RESEARCH PAPER

Efficiency of humic acid from various organic sources for reducing hexavalent chromium in aqueous solutions

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ABSTRACT

In this study, efficiency of humic acids (HAs) derived from various sources to reduce Cr^{VI} in aqueous solutions was compared. HAs were extracted from leonardite, peat moss, peat, cocopeat, coal, common char, biochar, vermicompost and sewage sludge. Some chemical and spectral characteristics of the extracted HAs were measured. Then, the reducing efficiency of HAs was measured and its relationship with the determined properties was investigated. To measure the reducing efficiency of HAs, a concentration of 0.1 mM Cr^{VI} (as potassium dichromate) in a sodium nitrate solution (0.03M) with a pH of 2 and in the presence of 100 mg of HA per liter was used. The experiment was conducted in three ways (symbolized by E1, E2 and E3). They differ from each other with respect to the method of phosphate buffer addition to release Cr^{VI} ions adsorbed by HA. This buffer was added to an aliquot of final extract, to the whole volume of final suspension and to initial solution containing Cr^{VI} in experiments E1, E2 and E3, respectively. According to the results, the Cr^{VI} reducing efficiency depends not only on the nature of HA but on the method of experiment. The minimum reducing efficiency was observed for common char using experiment E2 and the maximum value was for biochar and cocopeat in all three experiments. The results showed that the two factors of ΔlogK and maximum reducing efficiency in the format of a multiple regression had a significant relationship with Cr^{VI} reducing efficiency of HAs.

KEYWORDS: Biochar, Cocopeat, Cr^{VI} reducing efficiency, Phosphate buffer

INTRODUCTION

Chromium is known as a redox-active element with oxidation numbers ranging from -2 to +6. The +3 and +6 states are most frequently found in the environment. Hexavalent chromium (Cr^{VI}) is a toxic strong oxidizing with high mobility. In contrast, trivalent chromium is an essential nutrient with low mobility (Prasad, 2013). The Cr^{VI} anions can be easily reduced with various reducing agents (Pakzadeh & Batista, 2011). In Cr^{VI} removal reactions, electron supply can occur by either mineral or organic matter (Kumar et al., 2007). Recently, researchers have focused their attention toward the use of environmentally friendly amendments such as humic substances for Cr^{VI} removal from contaminated waters (Espinoza-Quinones et al., 2009; Gao et al., 2008; Park et al., 2005). Humic substances are dark-colored polymers formed by biochemical reactions (Stevenson, 1994). The ability of such materials to ensure a successful remediation effort is influenced by their structural attributes (Quadri et al., 2008).

Humic acids (HAs) are well known amendments to remove Cr^{VI} from the environment (Palmer and Wittbrodt, 1994). Various sources such as leonardite (Zhilin et al., 2004), peat

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(Huang et al., 2012), compost (Haddad et al., 2015), sewage sludge (Amir et al., 2003), coal (Zhilin et al., 2004), municipal organic wastes (Salati et al., 2010) and soil (Palmer & Wittbrodt, 1994) have been used for extraction of humic materials. Oxygen containing groups are effective in Cr^{VI} reduction (Hsu et al., 2010). Formation of chromate ester (C-O-Cr) is reported to be the first step in reduction of Cr^{VI} (Aldmour et al., 2019). This is a way to transfer electron from HAs to Cr^{VI}. Different functional groups such as carboxylic groups (Huang et al., 2012), phenolic hydroxyl and carboxylic groups (Chen et al., 2011), phenolic hydroxyl and thiol (Scaglia et al., 2013), phenolic and hydroxyl groups (Zhang et al., 2018) as well as quinones and cuticles (Brose & James, 2010) were found to contribute in this reaction. It has been reported by several researchers that HAs with different sources have different efficiencies in remediation of environmental pollutants (Celano et al., 2008). However, some researchers showed no differences (Ashton Acton, 2013). Recently, Yang et al. (2020) reported the efficiency of a compost-derived HA in the reduction of Cr^{VI} under neutral pH and motionless conditions.

