



**HAL**  
open science

## Adsorption location of copper on hemp shives revealed by combination of K-edge subtraction X-ray microtomography and X-ray micro-fluorescence

Chiara Mongiovi, Xavier Gabrion, Jason Govilas, Wajih Akleh, Mario Scheel,  
Jonathan Perrin, Timm Weitkamp, Sylvie Durand, Léna Brionne, Camille  
Rivard, et al.

### ► To cite this version:

Chiara Mongiovi, Xavier Gabrion, Jason Govilas, Wajih Akleh, Mario Scheel, et al.. Adsorption location of copper on hemp shives revealed by combination of K-edge subtraction X-ray microtomography and X-ray micro-fluorescence. *Cellulose*, 2024, 31 (2), pp.809 - 822. hal-04528333

**HAL Id: hal-04528333**

**<https://hal.science/hal-04528333v1>**

Submitted on 1 Apr 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Adsorption location of copper on hemp shives**  
2 **revealed by combination of K-edge subtraction**  
3 **X-ray micro-tomography and X-ray micro-**  
4 **fluorescence**

5

6 Chiara Mongioví, Xavier Gabrion, Jason Govilas, Wajih Akleh, Mario Scheel, Jonathan  
7 Perrin, Timm Weitkamp, Sylvie Durand, Léna Brionne, Camille Rivard, Sebastian Schöder,  
8 Katharina Müller, Johnny Beaugrand, Vincent Placet, Grégorio Crini

9

10 *Chiara Mongioví, Grégorio Crini (✉)*

11 *Université de Franche-Comté, CNRS, Laboratoire Chrono-environnement, 16 route de*  
12 *Gray, 25000 Besançon, France*

13 *E-mail: gregorio.crini@univ-fcomte.fr, (G. Crini, ORCID: 0000-0003-2540-6851)*

14

15 *Xavier Gabrion*

16 *SUPMICROTECH, CNRS, Institut FEMTO-ST, Université de Franche-Comté, 25000*  
17 *Besançon, France*

18

19 *Jason Govilas, Wajih Akleh, Vincent Placet*

20 *Department of Applied Mechanics, CNRS, Institut FEMTO-ST, Université de Franche-*  
21 *Comté, 25000 Besançon, France*

22

23 *Mario Scheel, Jonathan Perrin, Timm Weitkamp, Camille Rivard, Sebastian Schöder*

24 *Synchrotron SOLEIL, L'Orme des Merisiers, Départementale 128, 91190 Saint-Aubin,*  
25 *France*

26

27 *Camille Rivard*

28 *Synchrotron SOLEIL, L'Orme des Merisiers, Départementale 128, 91190 Saint-Aubin,*  
29 *France*

30 *TRANSFORM, UAR 1008, INRAE, Imp. Yvette Cauchois, 44300 Nantes, France*

31

32 *Sylvie Durand, Léna Brionne, Johnny Beaugrand*

33 *BIA, UR1268, INRAE, 44316 Nantes, France*

34

35 *Katharina Müller*

36 *IPANEMA, UAR 3461 CNRS, site du Synchrotron SOLEIL, 91190 Saint-Aubin, France*

