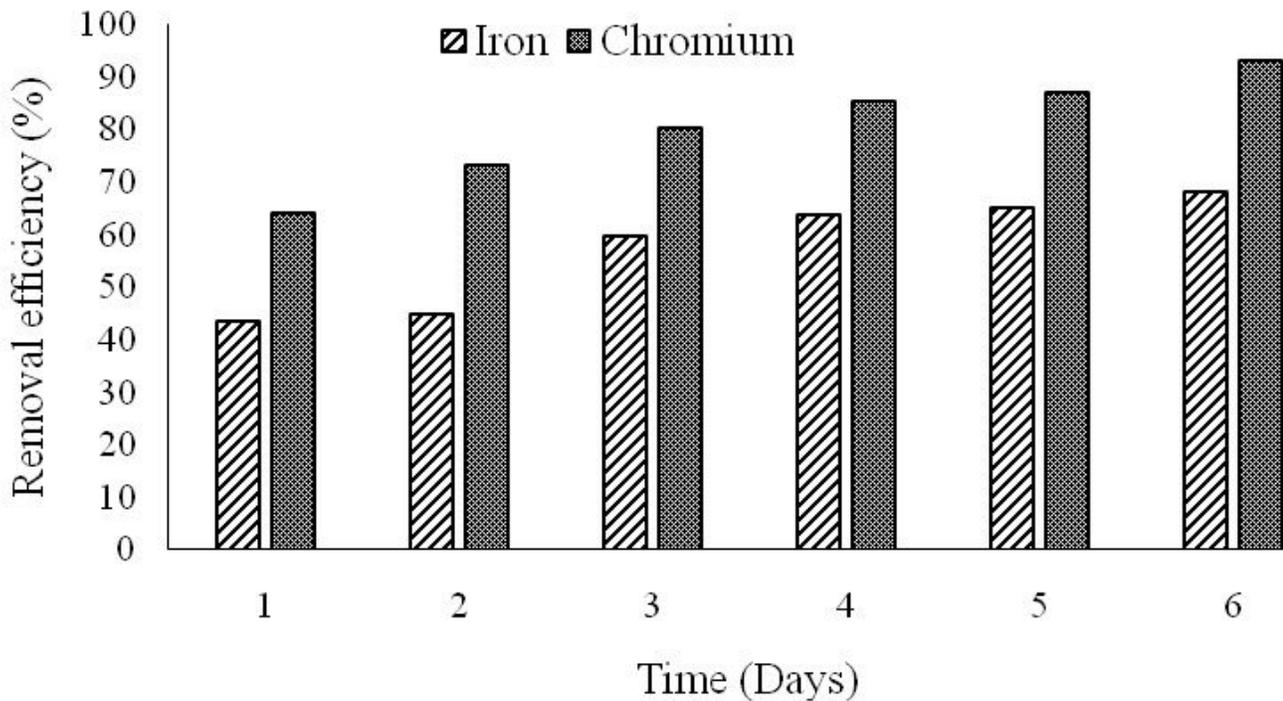


Figure 7

Comparison of removal efficiency of Fe<sup>2+</sup> and Cr<sup>6+</sup> (Qayyum et al., 2020)

### Simultaneous removal efficiency



## Figure 8

Slow sand filter 4 (Simultaneous treatment)