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Toxicity of hexavalent chromium to *Daphnia magna*: influence of reduction reaction by ferrous iron

Sang D. Kim*, Kyeong S. Park, Man B. Gu

Department of Environmental Science and Engineering, Kwangju Institute of Science and Technology, 1 Oryong-dong, Puk-gu, Kwangju 500-712, South Korea

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Abstract

The reaction kinetics of hexavalent chromium with ferrous ions were studied to determine the influence of reduction on the toxicity of chromium to aquatic organisms. The changes in chemical forms of the chromate in the presence of ferrous ions were examined in a bioassay system using *Daphnia magna* as a test organism. This study demonstrated that the reaction kinetics of chromate with ferrous ions showed a significant decrease of chromate concentration with the second-order rate coefficient (k) for the reduction of Cr(VI) being determined as $55.2\,M^{-1}\,s^{-1}$. The concentration of Cr(VI) remaining in the solution decreased as the ratio of ferrous ion to chromate increased, revealing a non-stoichiometric reaction due to oxygenation and the moderately alkaline pH of the solutions. The toxicity test indicated that the bioavailability of chromate to *D. magna* was reduced in the presence of Fe(II) and that it decreased further with increasing Fe(II) concentrations. However, the toxic effect of chromate to aquatic organisms was not controlled kinetically in the presence of ferrous ions. It was also found that LC_{50} of chromate to *D. magna* decreased about 1.5-fold as the test period increased from 24 to 48 h in the presence of Fe(II).

Keywords: Hexavalent chromium; Reduction; Ferrous ion; Toxicity; *Daphnia magna*

1. Introduction

A significant amount of environmental literature has demonstrated that hexavalent chromium is directly correlated to a human carcinogen and to acute toxicity of aquatic organisms, while its reduced form, Cr^{3+}, is an essential element for animals [1]. Cr(VI) exists in soils and natural waters predominantly as a soluble anion that may be formed via oxidation of soluble and insoluble forms of less-toxic Cr(III). Chromate is 100 times more mobile and

* Corresponding author. Tel.: +82-62-970-2445; fax: +82-62-970-2434.
E-mail address: sdkim@kjist.ac.kr (S.D. Kim).

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more toxic than Cr^{3+} [2]. In the body, Cr(V), one of the reduction products of Cr(VI), is a known carcinogen and will lodge in any tissue to form cancerous growths. The annual chromate discharge worldwide is 239×10^3 t [3] as a result of numerous industrial activities such as the preservation of wood, leather tanning, metal finishing, etc. Cr(VI) emitted from these industries is transported to aquatic environments and thereby the ecosystem in natural waters is toxically affected. However, the form of chromium is changed by natural processes, particularly by oxidation and reduction with naturally occurring redox agents such as iron and Mn oxides [4]. Many studies have shown that hexavalent chromium can be reduced to the trivalent form by reduction reaction with organic and inorganic ions (e.g. elemental iron, divalent iron, sodium bisulfite) and humic substances [4–7] and thereby the toxicity of chromium is reduced due to the decrease of toxic Cr(VI) concentration.

A variety of Cr(VI) forms exist as different species, $HCrO_4^-$, CrO_4^{2-} and H_2CrO_4, in various pH conditions and total Cr(VI) concentrations. In this study, the dominant form is CrO_4^{2-} at pH 7.9 [8]. The mechanism for the reduction reaction of Cr(VI) to Cr(III) using ferrous sulfate as the reducing agent is [4] as follows:

$$6Fe^{2+} + 2CrO_4^{2-} + 13H_2O \rightarrow 6Fe(OH)_3 + Cr_2O_3 + 8H^+ \quad (1)$$

The valence state and the mobility of chromium at environmental sites are controlled by the oxidation of Cr(III) and the reduction of Cr(VI). As a result, the oxidation state of chromium enables the prediction of its toxicity to the ecosystem and human health. The speciation of metals in natural waters is important with respect to their bioavailability and therefore to toxicity and water quality criteria [9,10]. Therefore, the reduction reaction and its kinetics need to be discerned to estimate the bioavailability of Cr(VI) and an understanding of the fate of chromium in the environment is very important to evaluate the potential risk of chromium. Few studies have investigated the bioavailability of chromium from a toxicological point of view [11–13]. Most studies have concentrated mainly on chemical reduction kinetics of Cr(VI) to Cr(III) using various reducing agents and have identified the reduction capability and mechanisms, but have not considered and not verified the biological toxic effect of Cr(VI) on aquatic organisms after chemical reduction reaction.

