Assessment of Aquatic Toxicity of the Caffeic Acid Complexed with Cr(III) and Pb(II) in the Flotation Process

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The use of the metals collectors reagents in the flotation process, applied in the aim of the aqueous systems remediation, require an ecotoxicity assessment on aquatic organisms. For this purpose, laboratory experiments were performed in order to determine the toxicity indices expressed by: median lethal concentration values (LC50 / EC50), effects generated on the aquatic organisms and the estimation of biodegradability degree of the resulted effluents.The acute toxicity of caffeic acid (reagent collector) and the effluents obtained after two different flotation processes(ionic and precipitation) containing Pb(II) and Cr(III) was assessed.The biological material consisting in different aquatic organisms such as fish Cyprinus carpio, planktonic crustaceans Daphnia magna, green algae Selenastrum capricornutum, luminescent bacteria Vibrio fischeri and other gram + and gram - bacteria). The organisms were exposed to different concentrations of the caffeic acid (0 to 100 mg/L) and to various dilutions of effluents containing Pb(II) and Cr(III) respective (6.25% to 100% vol.). The caffeic acid shows no toxicity on the studied aquatic organisms(CL_{50}/CE_{50}> 100 mg/L). In both flotation process, the effluents containing Pb(II) present a high toxicity compared to the effluents containing Cr(III) for crustaceans and bacteria. From the point of view of the flotation processes, it is noted that precipitate flotation effluents were less toxic, this process being more efficient in terms of the effects on aquatic organisms.The most sensitive aquatic organisms were crustaceans and luminescent bacteria. On the fish, algae and gram positive and negative bacteria no toxic effects were observed.

Keywords: toxicity, caffeic acid, flotation, Pb(II), Cr(III), EC_{50}/LC_{50}

In small amounts Cr(III) is essential for the body, but in high concentrations Cr(III) is toxic both for aquatic organisms and also for humans [1-5]. The sources of trivalent chromium include water, many fresh vegetables and fruits, meat, grains, and yeast [6,7]. The Pb(II) toxicity on aquatic organisms has been intensively studied by different researchers [8-16]. There were not found literature data on acute toxicity of caffeic acid on aquatic organisms.

The following study present the possibility of caffeic acid using as a collector reagent for removal of Pb(II) and Cr(III) metallic ions from aqueous systems by ion and precipitate flotation.In order to avoid additional pollution further study for toxicity evaluation on aquatic organisms both for the collector and the effluents resulted from the flotation processes were required. The toxicity tests were carried out on the homogeneous flotation effluents compared with the clear effluents, considering the Pb(II) and Cr(III) concentrations.

Experimental part

Materials and methods

Obtaining of the flotation effluents

Working effluents were obtained from the flotation proceses (ion flotation and precipitate flotation) performed for removal the Pb(II) / Cr(III) metallic ions from aqueous solutions with caffeic acid (acid 3-(3,4—dihydroxiphenyl) propenoic,$C_9H_8O_4$) as collector reagent. The flotation experiments were performed using $Pb(NO_3)_2$ (225 mg/L Pb(II)) and $Cr_2(SO_4)_3$ (250 mg/L Cr(III)) solutions each mixed with caffeic acid at different [caffeic acid]:[metallic ion] molar ratios. During the contacting of caffeic acid solutions ($1.25 \cdot 10^{-2}$M) with metallic ion solution, *p*H was adjusted with 0.1 M and 2M NaOH solutions. To favour the agglomeration of the precipitate, in the system was introduced a cation flocculant (Praestol 610 BC 0.02%). After the *p*H adsustment, the resulting mixture was introduced about 10 min in the flotation column. The effluents obtained from the flotation proceses has been tested in terms of toxicity.

In table 1 is presented the analytical characterization of the flotation effluents which are used in toxicity tests.

Toxicity assessment methods

In order to assess the acute toxicity on aquatic organisms were used effluents containing Pb(II) and Cr(III) resulted from the removal of the metals from aqueous solutions by flotation with caffeic acid collector (table 1), and synthetic solutions of caffeic acid in different concentrations (0.01 to 100 mg/L).

Assessment of acute toxicity of chemicals is performed in accordance with EC Regulation 440/2008 [17,18] with the subsequent additions / modifications from the EC Regulation 761/2009 [19] Chemical toxicity classification was performed in accordance with U.S. EPA American Norms [20], HG no. 1408/2008 - Annex 1, section 5.2.1. [21].