37

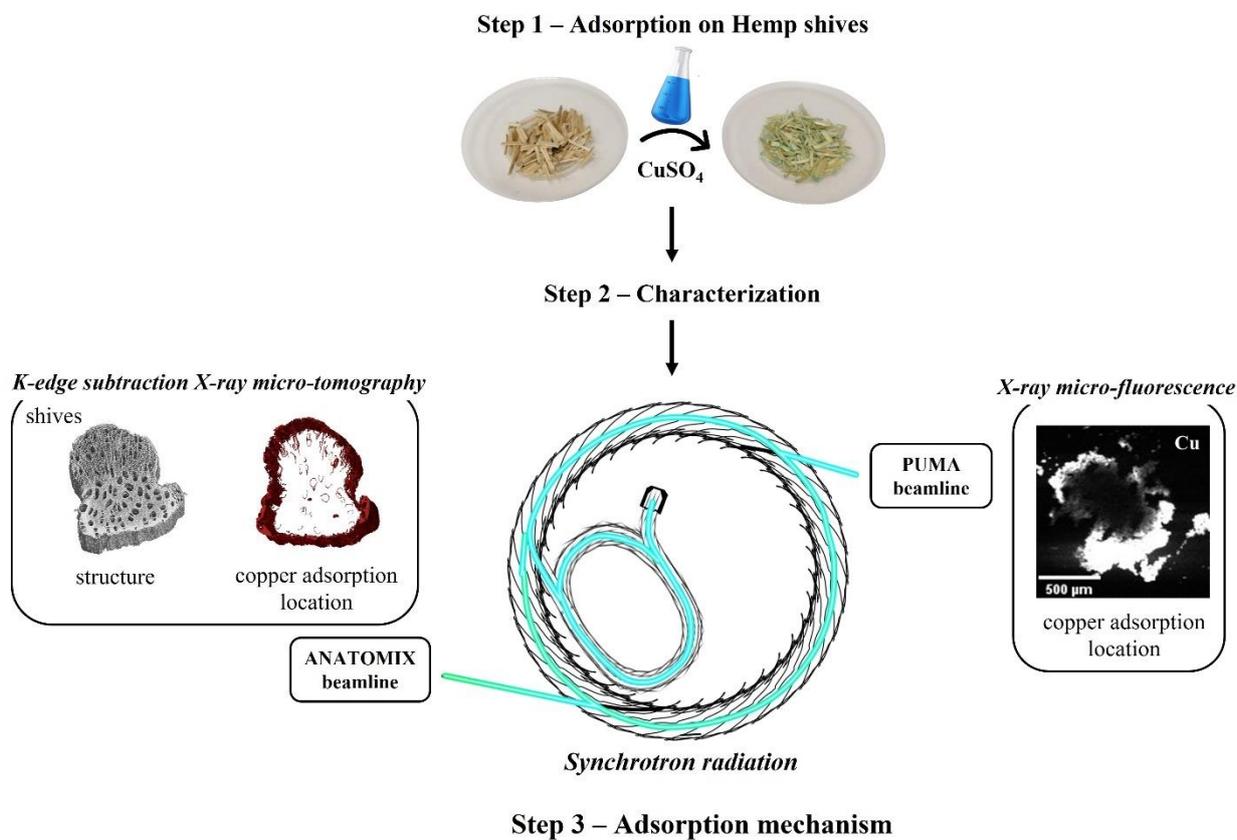
38 **Abstract** In the literature, hemp shives have been proposed as adsorbents capable of  
39 effectively complexing metals in aqueous solution. However, studies on the localization of metals  
40 in these multicell type lignocellulosic materials are scarce and further description of adsorption  
41 sites are required for better optimization of adsorbent capacity. In this article, we show the  
42 complementarity of two synchrotron-radiation techniques, namely K-edge subtraction micro-  
43 tomography and X-ray micro-fluorescence, to visualize the spatial distribution of the copper  
44 complexed by hemp shives. Three materials have been studied: a sample washed in water (SHI-  
45 W), a sample activated using sodium carbonate (SHI-C), and a sample chemically modified by  
46 1,2,3,4-butanetetracarboxylic acid in order to introduce carboxylic groups (SHI-BTCA). Tomography  
47 provides 3D information and only locates areas where copper is present in significant proportions.  
48 X-ray fluorescence imaging allows for a lower detection limit, but only provides 2D information.  
49 Data analysis confirmed that the Cu distribution is totally different for the three samples, in  
50 agreement with the proposed adsorption mechanism: adsorption surface and diffusion for SHI-W  
51 and SHI-C and chemisorption and ion-exchange for SHI-BTCA.

