

# Molecular Spectroscopic Study of Water Hyacinth Dry Matter

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**Abstract:** The potential use of water hyacinth dry matter for removing heavy metals from wastewater is studied by combining various spectroscopic techniques and quantum chemical ab initio calculations. The aquatic plant water hyacinth was treated with 0.025, 0.050, 0.100, 0.200, and 0.400 M acetic acid for different times up to 19.0 hours. The plant was divided into root and shoot then dried and grinded into pieces of 200  $\mu\text{m}$  in diameter. FTIR measurements revealed that acetate finds its way to the plant root and is then transported to the shoot. Furthermore, it is shown that the treated and dried plant can be used to mediate Cd from wastewater. FAAS results indicate that 55% of Cd (in case of low concentration) and 80% of Cd (in case of higher concentration) could be removed from wastewater within 2.0 hours. The coordination of acetate to the plant was assumed to happen *via* the cellulose of the plant where also the Cd uptake takes place. This is confirmed by studying changes in the absorption of the C=O stretching band of cellulose acetate subject to  $\text{CdCl}_2$  by FTIR and by a cellulose monomer quantum chemical model.

**Keywords:** FAAS, FTIR, water hyacinth, phytoremediation, density functional theory.

## INTRODUCTION

The phenomena of pollutants uptake by aquatic plants were studied by Sheffield and Orens decades ago [1, 2]. Later on water hyacinth among other aquatic plants showed a potential to remove pollutants when being used as a biological filtration system [3, 4]. It could remove several heavy metals and other pollutants as reported in Refs [5-12]. This triggered efforts directed towards the utilization of water hyacinth as an effective phytoremediation tool [13-16]. As a caveat we note, that it has been reported that water hyacinth could be a serious environmental problem on its own. It reduces sunlight penetration and lowers the oxygen content of water, which has a great impact on the water ecosystem [17-20].

Fourier Transform Infrared (FTIR) among other spectroscopic methods was employed extensively in cellulose research, e.g., it gives access to the structural modifications of cellulose [21]. Molecular modeling can provide valuable information concerning the optimization of applications of cellulose through explaining its molecular as well as other properties. For instance, the interaction of cellulose with dye fibers was studied by AM1 semiempirical molecular orbital calculations [22]. Polycyclic dyes were used by Wojciechowski and Wolska for dyeing cellulose fibers and with

AM1 computations sulpho group substitution sites were predicted [23]. Higher levels of theory like Density Functional Theory (DFT) was used by Terzyk in order to characterize the microporosity of carbon films which are obtained from cellulose [24]. DFT was also applied to study the effect of hydroxyl propyl cellulose on pore structure of carbons coated on ceramic particles [25]. Two carbenium-iminium ions are derived from the widely used oxidant and cellulose N-methylmorpholine-N-oxide by heterolytic degradation. The corresponding reaction mechanism was investigated in Ref [26]. Finally, we mention that cellulose esters are part of cellulose derivatives that have long history in industrial as well as pharmaceutical applications [27-29].

In our previous work atomic emission spectroscopy was used to study the concentrations of Cu, Pb, and Ni in water hyacinth root [30]. Further, both the dielectric constant and dielectric loss were estimated for plant roots collected from the Nile river [31]. In addition, studying the interaction between organic and inorganic structures through the acetate group has been subject of our molecular modeling work [32, 33]. Moreover, we have demonstrated that dry water hyacinth plant could remove Cd from polluted water [34]. The process is described in terms of the interaction between acetate and hexahydrated Cd. However, no explanation for the fate of acetate in the plant could be given so far.

In the present paper we will further elaborate on the benefits of water hyacinth dry matter. The plant has been subjected to acetic acid at different molarities and times. The molecular structure as well as the degree of acetylation was studied using

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FTIR spectroscopy. Since for the process under investigation the plant can be considered as cellulose like material, we have chosen to study cellulose acetate as a minimum model which is also amenable to ab initio quantum chemical calculations. This also allows investigating the binding sites of Cd in the acetylated cellulose. From the comparison with treated plants immersed in a wastewater containing Cd we can draw conclusions on the Cd uptake which is measured using Flame Atomic Absorption Spectroscopy (FAAS).