The purpose of this study was to investigate the reduction reaction of Cr(VI) with ferrous ions and thereby the changes of Cr(VI) bioavailability. These reactions were studied by determining the changes in the chemical forms of the chromium following the addition of ferrous sulfate as a reducing agent and by determining the toxicity of the chromate to *Daphnia magna* as a function of speciation.

2. Materials and methods

All the reagents were analytical grade and were used without further purification. $K_2Cr_2O_7$ (99.5%, Aldrich Chemical Co., Milwaukee, WI) was used as a toxicant exposing to the test organisms and $FeSO_4 \cdot H_2O$ was used as a reducing agent in the experiment. All the glassware, as well as the polyethylene and polypropylene laboratory ware, was soaked in 10% HNO_3 (v/v) for at least 48 h before use. Deionized (DI) water from a Barnstead NANOpure ultrapure water system was used throughout the study.

2.1. Test organisms and culture conditions

The test organism used in this study, *D. magna*, was obtained from the Korea Research Institute of Chemical Technology (Taejon, South Korea) and the food, *Selenastrum capricornutum* and yeast, trout chow and Cerophyll® (YTC) mixture were purchased from Aquatic BioSystem Inc. (Fort Collins, CO). The organisms were cultured and handled according to the procedures outlined in the EPA manual [14]. The detailed culturing and toxicity testing conditions followed those recommended by Ma et al. [15]. Test water throughout this study was reconstituted hard water ($MgSO_4$, 9.98×10^{-4} M; $CaSO_4$, 6.98×10^{-4} M; $NaHCO_3$, 2.28×10^{-3} M; KCl, 1.07×10^{-4} M) having hardness of $170 \pm 5\,mg\,l^{-1}$, alkalinity of $110 \pm 5\,mg\,l^{-1}$ as $CaCO_3\,mg\,l^{-1}$, and pH of 7.8 ± 0.2, prepared according to the constituents given in the EPA manual [14].

2.2. Cr(VI) reduction experiment

Kinetic experiments were performed at constant pH 7.1. Potassium dichromate ($K_2Cr_2O_7$) was added to the dilution water to achieve to a chromium concentration of 3×10^{-6} M. The solution was continuously stirred at constant temperature ($25 \pm 0.5\,^{\circ}C$). Freshly prepared $FeSO_4$ solution was added to the mixture of $K_2Cr_2O_7$ and dilution water to achieve 6×10^{-6} M $FeSO_4$. In order to investigate the pH effect on the reduction kinetics of Cr(VI) with Fe(II), the experiment was repeated at the same conditions as above except at different pH levels of 6.2, 7.1 and 7.5. The pH was adjusted using a pH-stat apparatus with concentrated H_2SO_4 and NaOH. Samples were taken at desired times to measure Cr(VI) concentrations. A similar set of experiments was conducted at various concentrations of $FeSO_4$ to determine the effect, on chromate reduction at pH 7.8, of the mole ratio of Fe(II) to Cr(VI) being varied from 0 to 10. Each solution contained 5×10^{-6} M $K_2Cr_2O_7$ and the appropriate proportion of $FeSO_4$. Solutions were equilibrated to reach a steady state for 2 h after the addition of $FeSO_4$ to the mixture of $K_2Cr_2O_7$ and dilution water. The concentrations of Cr(VI) and total Cr were determined using a colorimetrical method by mixing a 25 ml sample with 0.5 ml of 1,5-diphenyl carbazide (DPC) solution, which was prepared by dissolving 250 mg of DPC in 50 ml acetone, and 1–2 drops HNO_3 to maintain a pH of 1.0 ± 0.3 [16]. This solution was allowed 5 min for full color development and then the absorbance was measured at 540 nm. The Fe(II) concentration was also measured by using a 1,10-phenanthroline method [17].

2.3. Bioassay

The effect of Fe(II) on the toxicity of hexavalent chromium was examined in a static bioassay test, in which the test organism was exposed to 15 ml of test water. The first set of bioassay chambers contained only dilution water without $FeSO_4$. For the second set, *D. magna* was introduced to the test water, in the presence of $FeSO_4$, immediately after the addition of Cr(VI); for the third set, $FeSO_4$ and Cr(VI) were allowed to equilibrate for 2 h before the addition of the organisms. Each set comprised nine different Cr(VI) concentrations and a control. Four replicates were set up for each Cr(VI) concentration and five neonates of *D. magna* less than 24 h old were placed in each test cup. Total Cr(VI), pH

and DO were determined before and after the 24 h exposure period. The survival rates were determined to be the numbers of live organisms remaining after 24 h for the nine Cr(VI) concentrations and one control.

