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Humic and Fulvic Acids as Potentially Toxic Metal Reducing Agents in Water

Stavros Lalas,* Vasilios Athanasiadis, and Vassilis G. Dourtoglou

Industrial activity has contributed to potentially toxic metal pollution in various ecosystems throughout the world. In this study, the ability of humic and fulvic acids (isolated from lignite with a simple, rapid, and inexpensive method) to reduce toxic metals' concentration of contaminated water is examined. More specifically, the effect of these compounds is tested on water contaminated (at various concentrations) with Cd, Cr, Cu, Ni, and Pb. The determination is performed by inductively coupled plasma-optical emission spectrometry. The maximum binding capacity is presented by fulvic acids in the following order Cr>Pb>Ni>Cu>Cd (97.8, 96.5, 95.4, 95.1, and 83.3%, respectively), while for humic acids it is Pb>Ni>Cr>Cu>Cd (81.3, 70.7, 68.6, 67.0, and 66.8%, respectively). The binding activity is also tested on water contaminated with two metals simultaneously. In these cases, the existence of two metals in water changes the reduction ability of humic and fulvic acids (positively or negatively).

1. Introduction

Environmental pollution by heavy metals has become an ecotoxicological hazard of prime interest and increasing significance. Heavy metal ions are released into the environment in a number of different ways and because of their accumulation in living organisms form a serious and complex problem that has been a focus of attention all over the world.^[1] Heavy metals can leach to ground and surface water and cause toxic effects when taken up in excess by soil organisms and plants.^[2]

Water pollution by heavy metals has become a public concern due to the development of high-technology industries around the world.^[3] The long-term intake of drinking water containing even low concentrations of heavy metals may cause fatal diseases by the accumulation of heavy metals in the internal organs of human body.^[4] Therefore, it is necessary to appropriately remove them through various water treatment processes for satisfying the water quality standard, such as

complexing agents ethylene diamine tetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA),^[5] potassium ferrate,^[3] total organic carbon,^[6] carbonate hydroxylapatite,^[7] nanoporous gold,^[8–10] humic and fulvic acids,^[1,11–14] and humic/fulvic acids by miscellaneous nanomaterials.^[15]

Humic and fulvic acids make up an important part of soil organic matter, and their binding capacity affects the fate of metal ions and plays an important role in their mobility.^[16] The fulvic acid fraction is assumed to form more soluble metal complexes because it is soluble in water, whereas humic acids tend to produce more insoluble metal complexes.^[12,17] Due to their higher molecular weight and low content of acidic functional groups, metal complexes of humic acids are less soluble, mobile, and bioavailable than those of fulvic acids^[18] and additionally have

less proton binding sites.^[14] Humic substances are heterogeneous macromolecular aggregates that comprise the main part of natural organic carbon in soils, water, and sediments.^[19]

Lignite, the youngest type of coal located between the peat and brown coal on the caustobiolites scale,^[20] contains large amounts of humic and fulvic acids, with the former predominating.^[11] It is assumed that humic substances are the main components responsible for sorption of metals on lignite (particularly, their carboxylic and hydroxyl-phenolic groups). These groups are the active center of the ion-exchange. So, the lignite-based materials can be used as an alternative cation-exchanger,^[11] and the fact that they are plentiful and inexpensive makes them an attractive option for the removal of metals from water. Therefore, lignite represents a potential low-cost sorbent of toxic metals for use in water treatment, groundwater remediation, and construction of active geochemical barriers.^[21]

Although, humic and fulvic acids have previously reported to have the ability to bind potentially toxic metals, their use for the purification of water has not yet been exploited. Furthermore, the isolation of humic and fulvic acids from lignite from Ptolemaida (Greece) has not reported previously. Additionally, the complexation ability and selectivity of those compounds under simultaneous existence of various potentially toxic metals has not yet been studied. The above were the aims of the present work.

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2. Experimental Section

2.1. Lignite

The sample of lignite used in this study was obtained from lignite mines of Ptolemaida (Komanos area), West Macedonia, Greece.

2.2. Chemical and Reagents

All the reagents used were of analytical grade. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Panreac (Barcelona, Spain). Nitric acid was obtained from Merck (Darmstadt, Germany). Cadmium nitrate, chromium nitrate, copper nitrate, nickel nitrate, and lead nitrate were purchased from Carlo Erba (Milano, Italy). Double distilled water was used in all experiments.