## MATERIALS AND METHODOLOGY

### Reagents

Cadmium chloride dried LR (MW 183.32) was purchased from S.d. Fine-Chem Ltd., Cellulose acetate with 39.7wt% acetyl content was purchased from Aldrich chemical company, Inc., USA. Both potassium bromide and acetic acid anhydride (which was used without further purification) were obtained from Aldrich-Chemicals. Cadmium standard solution was purchased from Merck, Germany.

### Sample Collection and Preparation

Water hyacinth plant was collected (10 cm length) from the river Nile at Rod El-Farag, north of Cairo. It was divided into groups; each group was immersed in 0.025, 0.050, 0.100, 0.200, and 0.400 M acetic acid, respectively, for 19.0 hours. Other groups were subjected to 0.100 M acetic acid for 0.5, 1.0, 2.0, 4.0, 12.0, and 19.0 hours.

Water hyacinth was washed thoroughly with doubly distilled water and was then divided into shoot and root; dried separately at 65 °C till constant weight. CdCl<sub>2</sub> was mixed with cellulose acetate as 1:1 and 1:2 in powder form and grinded into pieces of 200 μm in diameter carefully. Acetylated water hyacinth root and shoot were separately treated with 0.10% CdCl<sub>2</sub> solution for 24.0 hours then studied by FTIR. A synthetic wastewater containing 0.2 and 1.2 mg/l Cd was prepared. Then 2.5 g of root shoot and mixtures of root and shoot separately were immersed in 1.0 liter of the synthetic wastewater samples. Water samples were collected at 0.0, 1.0, 2.0, 3.0, 6.0, and 9.0 hours respectively. Both the standard and the synthetic solutions of Cd were prepared by dilution from 1000.0 mg/l stock solution.

### Instrumentation

The FTIR spectra were collected for the studied samples using the spectrometer JASCO, FTIR-300 E at the Spectroscopy Department, National Research Centre, Egypt, which covers the range from 400 to 4000 cm<sup>-1</sup>. The KBr disc technique was followed for the FTIR study, that is, each sample (plant root, plant shoot, cellulose acetate/CdCl<sub>2</sub> mixture and root/shoot mixture) was mixed with KBr (1.0 % w/w) and pressed in pellets.

The NovAA 400 Jena Flame Atomic Absorption Spectrometer, at the Hydrogeology Department, TU Berlin, Germany, was used for the determination of Cd concentration. The operational conditions were as follows: 3.0 mA lamp current, 228.8 nm spectral line, 8.0 mm burner height, 5.0 s measuring time using air acetylene flame [34].

## Calculation Details

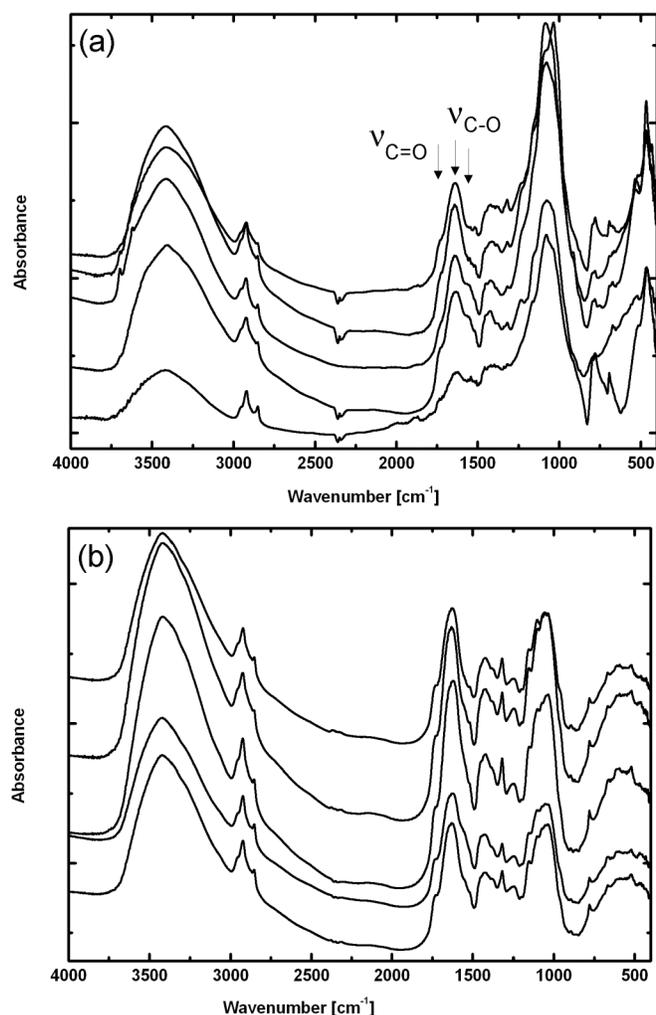
A cellulose monomer (4-1 linkage) was chosen as a model molecule as shown in Fig. (5) below. Since sites 1 and 4 are blocked, we only considered substitutions at sites 2, 3, and 6. In principle cellulose acetate contains the acetyl group, -(C=O)CH<sub>3</sub>. Below we will argue that such a model is not in accord with the experiments. However, a structure which does conform with the observations should contain carboxyl groups, -(C=O)OH, replacing the hydroxyl groups at sites 2, 3, and 6. Therefore, in order to get some general idea about the correlation of structural and spectral changes we have chosen to study in fact cellulose carboxylates. Geometry optimization and harmonic frequency analysis has been performed using DFT with the B3LYP functional and the effective core LANL2DZ basis set as implemented in the Gaussian 03 program package [35].