52 **Keywords** *Hemp shives . Copper . Adsorption . X-ray micro-fluorescence . K-edge*  
53 *subtraction imaging . XRF mapping*

54

55

56 **Graphical abstract**



58 **Introduction**

59 In the field of water treatment, by-products of agricultural origin and from forest  
60 industries are proposed as adsorbents for metal removal using adsorption-oriented  
61 processes. These natural materials are lignocellulosic complexes mainly consisting of  
62 cellulose, hemicelluloses and lignin and showing appropriate adsorption properties due  
63 to their multicomponent composition, particular structure, and the presence of various  
64 active ligands. The main advantages of adsorption using by-products of agricultural origin  
65 are the availability of the raw materials, the simple operation, cheapness of the processes,  
66 high efficiency in metal removal, and easy elimination of spent materials by incineration  
67 (Halysh et al. 2020; Sjahro et al. 2021; Sharma et al. 2022).

68 The adsorbents proposed are, for example, sugarcane bagasse, corn cobs, corn stalks,  
69 coir, jute, date pits, rice straw, fruit peel waste, walnut shells, shells of almond, cotton  
70 waste, bark, sawdust, flax, hemp, and cellulose nanocrystals (Junior et al. 2012; Asberry  
71 et al. 2014; de Quadros Melo et al. 2016; Furlan et al. 2018; Morin-Crini et al. 2019; Shen  
72 et al. 2019; Wang 2019; Halysh et al. 2020; Mokgehle et al. 2021; Santander et al. 2021;  
73 Sjahro et al. 2021; Ahmed et al. 2022; Basnet et al. 2022; Cruz-Lopes et al. 2022; Mergbi  
74 et al. 2023; Akköz and Coşkun R 2023; Pinsard et al. 2023; Zeng et al. 2023). They are used  
75 both in raw and modified form and in particles (shives), fibers, felts and powder forms.  
76 Indeed, chemical modification can improve the adsorption properties of the materials  
77 (Lee and Rowell 2004; Chandraghatgi and Englezos 2008; Sjahro et al. 2021). Regarding  
78 hemp, some studies concluded that hemp-based materials could be low-cost and viable

79 adsorbent for metal removal (Morin-Crini et al. 20219; Pinsard et al. 2023). Empirical  
80 mathematical models are often used to fit the adsorption experimental data and to draw  
81 conclusions, from the assumptions of these models, in order to identify the interactions  
82 involved in the liquid-solid adsorption mechanism (Halys et al. 2020). Another  
83 complementary and necessary approach is to couple different microscopic and  
84 spectroscopic techniques to obtain information on these mechanisms. For example, to  
85 characterize materials before and after use, techniques such as scanning electron  
86 microscopy (SEM), energy-disperse X-ray (EDX), Fourier transform infrared spectroscopy  
87 (FTIR), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), X-ray absorption  
88 near-edge structure (XANES) spectroscopy, computed micro-tomography (micro-CT), and  
89 density functional theory (DFT) calculations are used. These techniques can provide  
90 information on the type of functional groups present in the materials, their distribution in  
91 the structure, and their role and involvement in the adsorption mechanism (Biziks et al.  
92 2016; Jiang et al. 2017, 2018; Mongioví and Crini 2023; Mongioví et al. 2021, 2022a,  
93 2022b; Sun et al. 2021; Müller et al. 2021; Maceda et al. 2022; da Rocha Rodrigues et al.  
94 2023). Information on the precise location of the distribution of metals in materials is  
95 however rare.

96 Since the last decade, hemp-based materials in felt and shives forms, as co-products of  
97 the hemp industry, have also been proposed by our research group as low-cost, available  
98 and efficient adsorbents for the complexation of various metals, e.g., Al, Co, Cu, Mn, Ni  
99 and Zn, present in aqueous solutions (Crini et al. 2021; Morin-Crini et al. 2020). These  
100 materials had high adsorption capacities towards metals depending mainly on the type of  
101 material used. A recent study revealed the adsorption mechanism of materials prepared  
102 from hemp shives, namely sodium carbonate-activated (SHI-C) and polycarboxylic agent-  
103 grafted (SHI-BTCA) hemp shives, using microscopic and spectroscopic techniques  
104 (Mongioví et al. 2022b). The main difference between the two materials is the amount of  
105 carboxylate groups, which is much higher in the case of the SHI-BTCA sample. The  
106 combination of the SEM, EDX, FTIR, Raman, XANES, and XPS techniques allowed us to  
107 characterize and distinguish the type of interactions involved in the liquid-solid adsorption  
108 phenomena for copper complexation by each sample. The results showed that the Cu  
109 adsorption mechanisms on the hemp shives SHI-C and SHI-BTCA were remarkably  
110 different. For the first adsorbent, surface adsorption of copper and diffusion into the  
111 structure of the material were predominant, whereas for the second material, copper  
112 adsorption was due to chemisorption (microprecipitation, complexation) and ion  
113 exchange, highlighting in the latter case the main role of carboxylate groups.