3. Results and discussion

3.1. Reduction kinetics

We studied the effect of the presence of Fe(II) on the reduction kinetics of Cr(VI) with dilution water. $FeSO_4$ at a concentration of 9×10^{-6} M was added to 3×10^{-6} M $K_2Cr_2O_7$ and the change of its concentration was observed over 24 h (Fig. 1a). The hexavalent chromium titration of ferrous ion in the dilution water at pH 7.1 and at the ionic strength $I \cong 0.01$ M (which were the same as in other kinetic experiments and bioassays) showed significant decrease of Cr(VI) concentration due to the reduction of Cr(VI) to Cr(III) in the presence of Fe(II) and reached a steady state in 60 min. However, the concentrations of Cr(VI) were not changed in blank solution with dilution water used in the bioassay test. It was confirmed that there were no other possible reducing agents in the dilution water. Assuming the reaction of Cr(VI) reduction with dissolved Fe(II) follows stoichiometric model, the reduction kinetics is an overall second-order reaction in 60 min before the Cr(VI) reduction reached a steady state. Using non-linear regression fitting of the data, the results were obtained by

$$[\mathrm{Cr(VI)}]_t = \frac{[\mathrm{Cr(VI)}]_0}{1 + 3k[\mathrm{Cr(VI)}]_0 t} \tag{2}$$

where $[\mathrm{Cr(VI)}]_0$ indicates the initial Cr(VI) concentrations and $[\mathrm{Cr(VI)}]_t$ is Cr(VI) concentrations after a certain time t [18]. The second-order rate coefficient (k) for the reduction of Cr(VI) was calculated to be 55.2 $\mathrm{M^{-1}\,s^{-1}}$ ($r^2 = 0.91$) by SIGMAPLOT software (SPSS Science, Chicago, IL). Although the reduction reaction of Cr(VI) with Fe(II) generated acidity and decreased the pH instantly, it recovered to the initial pH due to the pH buffer capacity of synthetic dilution water. The effect of pH on the reduction of Cr(VI) was observed at different pH values in the range 6.2–7.5 (Fig. 1b). Although significant difference of reduction rates did not appear between pH in the near-neutral solutions, it was observed that the reaction rate of chromate with ferrous ions increased and faster Cr(VI) reduction occurred with increasing pH [19,20]. Meanwhile, the effect of dissolved oxygen on Fe(II) oxidation is not dominant but competitive in the solution containing chromate at neutral pH ranges [5]. It was also found that Cr(VI) is a more powerful oxidizing agent than oxygen [5,21] and the rate of Cr(VI) reduction by Fe(II) is very rapid even in the presence of dissolved oxygen at pH $\leq$ 8.0 [22]. This indicates that the reduction of Cr(VI) with ferrous ions in a natural water condition of pH range 6–8 is not changed significantly by dissolved oxygen over these pH values. In addition, it shows that chromate reduction can be achieved quantitatively by Fe(II) oxidation even in oxygenated solution. However, the rate of Fe(II) oxidation by dissolved oxygen becomes rapid enough if the pH $>$ 10 [5,23]. At all pH values tested, the Cr(VI) removal extent was found to be about 60%. Other researchers have reported that the reduction of Cr(VI) is observed at low acidic pH values using humic substances as reducing agents and is pH dependent [6,7].