2.3. Extraction of Humic and Fulvic Acids From Lignite

The method used was adapted from Janoš^[19] with some modifications. Initially, 150 g of lignite and 850 g of double distilled water were mixed. The pH value of the mixture was adjusted to 9 using NaOH (0.5 mol L^{-1}), left to stand for 24 h and then centrifuged (using a Digicen 20-R, Orto Alresa, Madrid, Spain) for 10 min at 5000 rpm. The supernatant was separated and its pH was adjusted to 1 using HCl (37%). Next, the mixture was centrifuged for 10 min at 5000 rpm. Finally, humic and fulvic acids (precipitate and supernatant, respectively) were separated and weighed after drying (using a Telstar Cryodos 80 freeze dryer, Telstar Industrial, S.A., Terrassa, Spain) for 12 h.

2.4. Determination of Water Content

The ASTM method D4959-07^[22] was used to determine the water content of lignite. Specifically, lignite sample was placed in a suitable container and then in an oven at 105°C until constant weight. After drying, the water content (%) of lignite was calculated.

2.5. Preparation of Samples

Toxic metal concentrations were chosen according to the European Council Directive 98/83/EC (quality of water intended for human consumption).^[23] Generally, 50, 100, and 150% of the parametric value limit of the Directive were used as concentrations of each of the standard solutions of Cd, Cr, Cu, Ni, and Pb. Specifically, solution concentrations of 2.5, 5.0, and $7.5 \mu\text{g L}^{-1}$ were used in the case of Cd, 25, 50, and $75 \mu\text{g L}^{-1}$ in the case of Cr, 1000, 2000, and $3000 \mu\text{g L}^{-1}$ in the case of Cu, 10, 20, and $30 \mu\text{g L}^{-1}$ in the case of Ni and, finally, 5, 10, and $15 \mu\text{g L}^{-1}$ in the case of Pb. 100, 200, 400, and 600 ppm of dried humic or fulvic acids were added to each of the above solutions. After agitation the solutions were left to stand for 15 min, filtered through paper and, finally, the % reduction of concentration of

toxic metals was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 5300 DV, PerkinElmer, Shelton, USA). One percent of concentrated HNO_3 was used for the preparation of solutions.

Additionally, 600 ppm of humic or fulvic acids were added to mixtures of two toxic metals which were prepared at a concentration of 150% of the parametric value limit of the European Council Directive.

Blank samples [pure double distilled water, double distilled water contaminated with toxic metals (single solution) or metals (binary solution) and double distilled water with humic or fulvic acids] were prepared and used for comparison.

2.6. Statistical Analysis

Results are represented as average of three simultaneous assays. Standard deviation in Tables is given in parenthesis. Statistical significance of the differences between mean values was assessed by ANOVA; $p < 0.05$ was considered as statistically significant.

3. Results and Discussion

3.1. Humic and Fulvic Acid Extraction and Analysis

A water content of 8.25% was determined in the lignite from Ptolemaida (Western Macedonia, Greece).

Following a simple extraction procedure humic and fulvic acids were separated (0.29 and 0.15 g/100 g lignite, respectively). The determined content of lignite in humic and fulvic acids appeared to be within the limits previously reported in the literature.^[24,25] It is known that lignite contains by nature heavy metals.^[26] Therefore, humic and fulvic acids extracted from lignite were analyzed in order to determine their metal content under consideration. During analysis Cd and Pb were not detected. In contrast, in humic acids and fulvic acids, respectively, Cr was detected at low concentration of 1.088 and $0.062 \mu\text{g L}^{-1}$, Cu at 24 and $14 \mu\text{g L}^{-1}$, and Ni at 0.531 and $0.867 \mu\text{g L}^{-1}$.

3.2. Single Metal Binding Ability

The binding ability of the various metals under consideration by humic and fulvic acids is represented in **Table 1**. Specifically, Table 1 is representing the % reduction of a single metal after addition of 100, 200, 400, and 600 ppm of humic or fulvic acids.

3.2.1. Cadmium (Cd)

The binding ability of humic and fulvic acids in the case of Cd is presented in Table 1. Humic acids have reduced the concentration of the metal from 44.2 to 66.8%. When the solution contained $2.5 \mu\text{g L}^{-1}$ of Cd, 200, 400, and 600 ppm of humic acids showed a significantly higher ($p < 0.05$) binding capacity compared with 100 ppm (44.2%). The binding capacity for

Table 1. % Reduction of toxic metals after the addition of 100, 200, 400, and 600 ppm of humic and fulvic acids.