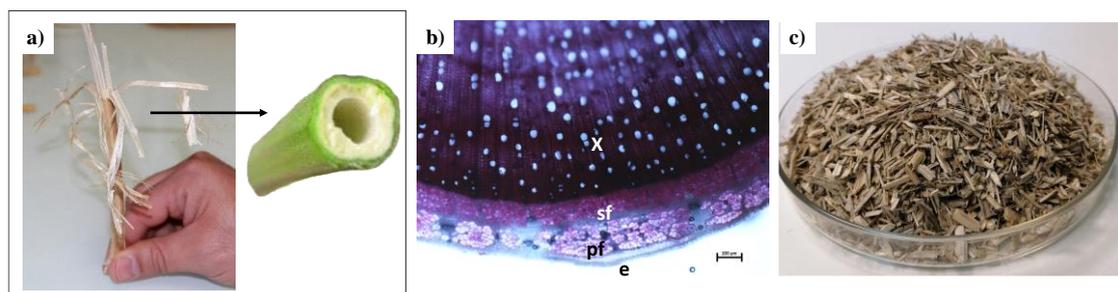
114 In this work, the location of copper adsorbed on the hemp shives was studied thanks to a  
115 combination of two synchrotron techniques, namely K-edge subtraction X-ray micro-  
116 tomography (KES tomography) and X-ray micro-fluorescence imaging, to better  
117 understand mechanisms involved during the copper adsorption.

## 118 **Materials and methods**

### 119 **Materials**

120 Hemp shives or also called Chènevotte, abbreviated SHI, were provided by Eurochanvre  
121 (Arc-les-Gray, France), an agricultural cooperative. These commercial shives (0.12-0.9  
122 euros/kg depending of the quality) (Pinsard et al. 2023) are constituted by parallelepiped

123 particles whose length varies from 5 up to 25 mm (Figure 1), being commercialized for  
124 plant and animal mulching, and insulation. In this work, three materials have been  
125 studied: a sample washed in water (abbreviated SHI-W), a sample activated using sodium  
126 carbonate (SHI-C), and a sample chemically modified by 1,2,3,4-butanetetracarboxylic  
127 acid in order to introduce carboxylic groups (SHI-BTCA). These modifications have been  
128 recently detailed (Mongioví and Crini 2023; Mongioví et al. 2021, 2022a, 2022b). The  
129 results showed significant changes in the surface morphology of each sample after  
130 treatment and their chemical composition. Briefly, for SHI-W, the washing eliminated  
131 water-soluble components. Alkaline treatment (SHI-C) increased the percentage of  
132 cellulose and reduced the ratio of hemicelluloses. When compared to the hemicellulose  
133 content, the amount of lignin did not vary. SHI-BTCA sample showed a completely  
134 different chemical composition due the grafting reaction. The volume of the pores,  
135 obtained from micro-tomography measurements, was comparable for all the washed and  
136 activated samples, while for the grafted sample a significant decrease was observed.  
137 However, for this later material, the grafting with BTCA led to an important increase in  
138 the ionic exchange capacity values due to the presence of carboxylic groups. SHI-W, SHI-  
139 C and SHI-BTCA samples were used as adsorbents for the adsorption of copper present in  
140 solutions using the batch method previously detailed (Mongioví and Crini 2023). In this  
141 work, experimental batch protocol is the following: 2 g of shives were added to 100 mL of  
142 copper solution (at a concentration of 200 mg/L); the solution was stirred for 2 h and the  
143 shives were then recovered and directly frozen.