The Cr^{VI} reduction experiments using HAs have been conducted in several ways, in which the step of releasing unreacted Cr^{VI} ions to solution is a determining step. The residual Cr^{VI} ions adsorbed on HA can be released by addition of different salts including Na_2SO_4 (Makino et al., 1998) and phosphate buffer (James & Bartlett, 1983). This addition can be before (Chen et al., 2011) or after (Lin et al., 2009) the filtration step and/or through co-addition of initial Cr^{VI} containing solution (Janoš et al., 2009). The different ways of addition of releasing anion may influence the reduction reaction and hence the results obtained. In this research, the efficiency of HAs extracted from different sources for reducing Cr^{VI} were examined using three ways of adding phosphate buffer as releasing agent.

MATERIAL & METHODS

Extraction and purification of Has

Nine feedstocks including leonardite, peat moss, peat, cocopeat, coal, common char, biochar, vermicompost and sewage sludge were prepared. The samples were air-dried at room temperature, and then sieved though a 100-mesh sieve. Humic substances were extracted from samples using 0.1 mol/L NaOH solution with a solid: solution ratio of 1:10. Extracted humic substances were then separated into humic acid (HA) and fulvic acid (FA) by acidifying the extract to pH 2.0 by addition of 6 M HCl. The precipitated HAs was separated from the suspended FAs by centrifugation (2500 rpm for 15 min). HA was purified by shaking in a mixture of HCl (0.1 M) and HF (0.6 M) for 24 h, followed by centrifugation and washing with deionized water several times (Swift, 1996). The HA samples were dried in a vacuum oven at 90°C and then sieved through a 60 mesh screen. Finally, the samples were kept in a dark place in the refrigerator (Ghabbour & Davies, 2001).

Characterization of Has

Yield

The yield of HAs was calculated using the following equation:

$$\text{Yield}\,(\%) = [\frac{M_h}{M_o}] \times 100$$

where, M_h (g) is the dry mass of the extracted HAs and M_o (g) is the dry mass of organic source.

Total acidity

To determine the total acidity of HAs using indirect titration method, 0.2 g of the samples was dispersed in 20 ml 0.2 N $Ba(OH)_2$ solution and shaken for 24 h, then filtered through a Whatman 42 filter paper. The filtrates were titrated with 0.5 N HCl to pH 8.4. Also, a blank was taken without HA sample. The total acidity was calculated from the following equation (Swift, 1996):

$$\text{Total acidity}\,(\text{meq}\,/\,\text{g}) = [(V_{blank} - V_{sample}) \times N_{acid}]\,/\,M_{sample} \qquad (1)$$

where, V_{blank} and V_{sample} (ml) are the volumes of titre for blank and sample, respectively, N_{acid} (meq/ml) is the acid normality and M_{sample} (g) is the mass of HA.

FTIR analysis

Fourier transform infrared (FTIR) spectra of HAs were recorded over the range of 4000–400 1/cm on KBr pellets. For this, HA samples were first dried at 60°C. Then, 1 mg of them were mixed with 400 mg of potassium bromide and homogeneously homogenized. Homogenized samples were pressurized by applying 10 tons pressure for 3 minutes. Then, the spectrum of humic acid samples was obtained using a FTIR spectrophotometer (Bruker Tensor 27) (Klavins and Purmalis, 2013).

Spectral ratios

Five mg of HAs were dissolved in 10 ml of 0.05 M sodium bicarbonate solution, then the spectral ratios were determined at 465 and 665 nm (E4/E6) and 205 and 350 nm (E2/E3) by UV/visible spectrophotometer (Ray Leigh 7200 G) (Chen et al., 1977; Peuravuori & Pihlaja, 1997). The values of $\Delta \log K$ index were calculated by the following formula (Kumada, 1987):

$$\Delta \log K = (\log E4 - \log E6) \qquad (2)$$

where, E4 and E6 are absorbance values at 465 and 665 nm, respectively.