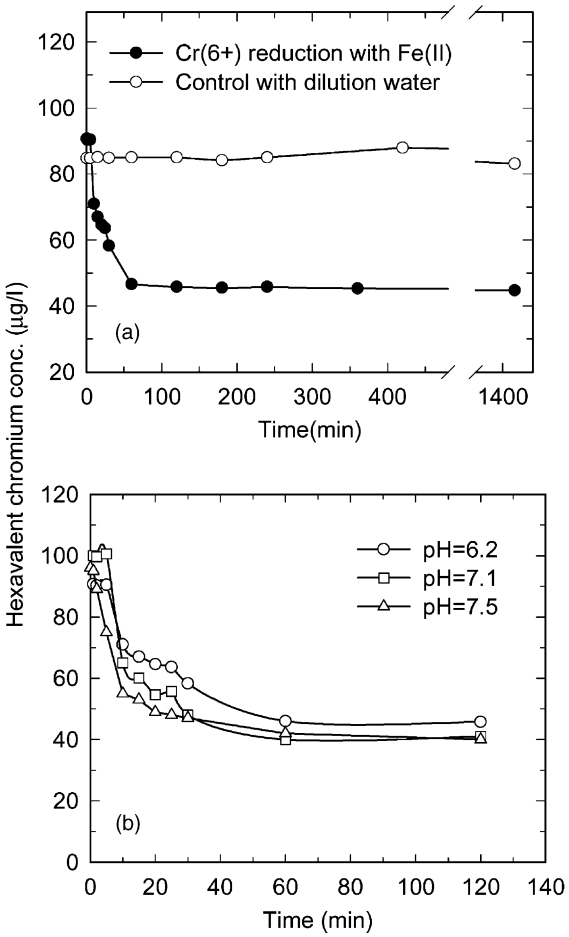

Fig. 1. (a) Effect of ferrous ions on the rate of Cr(VI) reduction in the synthetic dilution water at pH 7.1. Fe(II) concentration was 9×10^{-6} M and the ionic strength was about 0.01 M. (b) Effect of pH on the rate of Cr(VI) reduction.

We conducted experiments to determine the dependence of the Cr(VI) reduction rate on the changes in Fe(II) concentrations of the solution. The concentration of observed Cr(VI) versus the ratio of Fe(II) to Cr(VI) is shown in Fig. 2. The concentrations of Cr(VI) remaining in the solution decreased with the increasing ratio of ferrous ions to chromate. The significant non-linear relationship ($r^2 = 0.96$) between the Cr(VI) reduction and the mole ratio (Fe(II):Cr(VI)) was obtained at 95% confidence intervals. On the other hand, James [21] found that approximately 96% of the Fe(II) added to the system was oxidized within 20 min when stoichiometric amounts of Fe(II) and Cr(VI) were present (3:1). The non-stoichiometric reaction in this study may have been caused by the increase in the rate of Fe(II) oxidation by oxygenation [5]. The rate of Fe(II) oxidation by dissolved oxygen is known to be significantly dependent on pH [23]. Therefore, the moderately alkaline pH in

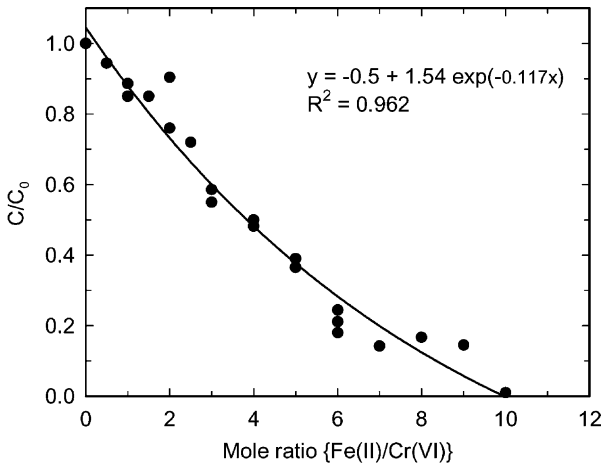

Fig. 2. Reduction of chromate by ferrous ions at various mole ratios of Fe(II) to Cr(VI). The solutions were allowed 2 h to reach steady state for reduction reaction. $[Cr(VI)]_0 = 5\,\mu M$, $I = 0.01\,M$, pH = 7.8 (same as in bioassays).

this experiment (pH = 7.8) affected to increase the rate of the competitive Fe(II) oxidation by dissolved oxygen and thereby the extent of reaction between Cr(VI) and Fe(II) was decreased.

3.2. Bioassay

The effect of Fe(II) on the toxicity of hexavalent chromium was determined by examining the survival rate of *D. magna* in static bioassay chambers after a 24 h exposure period (Fig. 3).

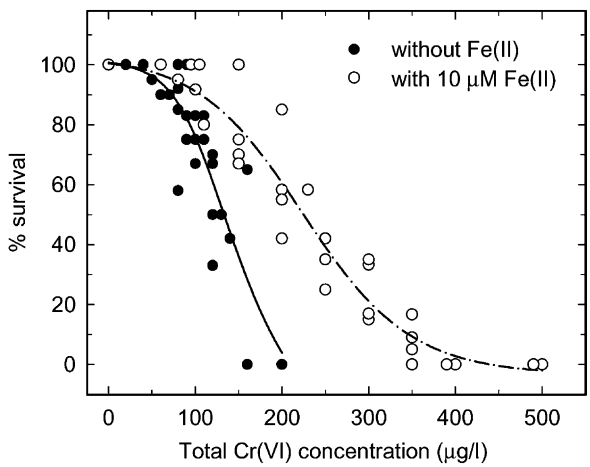

Fig. 3. Effect of ferrous ions on the survival of *D. magna* in static test. The data points were fitted to sigmoidal function in order to assist visualizing the trend.