Metal	Concentration ($\mu\text{g L}^{-1}$)	Humic acids				Fulvic acids			
		100 ppm	200 ppm	400 ppm	600 ppm	100 ppm	200 ppm	400 ppm	600 ppm
Cd	2.5	44.21* (0.90)	51.12 (1.00)	51.15 (1.10)	51.19 (1.20)	55.20 (0.78)	67.14 (0.96)	71.30 (1.02)	78.14 (1.16)
	5	49.02 (1.00)	52.14 (1.35)	56.25 (1.40)	56.28 (1.40)	66.69 (1.15)	73.25 (1.25)	80.17 (1.65)	88.67 (1.64)
	7.5	38.85 (0.80)	55.83 (1.00)	61.25 (1.10)	66.77 (1.40)	79.75 (1.54)	84.35 (1.65)	89.25 (1.87)	95.10 (1.90)
Cr	25	51.56 (0.90)	52.87 (1.00)	54.85 (1.20)	57.69 (1.40)	60.00 (1.25)	65.36 (1.45)	72.65 (1.94)	80.12 (2.42)
	50	54.10 (0.90)	58.68 (1.00)	61.12 (1.20)	63.20 (1.25)	67.20 (1.24)	75.90 (1.48)	81.40 (2.01)	90.30 (2.54)
	75	59.76 (0.80)	62.50 (1.00)	65.30 (1.10)	68.59 (1.40)	75.25 (1.47)	84.56 (1.64)	89.35 (1.88)	97.82 (2.14)
Cu	1000	51.40 (0.90)	54.16 (1.30)	57.49 (1.50)	66.97 (1.20)	68.44 (1.49)	72.25 (1.03)	75.36 (1.92)	83.34 (2.15)
	2000	30.61 (1.00)	35.98 (1.00)	45.11 (1.20)	48.90 (1.25)	61.90 (0.87)	64.90 (1.14)	70.20 (1.25)	78.40 (1.87)
	3000	23.53 (0.80)	29.14 (1.00)	37.51 (1.30)	42.15 (1.60)	55.14 (0.74)	61.14 (0.81)	63.35 (0.85)	66.14 (0.89)
Ni	10	70.73 (1.80)	65.25 (1.50)	61.59 (1.30)	55.58 (1.50)	95.36 (1.98)	88.25 (1.82)	76.14 (1.67)	67.25 (1.55)
	20	67.80 (1.60)	66.28 (1.50)	62.35 (1.50)	60.14 (1.30)	85.78 (1.79)	74.25 (1.69)	65.14 (1.64)	58.14 (1.58)
	30	69.76 (1.70)	67.97 (1.50)	62.03 (1.40)	61.10 (1.40)	75.16 (1.71)	69.35 (1.65)	60.14 (1.57)	52.36 (1.55)
Pb	5	52.31 (1.00)	56.30 (1.30)	58.20 (1.40)	61.30 (1.60)	72.65 (1.24)	81.11 (1.69)	91.20 (1.84)	96.54 (1.92)
	10	54.30 (1.30)	59.34 (1.60)	63.10 (1.60)	68.47 (1.80)	70.25 (1.25)	76.36 (1.47)	81.25 (1.51)	86.87 (1.69)
	15	61.35 (1.20)	67.25 (1.50)	75.34 (1.60)	81.26 (1.90)	68.36 (1.21)	74.58 (1.34)	78.36 (1.54)	83.35 (1.89)

*Values are means of triplicate determinations. SD is given in parenthesis.

concentrations of 200–600 ppm showed no significant differences (about 51.1% reduction in all concentrations). When the solution contained $5.0 \mu\text{g L}^{-1}$ of Cd the addition of 400 and 600 ppm of humic acids have not shown any significant difference in binding activity. However, they exhibited significantly greater ($p < 0.05$) binding ability (up to 56.3%) than the concentrations of 100 and 200 ppm (49.0 and 52.1%, respectively). Finally, 600 ppm of humic acids showed a significant higher ($p < 0.05$) binding capacity (66.8%) in the solution containing $7.5 \mu\text{g L}^{-1}$ of Cd in comparison to the concentrations of 100, 200, and 400 ppm (38.9, 55.8, and 61.3%, respectively) which in turn showed significant ($p < 0.05$) differences to each other.