## RESULTS AND DISCUSSION

### FTIR Study of Plant Acetylation

Figs. (1, 2) present the FTIR spectra of water hyacinth after acetic acid treatment with molarities up to 0.400 M and times up to 19.0 hours. In general the spectra of shoot and root could be assigned as follows [36, 37]: The OH bands of water are found at 3388.3 cm<sup>-1</sup> and 1422.2 cm<sup>-1</sup>. The CH<sub>2</sub> group contributes two bands at 2922.6 and 1371.1 cm<sup>-1</sup> while the CH<sub>3</sub> stretching vibration is located at 2852.2 cm<sup>-1</sup>. The C=O stretching band which is the characteristic band of the carbonyl group appears around 1736.5 cm<sup>-1</sup>; this band is found already in plant shoot but it becomes more intense as a result of treating the plant with acetic acid. Previously, we observed [36] that the interaction of hydrated divalent metals with organic acids leads to the appearance of the carboxylate band around 1620–1650 cm<sup>-1</sup>. In the present work this band occurs around 1634.4 cm<sup>-1</sup> and is assigned as a COH vibration. This assumption is confirmed by the observation of the asymmetric stretching vibration of COO at 1516.7 cm<sup>-1</sup>. The latter band is regarded as a weak band, so that one can conclude that the COH band at 1634.4 cm<sup>-1</sup> is coupled to the bending vibration of water and not only attributed to metal uptake. However, in general the 1634.4 cm<sup>-1</sup> band is an important marker for the uptake of hydrated metals by water hyacinth in Nile water. The C-O stretching vibration of the cellulose backbone is at 1035.6 cm<sup>-1</sup>.

The FTIR technique is highly sensitive and reliable for the determination of the degree of carboxylation. The latter is obtained from the ratio between the intensity of the C=O stretching band at 1736.5 cm<sup>-1</sup> and the intensity of the backbone C-O stretching vibration at 1035.6 cm<sup>-1</sup> [38]. Table 1 presents the degree of carboxylation for shoot and root after treating the plant with 0.025, 0.050, 0.100, 0.200, and 0.400 M acetic acid. Results show that this process takes place for all the studied acid molarities with no significant variation even in terms of time which could be due to the limited time resolution. We further find a decrease in the degree of carboxylation in the root followed by a corresponding increase in the shoot. The latter effect could be attributed to a possible transport from root to shoot which may be enhanced at 0.050