Acute toxicity classes corresponding to classification system of the effluents discharged into the aquatic environment were established according to [22].The classification system involves determination and

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Effluent	Flotation process type	Initial characteristics of the sample		Molar ratio CA : Pb(II) / Cr(III)	$C_{f,\ metalic\ ion}$, [mg/L] in clear effluent	$C_{f,\ metalic\ ion}$, [mg/L] in homogeneous effluent
		C_i, metalic ion, [mg/L]	pH_i			
CA :Pb(II)	Ion flotation	225	6.00	1:1	4.31	18.2
CA :Pb(II)	Precipitate flotation	225	11.00	0.05:1	0.16	9.03
CA :Cr(III)	Ion flotation	250	6.00	1.25:1	2.55	12.05
CA :Cr(III)	Precipitate flotation	250	7.00	0.01:1	0.18	9.10

Note: CA – caffeic acid; C_i – initial concentrations of metallic ion Pb(II) / Cr(III); C_f - final concentration after flotation process of metallic ion Pb(II) / Cr(III); pH_i – initial pH;

Table 1
CHARACTERISTICS OF THE FLOTATION EFFLUENTS

quantification of effluents acute toxicities using a microbioassays battery with different aquatic organisms (fish, crustaceans, algae and bacteria). According to this methodology the hazard is classified in five toxicity classes, each class having a weight score of the effects significance. Flotation effluents classification in one of the toxicity classes was performed based on the highest toxicity units (TU) values (equation (1)) that were recorded following acute toxicity tests performed in laboratory.

$$TU = \frac{100}{LC / EC50} \qquad (1)$$

Fish toxicity test

Acute toxicity bioassays applied in the laboratory to determine the median lethal concentration values - LC_{50} were conducted in accordance with the experimental method described by OECD 203 / C01 (Determination of Acute Toxicity on Aquatic Organisms - fish) [23] by applying a static test (without renewal test solutions during the test) with *Cyprinus carpio*; working solutions and selection of fish species have fulfilled the conditions of the method.

Crustaceans toxicity tests

Acute toxicity bioassays applied in the laboratory to determine the lethal / immobilization concentration values - EC_{50} were performed in accordance with the experimental method described by OECD 202 / C02 (*Daphnia sp.* - Acute immobilization test, using Daphtoxkit F magna kit from MicroBioTests Inc., Belgium) [24, 25].

Algae toxicity test to algae

In order to determine the toxicity on the green algae *Selenastrum capricornutum*, Algaltoxkit test (MicroBioTests Inc., Belgium) was used. The test is according to the experimental procedure of the OECD201 (Algal growth inhibition test) [26] and ISO / DIS 8692 (Water quality - Test for inhibition of algal growth unicellular freshwater with green algae) [25, 27].

Luminescent bacteria test

To estimate the toxic effects of caffeic acid solutions and flotation effluents on luminescent bacteria (*Vibrio fischeri*), "Multi-Shot" bacterial kit with freeze-dried bacteria (Germany) and "BioFix Worlds" system testing (which comply with DIN EN ISO 11348-3 method) were used [25,28].

Microbial toxicity test

The acute microbial toxicity assessment was performed using the MARA test principle (Microbial Array for Toxicity Risk Assessment, NCIMB Ltd) [29]. The test was applied for evaluation of acute toxic effects of the studied solutions on 11 microbial strains (10 microbial lyophilized strains (Mycobacterium sp., Brevundimonas diminish, Citrobacter freundii, Comamonas testosterroni, Enterococcus casseliflavus, Delft acidovorans, Kurth gibsonii, Sthaphilococcus warnerii, Pseudomonas aurantiaca and Serratia rubidae) and a strain of yeast (Pichia fault).

Assessment of the degradability potential of the effluents. The effluent samples were tested in terms of the degradability potential through the followed quality indicators: COD (Chemical Oxygen Demand, ISO 6060:1996 [30]) and BOD (Biochemical Oxygen Demand, EN 1899-1:2003 [31]) and the Symons report was calculated [32].

Results and discussions

Toxicity tests

Freshwater fish - Cyprinus carpio

The toxicity experiments with fish (96 h test) showed lethal toxic effects for all the tested solutions (caffeic acid in 1 mg/L to 100 mg / L (table 2 and effluents in the range of 6.25% to 100% volume table 3). From the physiological point of view, there were observed no changes in behaviour and of the external organs at visual inspection.By interpreting the obtained results in relation to toxicity classification of the chemical substances [20, 21] and of the effluents discharged into the aquatic environment [22], it was established that: caffeic acid is not toxic and was estimated a LC_{50}>100 mg/L; flotation effluents containing Pb(II) and Cr(III), obtained after ion and precipitate flotation processes, are not harmful for aquatic vertebrates - fish *Cyprinus carpio.*