144

145 **Fig. 1** Raw hemp shives: a) stem; b) stem cross-section (X: xylem; pf: primary fiber; sf: secondary  
146 fiber; e: epidermis); and c) shives

### 147 **Preparation of samples for synchrotron measurements**

148 Transverse and longitudinal cryo thin sections of the shives were prepared using a  
149 cryomicrotome (CM 3050S, Leica, Nanterre). The shives, before and after copper  
150 adsorption, were placed wet in moulds and coated with OCT (Optimal Cutting  
151 Temperature, VWR France). The samples in their moulds were then fast frozen by plunge  
152 in an isopentane solution cooled with liquid N<sub>2</sub>. Several 20 µm thick sections were then  
153 collected from the sample blocks at different locations: longitudinal sections at the  
154 surface of the shives and transverse sections at two depths in the shives, one close to the  
155 surface and the other deeper. These sections were placed between two Ultralene films  
156 (SPEX SamplePrep) in a dedicated sample holder. Each section was observed under an  
157 optical microscope (AZ100M Macroscope, Nikon, Japan) prior to check its quality.

## 158 **X-ray micro-fluorescence (XRF)**

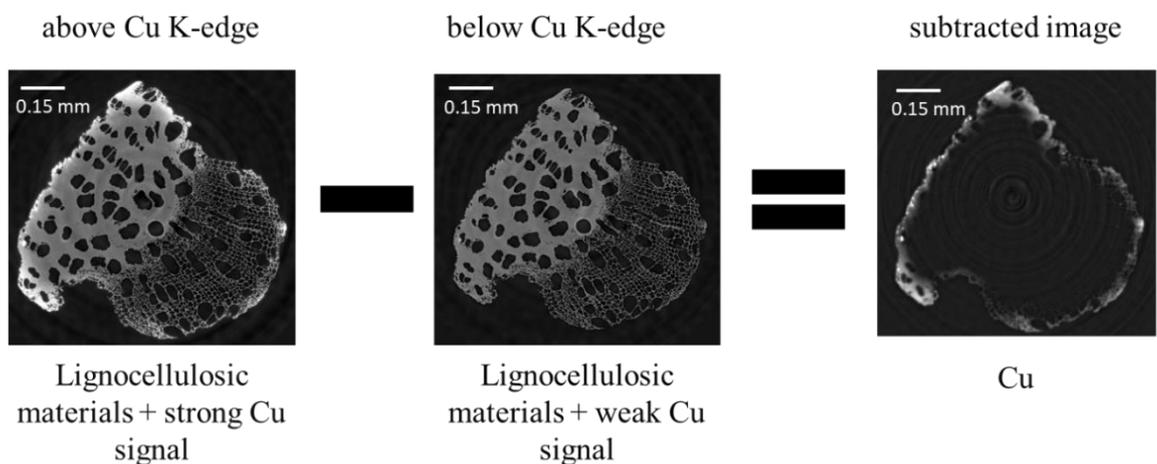
159  $\mu$ -XRF analysis was performed at the PUMA beamline of Synchrotron SOLEIL. A Si(111)  
160 double crystal monochromator (DCM) was used to monochromatize the beam and a  
161 Kirkpatrick-Baez (KB) mirror system focalized the X-rays on the surface of the samples in  
162 a diameter of 5  $\mu\text{m}$  (vertical) and 10  $\mu\text{m}$  (horizontal, including the footprint of the beam).  
163 The samples were mounted in a 45 degree geometry with the incoming beam, a silicon  
164 drift detector (RaySpec Ltd), placed at 90-degree geometry, was used to record the  
165 fluorescence spectra. A home-made diamond beam monitor was used to record the  
166 intensity of the primary beam (I0) at the exit of the KB mirror system. A video-microscope  
167 allowed to align the sample visually and to observe it during the acquisition. The  
168 cartographies of 10  $\mu\text{m}$  x 5  $\mu\text{m}$  were made with an incident X-ray beam energy of 10 keV  
169 and acquired in continuous scanning mode. A spatial resolution of 5, 7 or 10  $\mu\text{m}$  was used.  
170 The obtained data were corrected for deadtime and I0 via a python script and then fitted  
171 with the PyMCA (ESRF) software package (Solé et al. 2007).