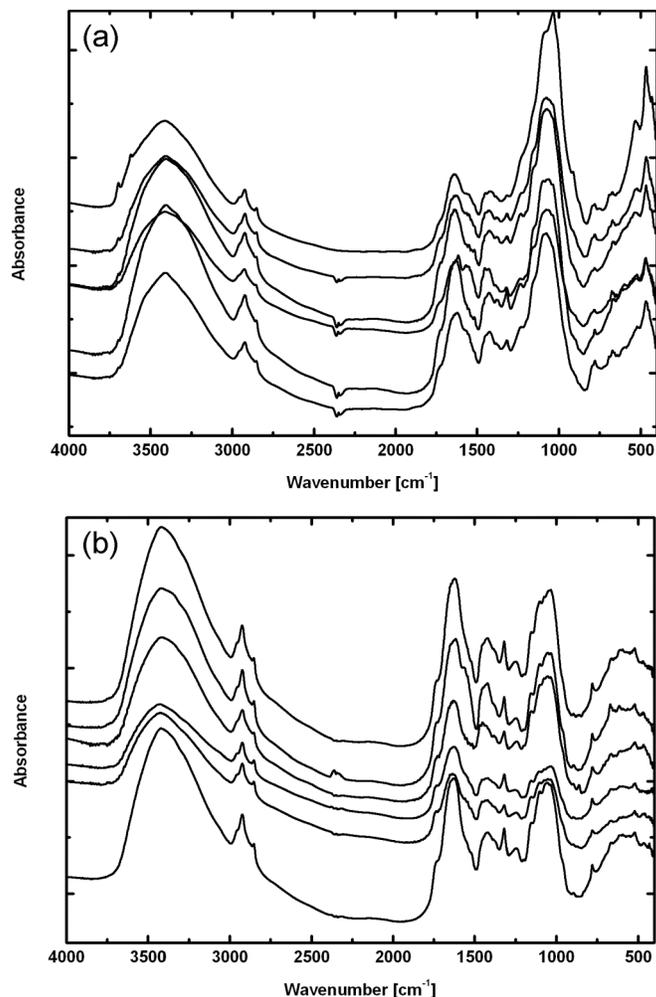
**Fig. (1).** FTIR spectra of water hyacinth root (a) and shoot (b) samples treated with different acetic acid concentrations (0.025, 0.50, 1.0, 2.0 and 4.0 M from bottom to top) at constant time (19 hours).

M. Additional support comes from the fact that the spectra show no absorption bands in the region of  $1840\text{--}1760\text{ cm}^{-1}$  which indicates that these samples are free of unreacted acetic anhydride. Furthermore, no bands were found at  $1700\text{ cm}^{-1}$  which gives evidence that the treated samples are free of acetic acid by-products. Overall we should also point out that our observations are in good agreement with previous results reported in Refs [38, 39]. Therefore, one can conclude that treating water hyacinth with acetic acid gives rise to C=O absorption even at high acid molarities. This supports the assumption that treating the plant leads to carboxylation of its cellulose content. Furthermore, the underlying process sustained irrespective possible deacylation processes.

#### Removal of Cadmium from Water

Treated water hyacinth root, shoot, and a mixture of root and shoot were immersed in a wastewater containing 0.2 and 1.2 mg/l Cd, respectively. Wastewater which is subjected to treated plants was collected and analysed with flame atomic absorption; the results are summarized in Table 2. They clearly indicate that treated water hyacinth root and samples. While at lower Cd concentration the removal by the

shoot could remove Cd from wastewater better than untreated



**Fig. (2).** FTIR spectra of water hyacinth root (a) and shoot (b) samples treated with 0.100 N acetic acid at different times (30 min, 1, 2, 4, 12, 19h, from bottom to top).

shoot is more effective, the roots perform better at higher concentration. The reason for this behaviour is not clear at the moment. We have also studied mixed root/shoot samples (1:1) which enhances the removal of Cd in the low concentration case, but falls below the performance of untreated root and shoot in the high concentration regime. Generally treated plants show higher ability to remove Cd from the studied solution as compared with untreated samples. Furthermore, we notice that the ability of plants to remove Cd is not only increased by treatment, but also the uptake rate increases. For instance, within 2 hours treated plants could remove about 55% of Cd in case of low concentration and 80% of Cd in case of higher concentration. On the other hand, the untreated plant needs 96 hours to mediate 40% of Cd in case of lower concentration and to mediate 85% of Cd in case of higher concentration.