Fulvic acids showed a binding capacity that was higher in all cases compared to humic acids (at the same level of addition) and ranged from 55.2 to 95.1%. When the solution contained $2.5 \mu\text{g L}^{-1}$ of Cd, fulvic acids in the concentration of 600 ppm exhibited a greater ($p < 0.05$) binding capacity (78.2%) than the concentrations of 100, 200, and 400 ppm (55.2, 67.1, and 71.3%, respectively) which in turn showed significant ($p < 0.05$) differences to each other. In the case of the solutions with 5.0 and $7.5 \mu\text{g L}^{-1}$ of Cd the results followed the same trend.

In the study of Pentari et al.,^[27] higher absorption of Cd (up to 98.0%) was observed. However, in this study, other kinds of lignite (rich in mineral matter and with low calorific value) were used. These lignites had significantly higher content in humic and fulvic acids than that used in the present study.

3.2.2. Chromium (Cr)

The binding ability of humic and fulvic acids in the case of Cr is presented in Table 1. Humic acids have reduced the

concentration of the metal by 51.6–68.6%. When the solution contained $25 \mu\text{g L}^{-1}$ of Cr, 400 and 600 ppm of humic acids showed a significantly higher ($p < 0.05$) binding capacity compared to 100 ppm (51.6%). There were no significant differences between the concentrations of 100 and 200 or 400 and 600 ppm. In the case of the solutions with 50 and $75 \mu\text{g L}^{-1}$ of Cr the results followed the same trend.

Fulvic acids showed a binding capacity that was higher in all cases than that of humic acids (at the same level of addition) and ranged from 60.0 to 97.8%. When the solution contained $25 \mu\text{g L}^{-1}$ of Cr, fulvic acids in the concentration of 600 ppm exhibited a greater ($p < 0.05$) binding capacity (80.1%) compared to 100, 200, and 400 ppm (60.0, 65.4, and 72.7%, respectively) which in turn showed significant ($p < 0.05$) differences to each other. In the case of the solutions with 50 and $75 \mu\text{g L}^{-1}$ of Cr the results followed the same trend.

Other researchers found higher absorption of Cr (up to about 97%).^[28] In this research, a commercially available pure humic acid crystalline powder in combination with bamboo bark-based activated carbon was used; a rather more complicated absorption procedure than that described in the present study.

3.2.3. Copper (Cu)

The binding ability of humic and fulvic acids in the case of Cu is presented in Table 1. Humic acids have reduced the concentration of the metal by 23.5–67.0%. When the solution contained $1000 \mu\text{g L}^{-1}$ of Cu, 400 and 600 ppm of humic acids showed a significantly higher ($p < 0.05$) binding capacity than the concentration of 100 ppm (51.4%). There were significant differences ($p < 0.05$) in binding between all concentrations. In the case of the solutions with 2000 and $3000 \mu\text{g L}^{-1}$ of Cu the results followed the same trend.

Fulvic acids showed a binding capacity that was higher in all cases compared with humic acids (at the same level of addition) and ranged from 55.1 to 83.3%. When the solution contained $1000 \mu\text{g L}^{-1}$ of Cu, fulvic acids in the concentration of 600 ppm exhibited a greater ($p < 0.05$) binding capacity (83.3%) than for concentrations of 100, 200, and 400 ppm (68.4, 71.3, and 75.4%, respectively) which in turn showed significant ($p < 0.05$) differences to each other. In the case of the solutions with 2000 and $3000 \mu\text{g L}^{-1}$ of Cu the results followed the same trend.

In the study of Pentari et al.,^[27] higher absorption of Cu (up to 97.9%) was found. However, during their experiments, they have used other kinds of lignite with significantly higher content in humic and fulvic acids in comparison to that used in the present study.

3.2.4. Nickel (Ni)

The binding ability of humic and fulvic acids in the case of Ni is presented in Table 1. Humic acids have reduced the concentration of the metal from 52.4 to 70.7%. When the solution contained $10 \mu\text{g L}^{-1}$ of Ni, 100 ppm of humic acids showed a significantly higher ($p < 0.05$) binding capacity than the concentration of 200, 400, and 600 ppm (70.7%). There were significant differences ($p < 0.05$) between all concentrations. In the case of the solutions with 10 and $15 \mu\text{g L}^{-1}$ of Ni the results followed the same trend. However, for this metal it appeared that the increase in concentration of humic acids decreased the % of reduction. Similar behavior was previously observed by Ji^[29] who found that the absorption of Ni decreased when the concentration of humic acid increased to $>6 \mu\text{g L}^{-1}$. In addition, Guthrie et al.^[30] noted that only 1% of the available binding sites of humic acids can bind potently Ni.