## 172 **Micro-tomography**

173 For this analysis, we chose shives with a dimension of approximately 1 mm<sup>2</sup> in section  
174 and 10 mm length. K-edge subtraction tomograms were acquired at ANATOMIX beamline  
175 of Synchrotron SOLEIL (Weitkamp et al. 2022). The shive samples were mounted on the  
176 rotation stage; motorized XYZ translation stages were used to center the sample on the  
177 tomography rotation axis and align it with the field of view of the detector. The scans were  
178 conducted with an electron beam current of 500 mA in the storage ring. Using the Si(111)  
179 double-crystal monochromator of the beam-line (energy bandwidth  $\Delta E/E \approx 10^{-4}$ ), the X-  
180 ray beam used for the measurements was set to photon energies of 8.975 and 9.003 keV  
181 for measurements, respectively, below and above the copper absorption edge. These  
182 energies were chosen using tabulated values from literature. To increase the X-ray flux  
183 density on the sample, a collimating compound refractive X-ray lens consisting of three  
184 biconcave beryllium lenses (RXOPTICS GmbH & Co. KG, Monschau, Germany) with a  
185 surface curvature radius of 1.0 mm in the apex and a circular aperture of 1.9 mm was  
186 inserted into the X-ray beam at a position 37 m from the X-ray source and 133 m from the  
187 sample position. The tomography data were recorded with an indirect detector system  
188 (Desjardins et al. 2018) consisting of a single-crystal lutetium aluminum garnet scintillator  
189 screen, a CMOS-based scientific-grade camera (Hamamatsu Orca Flash 4.0 V2) with  
190 2048x2048 pixels (physical pixel size 6.5  $\mu\text{m}$ ) and microscope optics with a magnification  
191 of 10 (using an objective 10x/NA 0.28 from Mitutoyo) that projected the visible-light  
192 image from the scintillator onto the sensor of the camera, resulting in an effective pixel  
193 size of 0.65  $\mu\text{m}$  and a field of view (FOV) of the detector of (1.3 mm)<sup>2</sup>. The exposure time  
194 was set to 20 ms per projection radiograph. In each tomography scan, 2000 projection  
195 radiographs were acquired on the fly (i.e., without stop of the rotating table between  
196 projections) over a scan range of 180 degrees.

197 The volume reconstruction was performed with the PyHST2 computer program (ESRF,  
198 Grenoble, France) (Mirone et al. 2014) at a voxel size of 0.65  $\mu\text{m}$ ; the reconstructed  
199 cylinder-shaped volumes have a diameter and height of 1.3 mm, corresponding to the  
200 detector FOV. To visualize the copper present in the shives, KES tomography was  
201 performed. The general principle is described in Figure 2. The same sample was irradiated  
202 by X-rays at two different energies around the copper K absorption edges as was already  
203 used with nanotomography (TXM instrument) at the ANATOMIX beamline (Scheel et al.

204 2014). The photoelectric absorption (i.e., attenuation) of X-rays by any chemical element  
 205 increases abruptly as the X-ray photon energy is slightly increased from a value below the  
 206 binding energy of electrons in a given electron shell of that element's atoms to a value  
 207 just above that binding energy; the binding energy value is therefore also referred to as  
 208 an "X-ray absorption edge" for that element. For Cu, the electron binding energy for the  
 209 K shell (i.e., the 1s shell) is 8979 eV (Thompson et al. 2009). Acquiring two sets of X-ray  
 210 image data, one below and one above this energy, results in different signals between  
 211 both data sets only at those positions in the images where Cu is present. While both  
 212 contain signal from the lignocellulosic material and the copper adsorbed on it, the copper  
 213 signal is much stronger in the second type of images than in the first, while the signal from  
 214 the lignocellulosic material is essentially identical for both types of images. By subtracting  
 215 both types of images from one another, it was possible to isolate the copper signal (areas  
 216 of high brightness) and obtain a third image showing only the spatial distribution of the  
 217 metal (Figure 2). This method can detect the copper in the volume if the quantity is large.  
 218 The post-processing of the KES tomography data was carried out using VG StudioMax  
 219 2023-1 and Fiji softwares. The two volumes were aligned with a best fit algorithm  
 220 implemented in the software, and the subtraction method is applied (volume acquisition  
 221 above Cu K-edge minus volume acquisition below Cu K-edge).