#### FTIR and Quantum Chemical Study of a Cellulose Model

##### Experiment

In order to develop some microscopic understanding of these findings we have studied a model system, that is,

**Table 1. Calculated Degree of Carboxylation ( $A_{1740}/A_{1035.6}$ ) for Water Hyacinth which Treated with Acetic Acid at Different Molarities (19h) and Times (0.1M)**

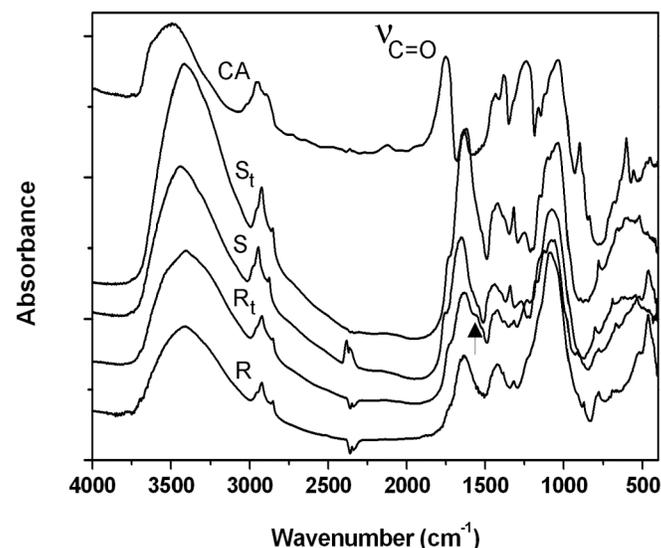
Acetic Acid	Shoot	Root
<b>at Different Acetic Acid Molarities</b>		
0.025	0.6787	0.5753
0.050	0.7390	0.5134
0.100	0.6262	0.3969
0.200	0.6215	0.4652
0.400	0.9630	0.4782
<b>at Different Times</b>		
0.500	0.6577	0.5119
1.000	0.7022	0.4844
2.000	0.8126	0.5490
4.000	0.8625	0.4630
12.000	0.6439	0.4545
19.000	0.6262	0.3969

**Table 2. Concentration of Cd (in mg/l) as a Function of Time**

Time, Hours	Root (R)		Shoot (S)		R+S
	Untreated	Treated	Untreated	Treated	
<b>Starting with 0.2 mg/l Cadmium</b>					
0.0	0.1993	0.1993	0.1993	0.1993	0.1993
1.0	0.2215	0.1033	0.1445	0.0932	0.1046
2.0	0.1584	0.0953	0.1291	0.0918	0.1050
3.0	0.1432	0.1040	0.1696	0.0978	0.1068
6.0	0.1281	0.0947	0.1593	0.0978	0.1073
9.0	0.1270	0.0989	0.1590	0.0887	0.1017
96.0	0.1020		0.1181		
<b>Starting with 1.2 mg/l Cadmium</b>					
0.0	1.1700	1.1700	1.1700	1.1700	1.1700
1.0	0.6288	0.2874	0.6502	0.3674	0.2980
2.0	0.5266	0.2169	0.3816	0.3272	0.2986
3.0	0.3040	0.2156	0.4118	0.2987	0.2751
6.0	0.3263	0.2609	0.2838	0.3007	0.2971
9.0	0.2755	0.2834	0.2375	0.3138	0.3078
96.0	0.1376		0.2434		

cellulose acetate (CA) which is also amenable to quantum chemical calculations. FTIR spectra of the untreated and treated plants are compared with that of CA in Fig. (3). The different spectra indicate that there should be similarities in the molecular structures. In particular there is a strong band

at  $1750\text{ cm}^{-1}$  which corresponds to a C=O vibration,  $\nu_{\text{C=O}}$ , and correlates to the band observed at  $1736\text{ cm}^{-1}$  in the plant. Hence, CA should be a suitable model system for studying the mechanistic details of the role of acetate in the Cd uptake of treated plants. In passing we note that one can in fact identify two bands upon taking the second derivative of the C=O band in the cellulose acetate spectrum. However, upon polymerization these two bands merge into a single broad band. The same broadening is observed for other bands such as those related to the OH or CH<sub>2</sub>OH vibrations. Finally, we notice that treated root and shoot samples have been subject to 0.10% CdCl<sub>2</sub> solution which leads to the appearance of a Cd carboxylate band,  $\nu_{\text{O-Cd-O}}$ , at  $1583.3\text{ cm}^{-1}$  especially prominent for the root sample (see arrow in Fig. 3).



**Fig. (3).** FTIR spectra for cellulose acetate (CA), acid treated water hyacinth root ( $R_t$ ) and shoot ( $S_t$ ) as compared with non treated plant shoot (S) and root (R). Acetylated water hyacinth root and shoot were treated with 0.10% CdCl<sub>2</sub> solution for 24.0 hours. The arrow marks the position of the Cd carboxylate band.