Organic carbon content

Organic carbon content was named as maximum Cr^{VI}-reducing efficiency by Wittbrodt and Palmer (1995). The parameter provides an estimate of the greatest reducing capacity of various HAs with different carbon content. For this, 5 ml of 0.5N $K_2Cr_2O_7$ and 20 ml of concentrated sulfuric acid were added to 10 mg HA. The reaction vessel was kept in 90℃ water bath for 1 hour. After cooling, the remaining dichromate was titrated with 0.1 N ferrous ammonium sulfate solution. Maximum Cr^{VI} reducing efficiency (MRE) of the sample was calculated from the following equation (Scaglia et al., 2013):

$$\text{MRE}\,(\text{meq}\,/\,\text{g}) = (N_d V_d - N_f V_f)\,/\,M_{sample} \qquad (3)$$

where, N_d and V_d are the normality and volume of the added dichromate solution, N_f and V_f are the normality and volume of the ferrous ammonium sulfate solution, respectively and M_{sample} (g) is the mass of HA.

Cr^{VI}-reducing efficiency of Has

The reducing efficiency of HAs was obtained in three ways (symbolized by E1, E2 and E3). These experiments differ from each other with respect to the method of phosphate buffer addition to release the adsorbed Cr^{VI} ions on HA. For this, 0 and 100 mg HA from various sources was added to 100 ml of 0.03 M NaNO$_3$ solution containing Cr^{VI} (as K$_2$Cr$_2$O$_7$) at the concentration of 100 μM. The pH of this solution was adjusted to 2.0 by addition of HNO$_3$. This pH was chosen to accelerate the reaction. After 24 hours of shaking (120 rpm) under fluorescent light at room temperature, the suspensions were filtrated. Then, 10 ml of 0.1 M K$_2$HPO$_4$/KH$_2$PO$_4$ (pH = 7) was added to 2 ml of the filtrate and shaken for 30 minutes. Cr^{VI} concentration was determined by the diphenylcarbazide method (USEPA, 1992). Spectrophotometric determination was carried out at 540 nm after Cr^{VI} reaction with 1,5-diphenylcarbazide. Cr^{VI} reducing efficiency was calculated from the following formula:

$$Cr^{VI} - reducing\,efficiency(\%) = [(C_b - C_s)/C_b] \times 100 \qquad (4)$$

where, C_s and C_b are the concentration of Cr^{VI} in sample and control, respectively. This experiment was symbolized by E1. In this method, the phosphate buffer was added to a given volume of final extract (Lin et al., 2009). In E2 and E3 methods, the buffer was added to the whole volume of final suspension (Chen et al., 2011) and to the initial solution containing Cr^{VI} (Janoš et al., 2009), respectively. All experiments were repeated three times or more and the average values were reported.

RESULTS & DISCUSSION

Characterization of Has

The characteristics of HAs derived from various sources are presented in Table 1. The highest and lowest yield was found in coal and biochar, respectively. The efficiency of humic acid extraction depends on the nature of organic matter, degree of humification and the extraction conditions (NaOH concentration, suspension concentration and contact duration) (Tao et al., 2011).

Table 1. Yield, total acidity, E4/E6 and E2/E3 values and ΔlogK of HAs

Sample	Yield (%)	Total acidity (meq/g)	E4/E6	E2/E3	ΔlogK
Leonardite	9.3	6.75	8.8	3.2	0.94
Peat moss	2.2	2.82	6.4	3.2	0.8
Peat	10	10.57	6.6	3.5	0.82
Cocopeat	4.9	7.07	8.2	3.2	1.91
Coal	20.9	10.82	1.1	1.6	0.04
Common char	3.8	5.57	1.4	2.3	0.16
Biochar	2.1	7.32	4.5	26	0.46
Vermicompost	5.7	11.57	4.9	3.4	0.69
Sewage sludge	4.8	6.32	5.3	6.2	0.72