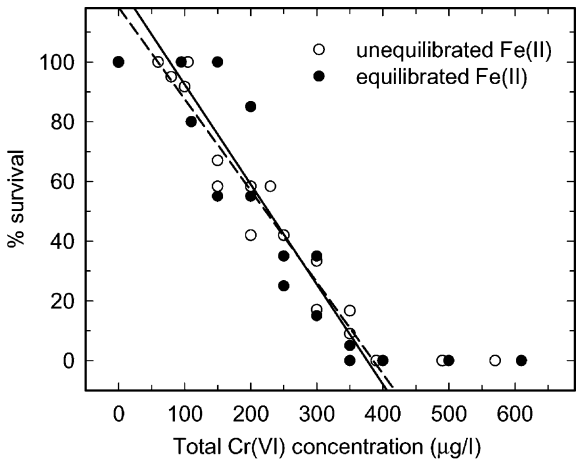

Fig. 4. Comparison of the toxicity curves between equilibrated ((—), $r^2 = 0.86$) and non-equilibrated ((- - -), $r^2 = 0.94$) Fe(II) solutions with Cr(VI). The Fe(II) concentration was 10 μM. The equilibrated Fe(II) solution was pre-stabilized with reconstituted dilution water for 2 h.

The results showed that the presence of Fe(II) decreased the toxicity of Cr(VI) due to reduction of Cr^{6+} to Cr^{3+} and displaced the toxicity curve to higher Cr(VI) concentrations. The static bioassay test revealed an increase of chromate LC_{50} values (lethal concentrations to kill 50% of test organisms), or a decrease of toxicity, from 128 to 215 $\mu g\,l^{-1}$ in the presence of 10 μM $FeSO_4$. However, the results indicated that the equilibration of Fe(II) with Cr(VI) solution for 2 h, the enough time required to reach steady state in reduction reaction of Cr(VI), prior to the addition of test organisms had no effect on Cr(VI) toxicity to *D. magna* (Fig. 4). This means that Cr(VI) toxicity to *D. magna* does not occur merely in the short period of exposure in the beginning of experiment but throughout the entire exposure time. That is, the toxic effect of chromate to aquatic organisms is not controlled kinetically in the presence of ferrous ions. In order to examine the effect of various Fe(II) concentrations on the Cr(VI) toxicity to *D. magna*, test organisms were exposed to four different ferrous sulfate concentrations having approximately 0:10 of stoichiometric ratio to Cr(VI) solution. The results clearly show that the toxicity of chromate decreases with increasing concentrations of Fe(II) (Fig. 5). The addition of 5 μM of Fe(II) to the test solution resulted in a shift of the curve to the right, and a decrease of toxicity with 220 $\mu g\,l^{-1}$ of Cr(VI) required to obtain 50% survival. To obtain the same survival rate, it was necessary to add about 350 and 470 $\mu g\,l^{-1}$ of chromate for 20 and 40 μM of Fe(II), respectively. It is interesting to note that there is no difference in the survival rate between 5 and 10 μM of Fe(II). This may attributed to the presence of Fe(II) at lower ratios of ferrous ions to chromate than that of stoichiometric amounts (3:1). On the other hand, the LC_{50} values of chromate at various Fe(II) concentrations were compared in two exposure times of 24 and 48 h (Table 1). The results indicated a significant difference in LC_{50} values between 24 and 48 h exposure time. A decrease of LC_{50} values, being an increase of Cr(VI) toxicity, to the *D. magna* was observed as the test period was increased from 24 to 48 h in the presence of

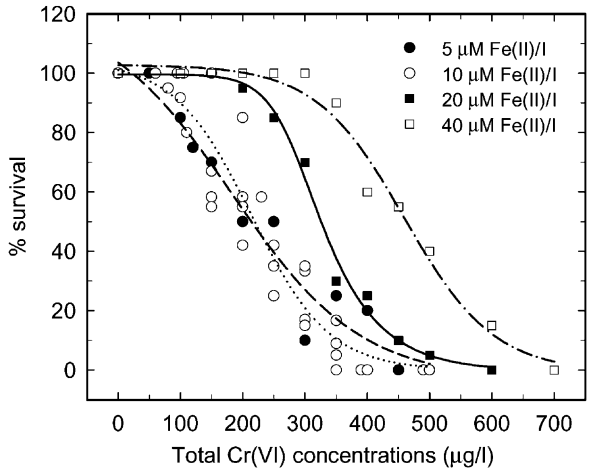

Fig. 5. Results of toxicity tests of Cr(VI) to *D. magna* with different Fe(II) concentrations: (- - -) 5 μM Fe(II); (· · ·) 10 μM Fe(II); (—) 20 μM Fe(II); (-·-·-) 40 μM Fe(II).