Following these lines we have also studied mixtures of CA and CdCl<sub>2</sub>. The FTIR spectra obtained for different mixtures are reported in Fig. (4). The spectra of CdCl<sub>2</sub>/Cellulose mixture indicate the appearance of a new band at  $1583.3\text{ cm}^{-1}$  and the simultaneous disappearance of the C=O band at  $1750\text{ cm}^{-1}$ . We argue that the band at  $1583.3\text{ cm}^{-1}$  arises from the coordination of Cd to the deprotonated COOH group, i.e., it could be assigned to the Cd-carboxylate band,  $\nu_{\text{O-Cd-O}}$ . Notice that this band would not be observed in the presence of an acetyl group.

The assumption is further supported by comparison with Fig. (3) which shows the Cd carboxylate band at the same frequency  $1583.3\text{ cm}^{-1}$ . This indicates that the plant behaves like cellulose when both of them interact with CdCl<sub>2</sub> which in turn leads us to treat water hyacinth in this removal process as cellulose material. Furthermore, the treated plant is acting as cellulose carboxylate in this case. However, the small difference in the spectra of the treated plant and cellulose acetate indicate that most likely not all cellulose units are carboxylated.

## Quantum Chemistry Results

Optimized structures for different models of cellulose carboxylate and as well as CdCl<sub>2</sub> coordinated species are shown in Fig. (5) and harmonic frequencies of relevant transitions are compared to the experimental data in Table 3. As a general trend we observe that the harmonic frequencies are too low by about 3%. This is not surprising given the simple monomer model which in particular neglects interactions with neighboring cellulose units which could lead to some sort of confinement of nuclear motions. Second, we observe that there is not much difference between the C=O vibrations at different sites; the biggest difference is in the di-carboxylate which also shows a small blue-shift attributed to a confinement effect. Since there is only one discernible  $\nu_{C=O}$  feature in the experiment from these data it cannot be concluded how many sites are carboxylated as the transitions coincide taking into account the expected error bar. CdCl<sub>2</sub> attaches to the carboxyl group as shown in Fig. (5e, f). Inspecting Table 3 we notice that this gives rise to the fundamental transition of  $\nu_{O-Cd-O}$  at 1523 cm<sup>-1</sup> which, similar to the  $\nu_{C=O}$  case above, underestimates the experimental value by about 4%. Interestingly, also the  $\nu_{C=O}$  transition is red shifted which is not in accord with the experiment. One possible interpretation would be that there is only one carboxyl group per monomer and the changes in the signal reflect the relative number of groups coordinated to CdCl<sub>2</sub>. However, an alternative possibility would have di-carboxylate with either the site 2 or 3 and site 6 carboxylated (see Fig. 5f). As seen from Table 3 this would not affect the position of the  $\nu_{C=O}$  fundamental transition.

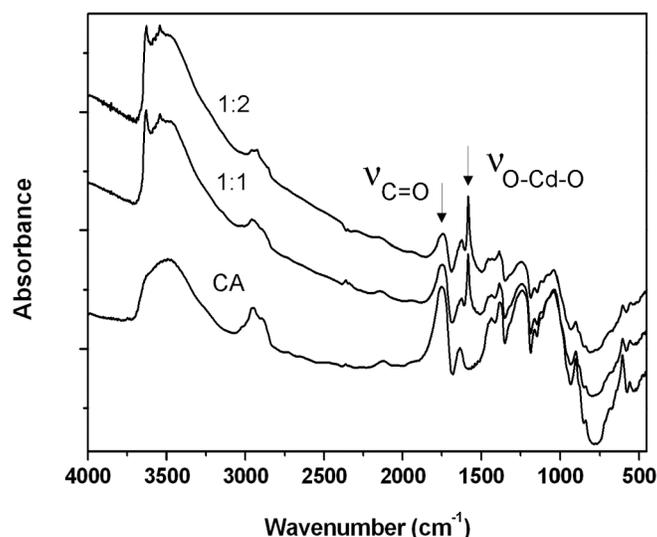
**Table 3. Harmonic Frequencies (in cm<sup>-1</sup>) of the Relevant Vibrations of Different Cellulose Models as Obtained Using DFT/B3LYP with a LANL2DZ Effective Core Potential (For Structures See Fig. 5).**