The highest and lowest total acidity was observed in vermicompost and peat moss, respectively. Asing et al. (2009) reported the values of 10.5 and 8.3 meq/g for the total acidity of HAs derived from coal and vermicompost, respectively. As one can see, there is a good consistency between the results. The E4/E6 ratio varied from 1.1 for coal to 8.8 for leonardite. This range is wider than expected by Chen et al. (1977) i.e. E4/E6=3-5. Such high ratios have also been found by Enev et al. (2014) and Szajdak et al. (2007). The reason for the increased

E4/E6 ratio could be contamination with proteins, carbohydrates or even fulvic acids. The very low values of E4/E6 ratio obtained for coal may be attributed to its very high aromaticity. The E2/E3 ratio varied from 1.6 for the coal derived HA to 6.2 for the sewage sludge derived HA. The lower values observed for coal might be related to its high molecular weight, while the reverse is true for sewage sludge. The degree of humification for humic acid extracted from sewage sludge was low, but its aliphaticity was high (Garcia-Gil et al., 2004). Klavins and Purmalis (2013) also reported the values between 2.6 and 3.4 for the E2/E3 ratio of a peat derived HA. The values of Δlog K varied from 0.04 for the coal to 1.9 for the cocopeat. This indicates high and low degree of humification for coal and cocopeat, respectively.

Figure 1 shows the measured values of maximum Cr^{VI}-reducing efficiency (MRE) for the extracted HAs. As it can be seen, the highest MRE was observed in coal (80% organic carbon), whereas the lowest was for sewage sludge (29% organic carbon). The values of MRE could be ranked in the following order:

Coal > Cocopeat = Peat moss > Vermicompost > Commom char = Leonardite = Biochar = Peat = Sewage sludge

Scaglia et al. (2013) reported the values of 77.1 and 71.5 meq/g for the MRE of humic acids extracted from compost and leonardite, respectively. This values were less than those obtained in this study.
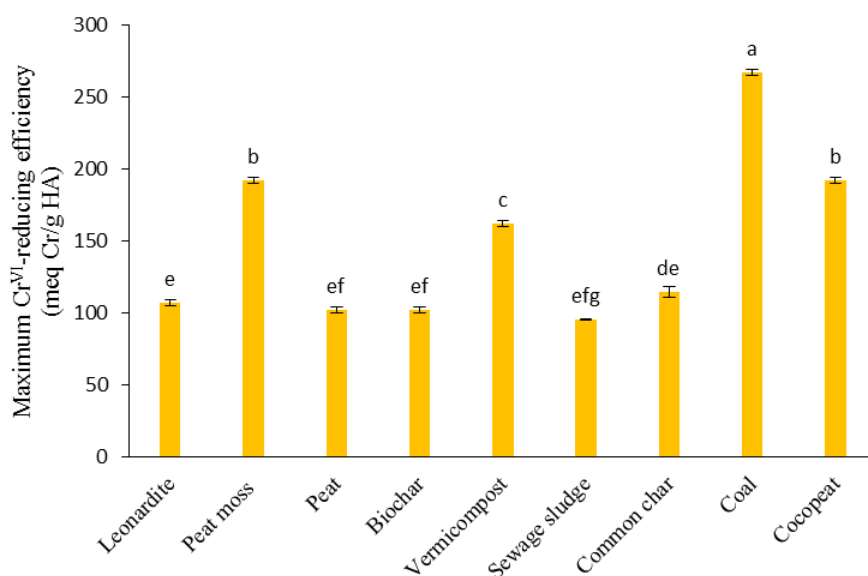

Figure 1. Maximum Cr^{VI}-reducing efficiency of HAs derived from various organic sources