Fe(II) and the ratio of LC_{50} (24 h) values to LC_{50} (48 h) decreased slightly with increasing Fe(II) concentrations. This may be attributed to consumption of dissolved oxygen for the oxidation of the remained ferrous ions in solution or adverse effect of iron. However, no difference of LC_{50} values between 24 and 48 h tests was observed in the absence of Fe(II).

On the other hand, the reduction of ferric to ferrous ions was investigated in the presence of commercial humic acid. We expected the reduction of Fe(III) by humic acid and then the reduction of Cr(VI) by the reduced ferrous ion. Wittbrodt and Palmer [24] reported that the Cr–Fe–soil humic substances (SHSs) system enhanced the rate of reduction of Cr(VI) within 5 min at low pH. However, no reduction of Fe(III) by humic acid and no change of Cr(VI) concentrations appeared due to the moderately alkaline pH of this system.

Until recently, research attention has not concentrated on studying the effect of chromium on aquatic systems because of the non-toxicity of trivalent chromium. However, potential risk occurs due to the release of Cr(VI) from industries into natural waters, such as rivers and lakes, or negatively the oxidation process of Cr(III) released into an aquatic system with unexpected environmental conditions in the presence of MnO_2 [25]. Meanwhile, it has been well known that the reduction of hexavalent chromium to trivalent chromium is the main reduction application in industrial wastewater treatment. Current technologies use

Table 1
Effect of ferrous ions on the LC_{50} values ($\mu g\,l^{-1}$) of *D. magna*

	Fe(II) concentrations (μM)				
	0	5	10	20	40
LC_{50} (24 h)	128	202	215	322	458
LC_{50} (48 h)	105	128	131	231	325
Ratio of LC_{50} (24 h) to LC_{50} (48 h)	1.2	1.6	1.6	1.4	1.4

various reducing agents for converting Cr(VI) to Cr(III) such as sodium bisulfite, ferrous sulfate, elemental iron and humic substances for the reduction of chromate. By quoting the patents and literatures, Eary and Rai [5] noted that ferrous sulfate is the most commonly used reagent and has more rapid rates of aqueous Cr(VI) reduction. It is also feasible to use zero-valence-state iron as a reducing agent for chromate removal in contaminated industrial sites, although Fe(II) solids are dissolved slowly over a 15 min to 6 h period [5]. Powell et al. [18] demonstrated that the presence of elemental or zero-valence-state iron can reduce significantly the Cr(VI) concentration by corrosion mechanisms. This suggests that the chemical forms of chromium are changed in the presence of iron or any reducing agents in the environment and thereby aquatic organisms are affected with different toxic units of chromium. In most Cr(VI) treatment technologies, however, it is assumed that the effluents from treatment using chemical technologies are safe without requiring biological verification, although in fact they may contain unknown or disregarded toxic components and undetected byproducts. Therefore, bioavailability may be more accurate if a bioassay is included in the chemical study of an ecosystem.

4. Conclusions

This study has shown that the use of ferrous sulfate for the removal of chromate through reduction reaction is applicable to Cr(VI) contaminated solutions. Furthermore, the results show that the reduction reaction of hexavalent chromium with Fe(II) in dilution water is fast and efficient. It is also apparent from bioassays that the reduction reaction affects the decrease of chromium toxicity to *D. magna*. However, Cr(VI) toxicity to *D. magna* is not affected by the equilibration of Cr(VI) with Fe(II) in the water prior to its introduction to the test chambers. In addition, the toxicity of chromium to the organisms is directly correlated to the Cr^{6+} concentration and there is a significant decrease of Cr toxicity when ferrous sulfate as a reducing agent is added to the solution. The results of our study on the chemical kinetics of chromate reduction with ferrous sulfate and toxicity tests demonstrate that the solution resulting from the chromate treatment by ferrous sulfate as a reducing agent decreases Cr^{6+} concentration and indicates much lower toxicity.

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