Fulvic acids showed a binding capacity that was higher in all cases compared with humic acids (at the same level of addition) and ranged from 52.4 to 95.4%. When the solution contained $10 \mu\text{g L}^{-1}$ of Ni, fulvic acids in the concentration of 100 ppm exhibited a greater ($p < 0.05$) binding capacity (95.4%) than in concentrations of 200, 400, and 600 ppm (88.2, 76.1, and 67.3%, respectively) which in turn showed significant ($p < 0.05$) differences to each other. In the case of the solutions with 10 and $15 \mu\text{g L}^{-1}$ of Ni the results followed the same trend. Again, similarly to the case of humic acids, it appeared that the increase in concentration of fulvic acids decreased the % of reduction.

3.2.5. Lead (Pb)

The binding ability of humic and fulvic acids in the case of Pb is presented in Table 1. Humic acids were able to bind 52.3–81.3% of the metal. When the solution contained $5 \mu\text{g L}^{-1}$ of Pb, 200, 400, and 600 ppm of humic acids showed a significantly higher ($p < 0.05$) binding capacity (56.3, 58.2, and 61.3%, respectively) than the concentration of 100 ppm (52.3%). There were significant differences ($p < 0.05$) between all concentrations. In the case of the solutions with 10 and $20 \mu\text{g L}^{-1}$ of Cr the results followed the same trend.

Fulvic acids showed a binding capacity that was higher in all cases compared to humic acids (at the same level of addition) and ranged from 68.4 to 96.5%. When the solution contained

$5 \mu\text{g L}^{-1}$ of Pb, fulvic acids in the concentration of 600 ppm exhibited a greater ($p < 0.05$) binding capacity (96.5%) than in concentrations of 100, 200, and 400 ppm (72.7, 81.1, and 91.2%, respectively) which in turn showed significant ($p < 0.05$) differences to each other. In the case of the solutions with 10 and $15 \mu\text{g L}^{-1}$ of Pb the results followed the same trend.

In the study of Pentari et al.,^[27] higher absorption of Pb (up to 99.7%) was observed. However, in this study, other kinds of lignite (rich in mineral matter and with low calorific value) were used. These lignites had significantly higher content in humic and fulvic acids than that used in the present study.

3.2.6. Conclusions on Single Metal Binding Ability

There is still a great debate about the way humic and fulvic acids bind potentially toxic metals. However, the major binding sites in those substances are usually attributed to the carboxylic and phenolic groups present.^[31] Cation binding to humic substances is assumed to occur through specific interactions between the cation and negatively charged surface functional groups and by nonspecific Coulombic binding to any residual negative charge.^[32] Free radicals were found to play a significant role in the binding of organochlorine pesticides and heavy metals with humic acid. Furthermore, the way humic acid molecules reacted with metals depend upon the type of metal ion and on the pH.^[31] However, Pehlivan and Arslan^[11] reported that very low pH functional oxidized groups (pH < 2) (hydroxyl, carboxyl, phenol, methoxyl, etc.) of humic acids are protonated.

As shown by the results the binding capacity of fulvic acids was higher in all cases compared to humic acids (at the same level of addition). This result was expected since fulvic acids have more proton binding sites.^[14]

Specifically, in the case of fulvic acids, the maximum binding capacity was presented for Cr (97.8%), followed by Pb (96.5%), Ni (95.4%), Cd (95.1%), and Cu (83.3%). Regarding the concentration of humic acids it appeared that the binding capacity is proportional to the concentration of the metals Cd, Cr, Cu, and Pb. On the contrary, nickel binding capacity reduced while the concentration of the humic acids increased.

Concerning humic acids, the maximum binding capacity was presented for Pb (81.3%), followed by Ni (70.7%), Cr (68.6%), Cu (67.0%), and Cd (66.8%). The results of this study are in agreement with those of Pandey et al.^[30] except in the case of copper where the authors found the highest binding capacity of humic acids compared to other metals. This difference is probably due to the fact that the pH of the solution was adjusted to different values (pH = 3.5) from those used in the present work. Additionally, the same authors^[31] analyzed their samples directly without destroying humic acid. However, during the present study, humic and fulvic acids were removed from the samples before the determination of potentially toxic metals concentration in water. Therefore, any direct comparison would not be applicable.