The FTIR spectra of HAs extracted from leonardite, cocopeat and biochar are shows in Figures 2 to 4, respectively. The FTIR spectrum of HA extracted from leonardite is briefly interpreted here. Absorption bands at wavenumber range of 3112-3439 1/cm are assigned to the O-H and N-H vibrations. The presence of absorption band at 2922 1/cm can be attributed to the C-H vibration of CH_2 in the aliphatic structures. The absorption band at 1694 1/cm is assigned to the C=O vibration of carboxylic groups. Absorption bands at 1643 and 1533 1/cm can be attributed to the C=C vibration in the aromatic structures. The absorption band at 1099 1/cm is responsible for the C-O vibration in carbohydrates or Si-O vibration in silicates (Enev et al., 2014, Chen et al. 2005). Other spectra show similar patterns. The difference between

these FTIR spectra was mainly in the content of carbohydrates. The order of the carbohydrate content was as follows: leonardite > cocopeat > biochar.
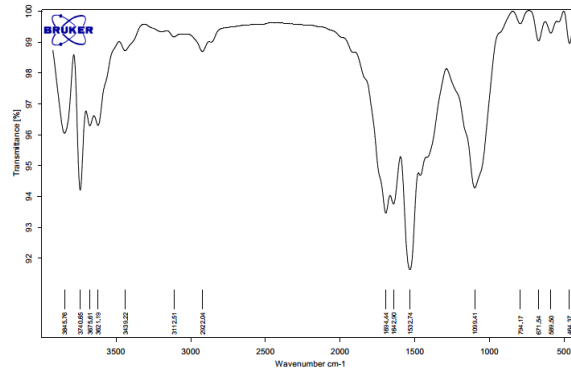

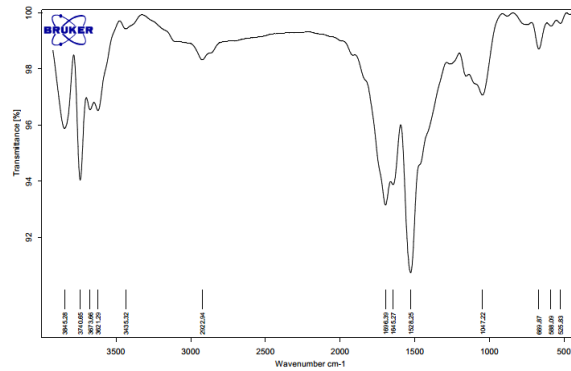**Figure 2.** FTIR spectrum of HA extracted from leonardite

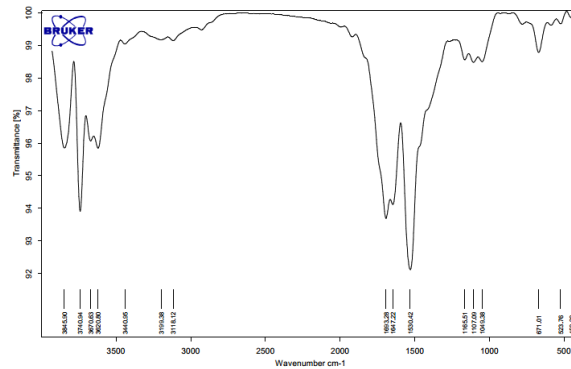**Figure 3.** FTIR spectrum of HA extracted from cocopeat

Figure 4. FTIR spectrum of HA extracted from biochar

Cr^{VI}-reducing efficiency of Has

According to the analysis of variance (Table 2) both main and interaction effects of the factors (including source of humic acid and type of experiment) on Cr^{VI}-reducing efficiency were significant ($P \leq 0.001$). This means that the main effects are not interpretable and the significant interaction is informative. In other words, the effect of source of humic acid depends on the level of the type of experiment and vice versa.

Table 2. Variance analysis of the effects of source of humic acid and type of experiment on Cr^{VI}-reducing efficiency

Sources of Variation	Degrees of Freedom	Mean Squares
Source of humic acid	8	15.78**
Type of experiment	2	1.13**
Source of humic acid × Type of experiment	16	1.02**
Error	27	0.15
CV	-	29.40

It is reasonable assumption that phosphate is able to remove almost all of the chromate adsorbed on the HA surfaces. This is in evidence by two ways: first, phosphate concentration is much greater than chromate, second, the value of "shared charge" for phosphate ($5/4 = 1.25$) is less than that of chromate ($6/4 = 1.5$). Consequently, phosphate is the most strongly adsorbed anion (McBride, 1994). This cancels out the role of adsorption in Cr^{VI} removal. The above mentioned assumption has been widely used by several workers (Janoš et al.; 2009, Lin et al., 2009; Chen et al., 2011). What makes the employed method different is the step of experiment at which the phosphate buffer is added.