TEST ORGANISM	Caffeic acid			
	CL/CE_{50} [mg/L]	NOEC [mg/L]	LOEC [mg/L]	Range of tested concentrations [mg/L]
Cyprinus carpio	>100	100	-	0 – 100
Daphnia magna	>100	0.01	0.1	0.01 – 1000
Selenastrum capricornutum	>100	0.01	0.1	0.01 – 100
Vibrio fischeri	96	4	6	0.01 – 100
Gram positive and gram negative bacteria	>100	0	3.1	0 – 100
Toxicity class [20,21,23,24,26]	**VERY LOW TOXICITY / PRACTICALLY NON-TOXIC**			

Table 2
CAFFEIC ACID TOXICITY ON AQUATIC ORGANISMS

Table 3
EFFLUENTS TOXICITY ON AQUATIC ORGANISMS

TEST ORGANISM	CA: Pb(II) ion flotation			CA:Pb(II) precipitate flotation			CA:Cr(III) ion flotation			CA:Cr(III) precipitate flotation		
	CL/EC50 %vol	**NOEC %vol**	**LOEC %vol**	**CL/CE50 %vol**	**NOEC %vol**	**LOEC %vol**	**CL/CE50 %vol**	**NOEC %vol**	**LOEC %vol**	**CL/CE50 %vol**	**NOEC %vol**	**LOEC %vol**
Cyprinus carpio	-	-	-	**>100** (>9.03 mg/L)	-	-	-	-	-	**>100** (>9.10m/L)	-	-
Daphnia magna	**41** (7.46 mg/L)	**6.25** (1.14 mg/L)	**12.5** (2.28 mg/L)	**51** (4.61 mg/L)	**6.25** (0.56 mg/L)	**12.5** (1.13 mg/L)	**59** (7.10 mg/L)	**6.25** (0.75 mg/L)	**25** (3.01 mg/L)	**69** (6.27 mg/L)	**6.25** (0.56 mg/L)	**50** (4.55 mg/L)
Selenastrum capricornutum	-	-	-	**>100** (>9.03 mg/L)	-	**6.25** (0.56 mg/L)	-	-	-	**>100** (>9.10 mg/L)	-	**6.25** (0.56 mg/L)
Vibrio fischeri	**30** (5.46 mg/L)	**4.2** (0.76 mg/L)	-	-	-	-	**73** (9.06 mg /L)	**33.5** (4.03mg/L)	**50** (6.25 mg /L)	-	-	-
Bacterii gram"-" and gram "+"	-	-	-	**90.33**	-	-	-	-	-	**91.66**	-	-
Comparison with literature data / toxicity range to Pb(II) and Cr(III)	**Fish** ($LC_{50\text{-}96h}$) *Cyprinus carpio* 2.624 mg/L ($Pb(NO_3)_2$); 1.64 mg/L Pb(II) [11]; 77 mg/L ($Pb(NO_3)_2$); 48.36 mg/L Pb(II) [33] *Labeo rohita* 15 mg/L ($Pb(NO_3)_2$); 9.38 mg/L Pb(II) [12] *Pangasius hypothalmus* 48.06 mg/L ($Pb(NO_3)_2$); 30.06 mg/L Pb(II) [33] **Crustaceeans** ($EC_{50\text{-}24h}$) *Daphnia magna* 0.44 mg/L ($Pb(NO_3)_2$); 0.28 mg/L Pb(II) [16] *Daphnia pulex* 4 mg/L ($Pb(NO_3)_2$); 2.50 mg/L Pb(II) [34] **Algae** ($EC_{50\text{-}72h}$) *Selenstrum capricornutum* 3181 mg/L($EC_{50\text{-}24h}$); 1989 mg/L Pb(II) [10] **Bacteria** ($EC_{50\text{-}15min}$) *Vibrio fischeri* 0.15 mg/L Pb(II) [9] EC_{50}(30min) 80-130 mg/L ($Pb(NO_3)_2$); 50-81 mg/L Pb(II) [8] IC50 (22h) 6.60 mg/L Pb(II) [35]						**Fish** ($LC_{50\text{-}96h}$) *Cyprinus carpio* 87.93-128.09 mg/L Cr(III) [14] *Cyprinus carpio* 17.05 mg/L ($CrCl_3$), 5.60 mg/L Cr(III) [33] *Pangasius hypothalmus* 7.46 mg/L ($CrCl_3$); 2.45 mg/L Cr(III) [33] **Crustaceeans** ($EC_{50\text{-}24h}$) *Daphnia similis* 3.24 mg/L Cr(III) [1] **Algae** ($EC_{50\text{-}72h}$) *Selenstrum capricornutum* 103 mg/L $Cr(NO_3)_2$); 30.43 mg/L Cr(III) [10] **Bacteria** ($EC_{50\text{-}15min}$) *Vibrio fischeri* 22.18 mg/L Cr(III) [9] EC_{50}(30min) 123 mg/L ($Cr(NO_3)_2$); 36.34 mg/L Cr(III [8] IC50 (22h) 1.78 mg/L Cr(III) [35] Other bacteria (MTC): 1500 mg/L Cr(III) [15]					