222

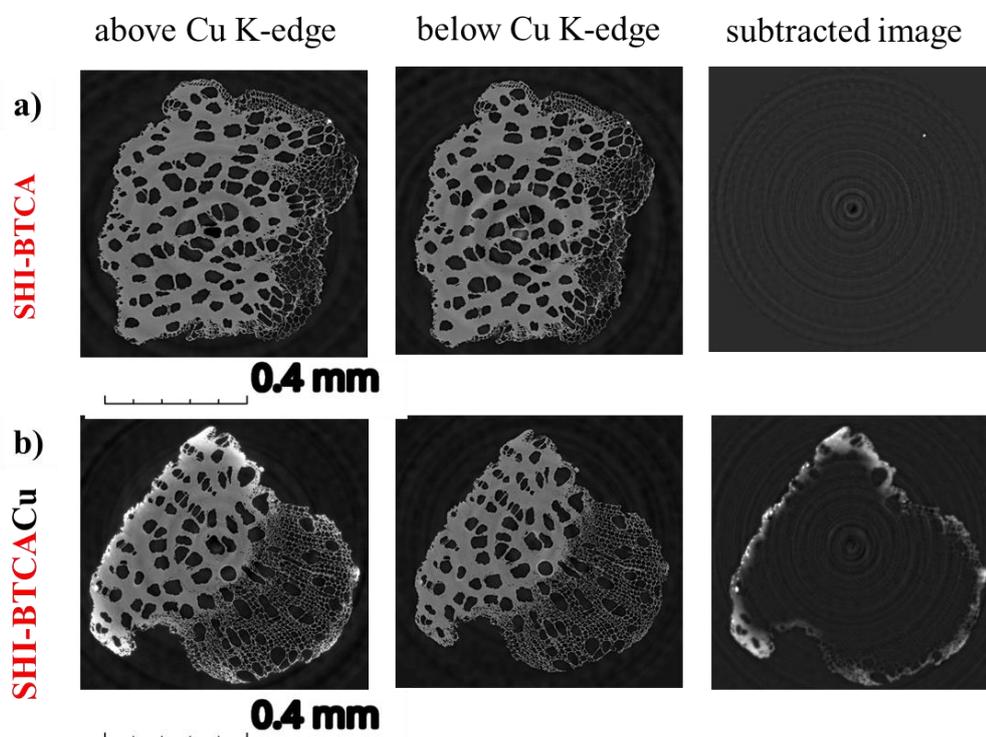
223 **Fig. 2** K-edge subtraction micro-tomography virtual cross-sections of shives taken at photon  
 224 energies above (left) and below Cu K-edge (middle) and resulting image after application of the  
 225 subtraction method (right).

226

## 227 Results and discussion

228 The KES tomography images in Figure 3 compare the presence of Cu before and after  
 229 adsorption for the SHI-BTCA sample. The absence of Cu before the metal adsorption  
 230 process is clearly visible. Indeed, the slice from the volume data set acquired below the  
 231 Cu K edge is very similar to the one taken above the edge. Consequently, the subtraction  
 232 of grey level of the slice above threshold minus the slice below the Cu K-edge does not  
 233 show any difference (other than ring artifacts from slight changes in the X-ray beam  
 234 profile, i.e., induced by the instrument), which confirms that this specimen has not been  
 235 exposed to copper (Figure 3a).