Figure 5 shows the Cr^{VI}-reducing efficiency of HAs derived from various sources by the three methods of phosphate buffer addition (E1, E2 and E3). The lowest reducing efficiency was found for common char using E2 method and the highest was for biochar and cocopeat in all three methods. Different HAs showed the following order of Cr^{VI}-reducing efficiency by different methods: Method 1: Biochar = Cocopeat > Peat > Leonardite = Peat moss > Vermicompost = Sewage sludge = Common char > Coal. Method 2: Cocopeat > Biochar > Peat moss = Peat > Coal = Leonardite > Sewage sludge > Vermicompost > Common char. Method 3: Biochar = Cocopeat > Peat moss = Peat = Vermicompost = Coal > Common char > Leonardite = Sewage sludge.

Because of extremely complex nature of HAs, it is very difficult to explain the exact causes behind the above different orders. Some possible explanations are discussed here.
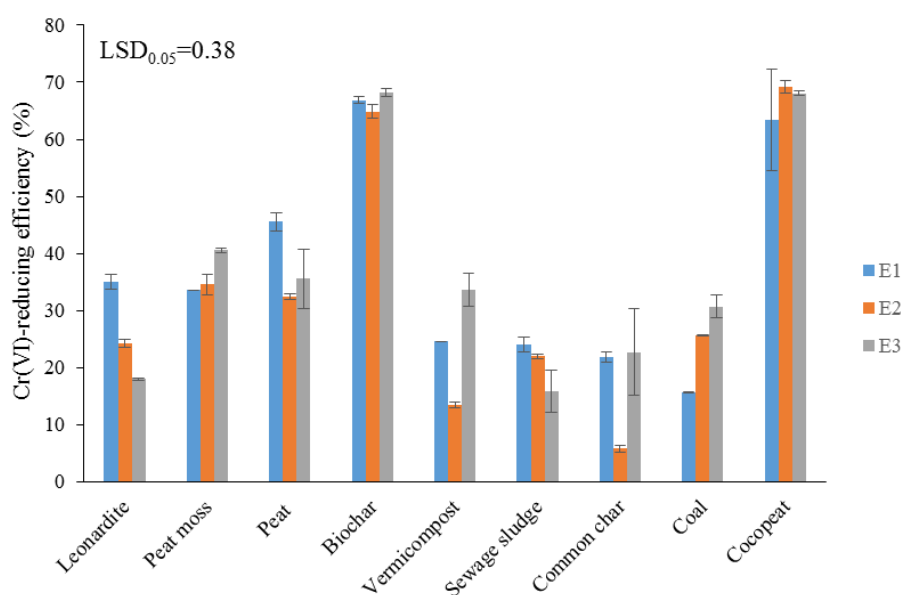
Figure 5. Cr^{VI}-reducing efficiency of HAs derived from various sources
(pH=2, $Cr^{VI} = 0.1$ mM, HA= 0.01 mg/L)

None of the above mentioned orders were in consistence with that found for MRE. This is attributed to the fact that only small part of total organic carbon is used to reduce Cr^{VI}. According to the results, most of organic carbon in coal is not effective as a reductant, but the reverse is true for cocopeat. Additionally, an important point is that three methods were not consistent for the HAs. Method 1 always exhibited greater or equal reducing efficiency than method 2 with only one exception but, the issue is more complex for E3 method.