Structure	Calculation	Experiment	Assignment
Cellulose carboxylate (2)	1697	1750	$\nu_{C=O}$
Cellulose carboxylate (3)	1695	1750	$\nu_{C=O}$
Cellulose di-carboxylate (2-3)	1710 1723	1750	$\nu_{C=O}$
CdCl <sub>2</sub> cellulose di-carboxylate (2-3)	1523 1661	1583 1750	$\nu_{O-Cd-O}$ $\nu_{C=O}$
CdCl <sub>2</sub> cellulose di-carboxylate (2-6)	1519 1716	1583 1750	$\nu_{O-Cd-O}$ $\nu_{C=O}$

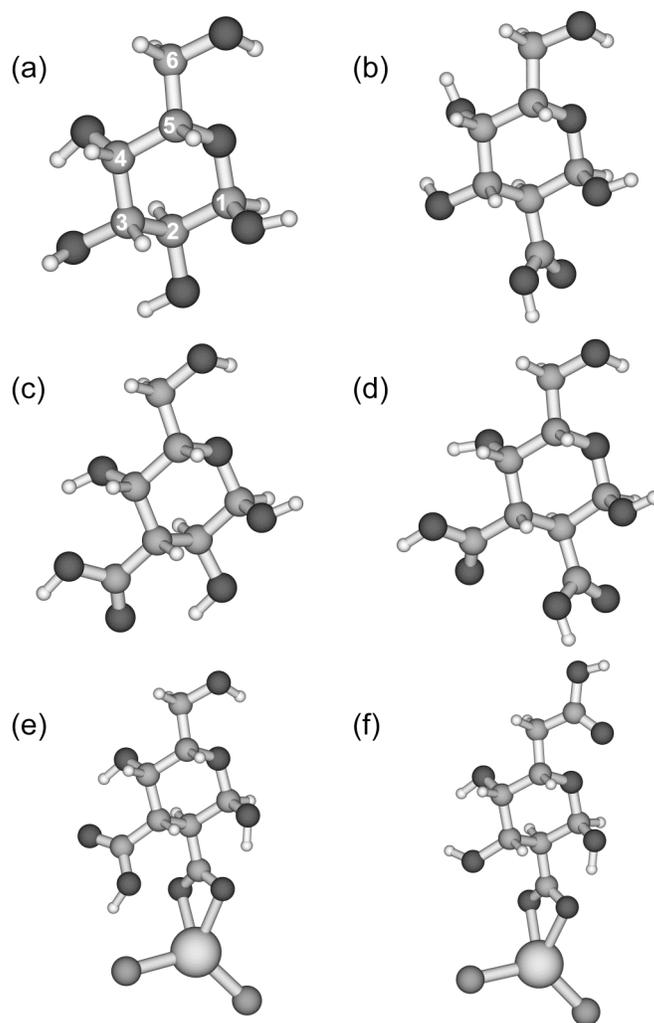
## CONCLUSIONS

In summary we have shown that water hyacinth which is subjected to acetic acid is able to absorb acetate which finds its way to the cellulose of the plant. The dried and treated system acts like carboxylated cellulose and in particular it can accelerate the removal of Cd from the aquatic environment. Although the details of the ongoing reactions are not known, some mechanistic aspects can be gathered by studying a cellulose acetate model. Combining spectroscopic and quantum chemical investigations we suggest a model where

Cd is coordinated to the deprotonated carboxyl group which replaces the hydroxyl group of the cellulose.



**Fig. (4).** FTIR spectra for cellulose acetate, cellulose acetate mixed with CdCl<sub>2</sub> as 1:1 and cellulose acetate mixed with CdCl<sub>2</sub> as 1:2.



**Fig. (5).** Cellulose monomer model assuming a 1-4 linkage as well as various carboxylated and CdCl<sub>2</sub> coordinated species. The structures have been obtained at the DFT/B3LYP/LANL2DZ level of theory.

The demonstration of the merits of treated and dried water hyacinth for remediation purposes has the additional advantage that it reduces or even eliminates the diverse impact of the water hyacinth on the aquatic environment. Our present finding together with our results from previous studies [40] strongly supports our suggestion that the cellulose of the plant plays an important role in the process of phytoremediation

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