3.3. Two Metal Simultaneous Binding Ability

The simultaneous binding ability of two metals by humic and fulvic acids is presented in Table 2 (% reduction of toxic metals

Table 2. % Reduction of toxic metals after addition of 600 ppm of humic or fulvic acids to the mixtures of two toxic metals prepared at a concentration of 150% (7.5 $\mu\text{g L}^{-1}$ Cd; 75 $\mu\text{g L}^{-1}$ Cr; 3000 $\mu\text{g L}^{-1}$ Cu; 30 $\mu\text{g L}^{-1}$ Ni; 15 $\mu\text{g L}^{-1}$ Pb).

% Reduction of metal combinations				
Humic acid (600 ppm)	Cd/Cr	Cd/Cu	Cd/Ni	Cd/Pb
	71.22/72.01*	12.63/31.10	85.20/69.14	65.04/71.54
	(1.10)/(1.30)	(0.80)/(0.89)	(1.50)/(1.21)	(1.20)/(1.32)
	Cr/Cu	Cr/Ni	Cr/Pb	–
	60.11/35.47	73.09/68.40	67.12/66.33	
	(1.00)/(0.85)	(1.31)/(1.22)	(1.29)/(1.21)	
Fulvic acid (600 ppm)	Cu/Ni	Cu/Pb	–	–
	31.65/15.92	34.41/82.54		
	(0.91)/(0.78)	(0.95)/(1.42)		
	Ni/Pb	–	–	–
	67.12/70.04			
	(1.27)/(1.33)			
	Cd/Cr	Cd/Cu	Cd/Ni	Cd/Pb
	81.10/64.31	90.91/70.39	93.76/55.67	74.56/15.47
	(1.65)/(1.44)	(1.00)/(1.54)	(1.95)/(1.25)	(1.74)/(0.58)
	Cr/Cu	Cr/Ni	Cr/Pb	–
	80.11/71.45	44.84/50.29	96.24/24.15	
	(1.85)/(1.47)	(1.15)/(1.14)	(1.87)/(0.98)	
	Cu/Ni	Cu/Pb	–	–
	74.73/58.46	82.57/54.58		
	(1.54)/(1.11)	(1.74)/(1.25)		
	Ni/Pb	–	–	–
	56.26/12.49			
	(1.55)/(0.68)			

*Values are means of determinations ($n = 3$). SD is given in parenthesis.

after addition of 600 ppm of humic or fulvic acids to the mixtures of two toxic metals).

As represented in Table 2, humic acids showed again binding activity on all studied metals. In the case of the mixture of Cd + Cr the binding of metals increased significantly ($p < 0.05$) for both of them in comparison to their binding in the corresponding single solution (71.2% instead of 66.8% in the case of Cd and 72.0% instead of 68.6% in the case of Cr). For this metal mixture the use of 600 ppm fulvic acids showed the opposite results. Specifically, the binding of Cd was reduced by 14.7%, while for Cr it was reduced by 34.3% (in comparison to the corresponding single solution).

In the case of the mixture of Cd + Cu the reduction of both metals was significantly ($p < 0.05$) decreased by 82.0% for Cd and 26.5% for Cu when humic acids were used (in comparison to their binding in the corresponding single solution). In the case of fulvic acids a 2.4% decrease (significant at $p < 0.05$) in the binding of Cd and a 6.2% increase in the case of Cu was observed.

Concerning the mixture of Cd + Ni and humic acids, there was a significant increase ($p < 0.05$) by 27.3% for Cd and 12.9% for Ni binding. When fulvic acids were used, a non-significant reduction of Cd was observed, while the reduction of Ni was significantly ($p < 0.05$) increased by 6.6%.

Humic acids in the mixture Cd + Pb produced a non-significant decrease in Cd, while in the case of Pb a significant decrease of

12.6% appeared. Fulvic acids showed a significant ($p < 0.05$) decrease in reduction by 21.7% for Cd and 81.5% for Pb.

In the case of the mixture Cr + Cu and humic acids, the binding of both metals was significantly ($p < 0.05$) reduced by 12.5% (Cr) and 17.0% (Cu). Fulvic acids showed similar results only in the case of Cr. Specifically, Cr presented a significant ($p < 0.05$) decrease in binding by 18.1%, whereas for Cu an 8.0% increase was observed.