Crustaceans - Daphnia magna

From the results obtained in acute toxicity tests with *Daphnia magna* was appreciated that the caffeic acid synthetic test solutions (0.01 mg/L to 1000 mg/L) was not toxic for this invertebrate, obtaining LC_{50} >100 mg/L (table 2). In both ion and precipitate flotation processes, the effluent containing the complex caffeic acid - metal has a moderate toxicity. The $LC_{50(Cr(III)}$ was about 59% vol.(in ionic flotation) and 69% vol. (in precipitation flotation) (table 3). The $LC_{50(Pb(II))}$ was 41% vol. (in ionic flotation) and 51% vol (in precipitation flotation) (table 3). The effluents containing Pb(II) have a higher toxicity compared with the effluents containing Cr(III), the $EC_{50\text{-}48h}$ for Pb(II) <$EC_{50\text{-}48h}$ forCr(III).Compared with ion flotation process, the precipitate flotation process determine obtaining of a less toxic effluents. At the concentrations of Pb(II) and Cr(III) analytically determined in effluent resulted after ion flotation processes (in supernatant) (4.31 mg/L Pb(II) and 2.55 mg/ L Cr(III)) it was estimated 30% lethal effects on the tested organisms. Regarding the effluents resulting from the precipitate flotation process is expected that they do not cause toxic effects, the concentrations analytically determined in the supernatant being <0.56 mg/L for both analyzed metals.

Algae – Selenastrum capricornutum

Given the current regulations and the results of aquatic toxicity tests conducted on freshwater green algae, have resulted that caffeic acid does not present acute toxicity for the phytoplankton, the $CEr_{50\text{-}72h}$ being > 100 mg/L (able 2). The effluents with Pb(II) and Cr(III) resulting from the precipitate flotation process, had no inhibitory effects on algal growth. For the effluents tested as 100% vol. were recorded insignificant growth inhibitions (<30%).

Luminescent bacteria - Vibrio fischeri

Caffeic acid highlight a low toxicity on the luminescent bacteria, the obtained $IC_{50(30\,min)}$ was 96 mg/L (table 2). In case of ion flotation process, the caffeic acid complexed with Pb and Cr determined a high toxicity ($IC_{50(30\,min)}$ 9.06 mg/L for Cr(III) and 5.46 mg/L for Pb(II)) (table 3). The effluents containing Pb(II) resulting after ion flotation process cause a higher toxicity compared to the effluents containing Cr(III) obtained from the same type of the process ($IC_{50Pb(II)}$ = 30% vol., $IC_{50Cr(III)}$ = 73% vol.).This toxicity is based on the presence of toxic metals that are absorbed through the cell membranes endangering the metabolic functioning of bacteria.

Microbial toxicity test

Caffeic acid does not show toxic effect on microbial strains because the minimum value of MTC (Microbial Toxic Concentration) was 98 mg/L (table 2). The effluents containing Cr(III) and Pb(II) respectively, resulted from the precipitate flotation process did not show toxic effect on bacterial strains, the recorded values of MTC being higher than 90% vol (table 3).

Toxicity classification of caffeic acid in the applied tests battery

Given the regulations, results that caffeic acid is slightly toxic, practically non-toxic, to aquatic organisms (fish, crustaceans, algae) as well as to bacteria (table 2).

Toxicity classification of flotation effluents

In both of the flotation processes, effluents containing Pb(II) present a greater toxicity for crustaceans and bacter could be correlated with the acute toxicity data specified in literature (table 3). From the point of view of the flotation process, it is noted that from the precipitate flotation process results less toxic effluents, this process being more efficient in terms of effects on aquatic organisms. The most sensitive aquatic organisms were *Daphniamagna* and *Vibrio fischeri*. On the fish, algae and gram positive and negative bacteria have not been registered toxic effects.