One disadvantage of E1 method is that some part of the unreacted Cr^{VI} ions bonded to HA may be remained on the filter paper and thus the reducing efficiency could be overestimated by this method. The mean reducing efficiency in E2 method (32.5%) was 4.2% less than that in E1 method (36.7%). This difference was about 13% for HAs extracted from common char, leonardite, peat and vermicompost, whereas a non-significant change was observed for other HAs. Consequently, HAs extracted from the former sources had a great potential to retain Cr^{VI} ions. Coal derived HA was the only exception, in which E2 method showed significantly greater reducing efficiency than E1 method. In E2 method, addition of phosphate buffer (pH=7) to the whole suspension increases the initial reaction pH (pH=2) to values near the point of zero charge (pzc) of coal extracted HA. This can cause twisting the high molecular weight HA chains which makes difficult the release of residual Cr^{VI}. The reducing efficiency of HAs extracted from leonardite and sewage sludge was considerably lower in E3 method than in both E1 and E2 methods. This may be due to competition of phosphate with chromate for adsorption that reduces the reducing efficiency of HA. In contrast, the reducing efficiency of HAs derived from common char and vermicompost was substantially greater in E3 method than in E2 method. This may be attributed to increasing ionic strength that favors the further Cr^{VI} reduction (Pagnanelli et al., 2013). Finally, the E2 method may be recommended because of its higher repeatability.

Influence of HA characteristics on Cr^{VI} reduction

Among the measured properties for HAs (aside from biochar extracted HA), only two of them including $\Delta logK$ and maximum reducing efficiency were found to be in a significant relation with reducing efficiency in the form of a multivariate regression. Regarding the standardized coefficient (β) of the regression equation, $\Delta logK$ played a more important role in describing the reducing efficiency (RE) than the maximum reducing efficiency (MRE). These two variables explained 83% of the variance:

$$RE = -13.742 + 29.504\,\Delta \log K + 0.127\,MRE \quad \left(P \leq 0.01 \right) \tag{5}$$

The higher value of $\Delta logK$ means the lower degree of humification of HA. Therefore, results show that HAs with a lower degree of humification (low molecular weight HAs) exhibit a higher efficiency for reducing Cr^{VI}. This is in agreement with the findings of Chen et al. (2012), but is contrary to the results of Cerqueira et al. (2012). The FTIR spectra of the HAs extracted from leonardite, peat, peat moss, vermicompost and cocopeat (Figures 1-3) show the presence of carbohydrate units that may be responsible for the irreversible reduction of Cr^{VI} (Zhilin et al., 2004). These HAs also have greater values of $\Delta logK$. HA extracted from sewage sludge has a high value of $\Delta logK$, but no evidence of carbohydrate units was found in its FTIR spectrum. Additionally, HA extracted from biochar has a low value of $\Delta logK$, indicating a high molecular weight HA, without the presence of carbohydrate. However, biochar derived HA showed a high reducing efficiency. Therefore, reduction of Cr^{VI} may be related to the reversible transformations of quinone-hydroquinone units (Zhilin et al., 2004). Moreover, participation of the maximum reducing efficiency which is in association with the

organic carbon content highlights the role of other mechanisms in reduction of Cr^{VI} by HAs. The contribution of reduction efficiency (obtained by the E2 method) to the maximum reducing efficiency was from 3.3% for common char to 42.2% for biochar. This indicates that the relationship between the factors associated with Cr^{VI} reduction and the organic carbon content of HAs was the lowest and the highest for biochar and common char, respectively.

CONCLUSION

This study indicates that not only the nature of humic acid (HA), but also the experimental method can influence the results of investigations designed for Cr^{VI} removal by HAs. In this research, three methods of phosphate buffer addition, in order to release the residual Cr^{VI} ions from HA, were compared. Among the tested methods, the method in which the phosphate buffer is added to the whole suspension was selected because of its high repeatability. HA properties including $\Delta logK$ and maximum reducing efficiency were in a significant relation with Cr^{VI} reducing efficiency. FTIR spectra of the HAs indicated the presence of carbohydrate units that may be responsible for the irreversible reduction of Cr^{VI}. However, there were some indications that other mechanisms such as reversible transformations of quinone-hydroquinone units, may involve Cr^{VI} reduction.

GRANT SUPPORT DETAILS

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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