The addition of humic acids to the mixture of Cr + Ni significantly ($p < 0.05$) increased the reduction of both metals (6.4 and 11.3% for Cr and Ni, respectively). For this mixture the use of fulvic acids showed different results. Specifically, Cr reduction presented a significant ($p < 0.05$) decrease in binding of 57.1%, while for Ni a significant ($p < 0.05$) decrease in binding (4.1%) was observed.

In the case of the mixture Cr + Pb and humic acids a significant ($p < 0.05$) decrease in reduction appeared only for Pb (18.8%). The same results were observed when fulvic acids were used. Specifically, a 71.1% significant ($p < 0.05$) reduction in binding of Pb appeared.

A significant ($p < 0.05$) reduction in binding of both metals was observed in the case of the mixture Cu + Ni and humic acids. Specifically, the binding was reduced by 26.5% for Cu and 75.4% for Ni. The use of fulvic acids showed opposite results. Specifically, the binding of Cu increased significantly ($p < 0.05$) by 12.9%, while that of Ni by 11.5%.

In the case of the mixture Cu + Pb and humic acids a significant ($p < 0.05$) decrease in binding (by 19.3%) appeared only for Cu. For this mixture the use of fulvic acids showed a significant ($p < 0.05$) increase in binding (by 24.7%) in the case of Cu, while Pb's binding was significantly ($p < 0.05$) reduced by 34.6%.

The addition of humic acids in the mixture of Ni + Pb significantly ($p < 0.05$) increased the reduction of Ni (by 9.7%) but reduced the binding of Pb (by 13.9%). For this mixture, the use of fulvic acids followed the same trend. Specifically, Cr reduction presented a significant ($p < 0.05$) increase in binding by 7.3%, while for Pb a significant ($p < 0.05$) decrease (by 85.1%) was observed.

3.3.1. Conclusions on Two Metal Binding Ability

As indicated by the results the existence of two metals in the solution changed, in most cases, the reduction ability (positively or negatively). Possibly, the binding of one metal to humic or fulvic acids modifies their structure and new positions in the molecule of the acid are becoming available for strong connection with metals or old positions previously offering unstable connection to metals become more stable. The opposite probably happens when the metals are connected in the same positions and so there is a competition, and, therefore, a reduction in the capture rate for one or both of them appears. In another study, the possible use of four Greek types of lignite was investigated for the removal of metal ions from wastewater.^[27] It was proved that among the elements studied (Cu, Pb, Cr, Zn), Cu appeared to be the only element whose absorption (by lignite) is considerably influenced by the presence of the other elements (Pb, Cr, Zn).

3.4. Further Research

Further research should be performed to optimize the conditions of pH and temperature and to find the optimal concentration of humic or fulvic acids in order to maximize the binding ability. Additional studies must also be carried out with more metals and combinations of them. Finally, further study is needed to find the chemical formulas of humic and fulvic acids which were isolated from the specific kind of lignite and especially for finding the position where the metals are linked in each molecule.

4. Concluding Remarks

The present work introduces a relatively low cost and highly efficient method to reduce the level of potentially toxic metals in water.

- The reducing agents were humic and fulvic acids isolated from lignite (an abundant and cheap coal) with a simple, rapid, and inexpensive procedure.
- The maximum binding capacity (for a single metal) was presented by fulvic acids in the following order $\text{Cr} > \text{Pb} > \text{Ni} > \text{Cu} > \text{Cd}$, while for humic acids was $\text{Pb} > \text{Ni} > \text{Cr} > \text{Cu} > \text{Cd}$.
- The binding activity of both acids on two metals (appearing simultaneously in water) was also examined presenting a change in their ability to reduce the metals under consideration.

Further research should be carried out for the optimization of the methods, not only of the extraction procedure of humic or fulvic acids, but also for the improvement of the binding ability (especially, for more than one metal). Finally, additional study is needed on lignite in order to determine the most appropriate origin which can give the most capable humic and fulvic acids for binding potentially toxic metals.

Abbreviations

DTPA, diethylenetriaminepentaacetic acid; EDTA, ethylene diamine tetraacetic acid; ICP-OES, inductively coupled plasma-optical emission spectrometry.

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Conflicts of Interest

The authors have declared no conflicts of interest.

Keywords

fulvic acids, humic acids, lignite, toxic metals

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