Performed acute toxicity test	CA: Pb(II) ion flotation		CA:Pb(II) precipitate flotation		CA:Cr(III) ion flotation		CA:Cr(III) precipitate flotation		Classification system for effluents discharged into the aquatic environment
	[a]TU	[b]W. s.	[a]TU	[b]W. s.	[a]TU	[b]W. s.	[a]TU	[b]W. s.	
Cyprinus carpio	0`	0	0	0	0	0	0	0	**TU < 0.4** **Class I** – there is no acute toxicity **0.4<TU≤1** **Class II** – low acute toxicity **1<TU≤10** **Class III** – acute toxicity **10<TU≤100** **Class IV** – high acute toxicity **TU>100** **Class V** – very high acute toxicity
Daphnia magna	2.43	2	1.69	2	1.96	2	1.44	2	
Selenastrum capricornutum	-	-	-	-	0	0	0	0	
Vibrio fischeri	3.33	2	1.36	2	-	-	-	-	
Other bacteria	-	-	-	-	2.33	2	1	2	
TU calculated for battery of tests	1.92		1.02		1.07		0.61		
Toxicity classification	Class III – acute toxicity		Class III - acute toxicity		Class III – acute toxicity		Class II– low acute toxicity		
Class weight score	2		2		2		1		

[a]calculated for each type of test on the basis of $CL(E)_{50}$ value; [b] weight score

Table 4
CLASSIFICATION OF THE EFFLUENTS TOXICITY

Analyzed sample	COD-Cr (mg O_2/L)	BOD_5 (mg O_2/L)	COD-Cr (mg O_2/mg)	BOD_5 (mg O_2/mg)	BOD/COD Effluent ratio
	Influent		Effluent		
Precipitate flotation CA:Cr(III)	576	222.5	60.95	23.55	**0.39**
Precipitate flotation CA:Pb(II)	1382	450.8	153	49.92	0.33
Ion flotation CA:Cr(III)	960	360.4	101.6	38	**0.38**
Ion flotation CA:Pb(II)	1834	627.7	203	69.5	0.34

Table 5
ASSESSMENT OF BIODEGRADABILITY

Table 4 shows the classification of effluents toxicity in microbiotests battery, according to the Persoon et al., 2003 [22].

Using Persoon et al. (2003) classification system revealed that the ion and precipitation flotation effluent containing CA:Pb(II), CA:Cr(III) and CA:Pb(II)are classified in Class III-acute toxicity, because it was obtained a mean of toxicity units value,situated in the range of 1-10. The effluents resulted from the precipitate flotation process containing CA:Cr(III) presents class II – low acute toxicity, with an average toxicity value situated in the range from 0.4 to 1.The toxicity of effluents is probably caused by the presence of Cr(III) and Pb(II), because the caffeic acid was not shown to be toxic to aquatic organisms.

Effluent degradability

From the experimental data showed in the table 5 can be seen that the effluents containing Cr(III) from the flotation processes are degradable (as $BOD_{5\ days}$/COD = 0.39), while the effluents containing Pb(II) obtained from the ion and precipitates flotation process are potentially degradable (as $BOD_{5\ days}$ /COD = 0.34). From precipitate flotation process were obtained effluents with a more pronounced biodegradability.

Conclusions

Within this research were evaluated the ecotoxicological characteristics of the caffeic acid (metal removal collector) and their complexes with Pb(II) and Cr(III) resulted from the ion and precipitate flotation processes. The samples showed different effects on the test organisms. The acute toxicity values varied with the type of organisms species. Therefore the planktonic crustaceans and bacteria were the most sensitive organisms, for these being highlighted acute toxic effects. The toxicity observed for the effluents was ccaused by the toxic metals Cr(III) and Pb(II), while caffeic acid has not proved any effect.

The effluents contained Cr(III) resulted from the precipitate flotation process present biodegradation capacity under activated sludgemicroorganisms action. Contrary, in case of the effluent containing Pb(II), resulted from ion and precipitates flotation processes, Pb(II) toxicity may influence the biodegradation efficiency, which was confirmed by toxicity studies performed on bacteria.

The performed studies revealed the efficiency of cafeic acid in removal of toxic metals (Pb and Cr) in flotation processes and also established the potentially additional toxicity caused by the final effluents.

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List of symbols

BOD - *Biochemical oxygen demand*
CA – Caffeic acid
COD -*Chemical Oxygen Demand*
EC_{50} - effective inhibitory concentration to 50% of the tested organisms
LC_{50} - lethal concentration for 50% of the tested organisms
LOEC - Lowest Observed Effect Concentration
MTC - Microbial Toxic Concentration
NOEC - No Observed Effect Concentration
TU - Toxicity Units

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