236 In contrast, the difference image from the SHI-BTCaCu sample shows the presence of  
 237 copper by high image intensity, highlighting the areas where copper is present (in white  
 238 in Figure 3b, right). This brightness is mostly localized in areas near the **surface** of the  
 239 sample. Similar results were obtained by analyzing the SHI-W and SHI-C samples, whose  
 240 images are shown in Supplementary files.

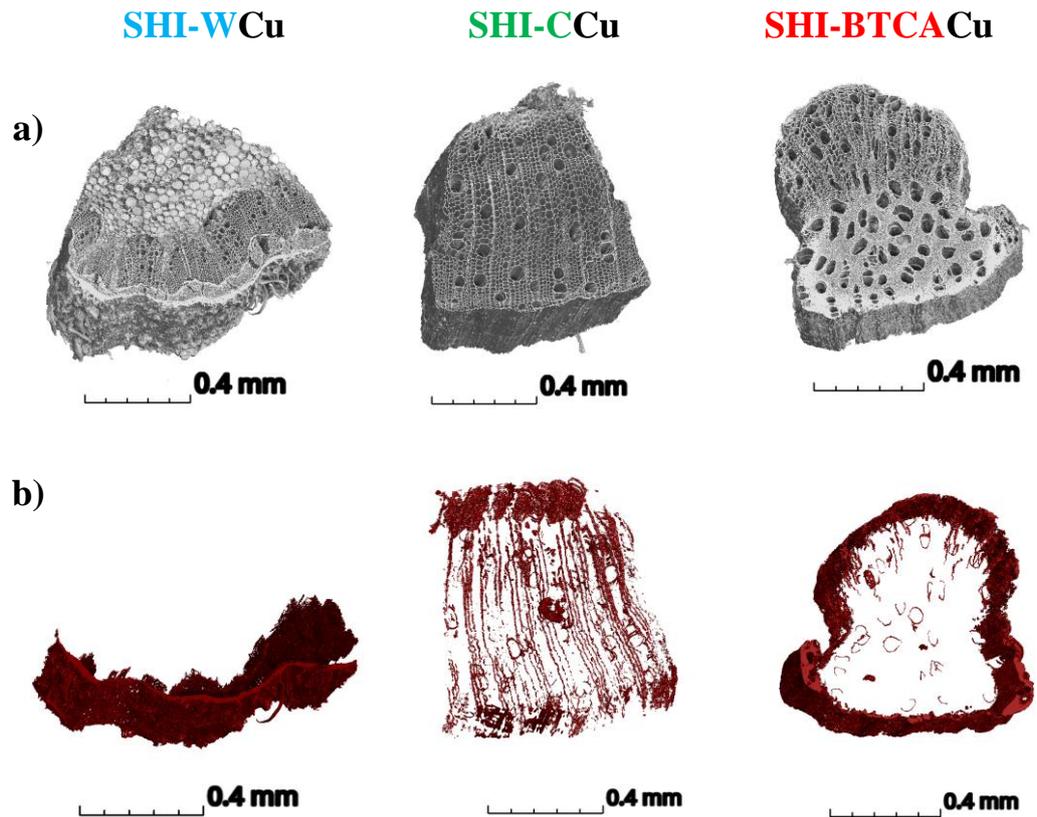


241

242 **Fig. 3** Micro-tomography virtual cross-sections (slices) taken at X-ray photon energies above  
 243 (left) and below the Cu K-edge (middle) and subtracted image (right) for the SHI-BTCA sample  
 244 before (a) and after (b) copper adsorption.

245 Figure 4 compares 3D tomography images for SHI-WCu, SHI-CCu and SHI-BTCaCu shives  
 246 taken above the Cu K-edge with the resulting 3D images after application of the  
 247 subtraction method. For SHI-WCu and SHI-CCu, the metal is localized both over the  
 248 external and internal surfaces of the material (which is consistent with the assumption of  
 249 a diffusion mechanism). In contrast, for SHI-BTCaCu, copper is located mainly on the outer  
 250 surface and around the surface of the vessels involving carboxylate functions, in  
 251 agreement with recent data (Mongiovi et al. 2022b).

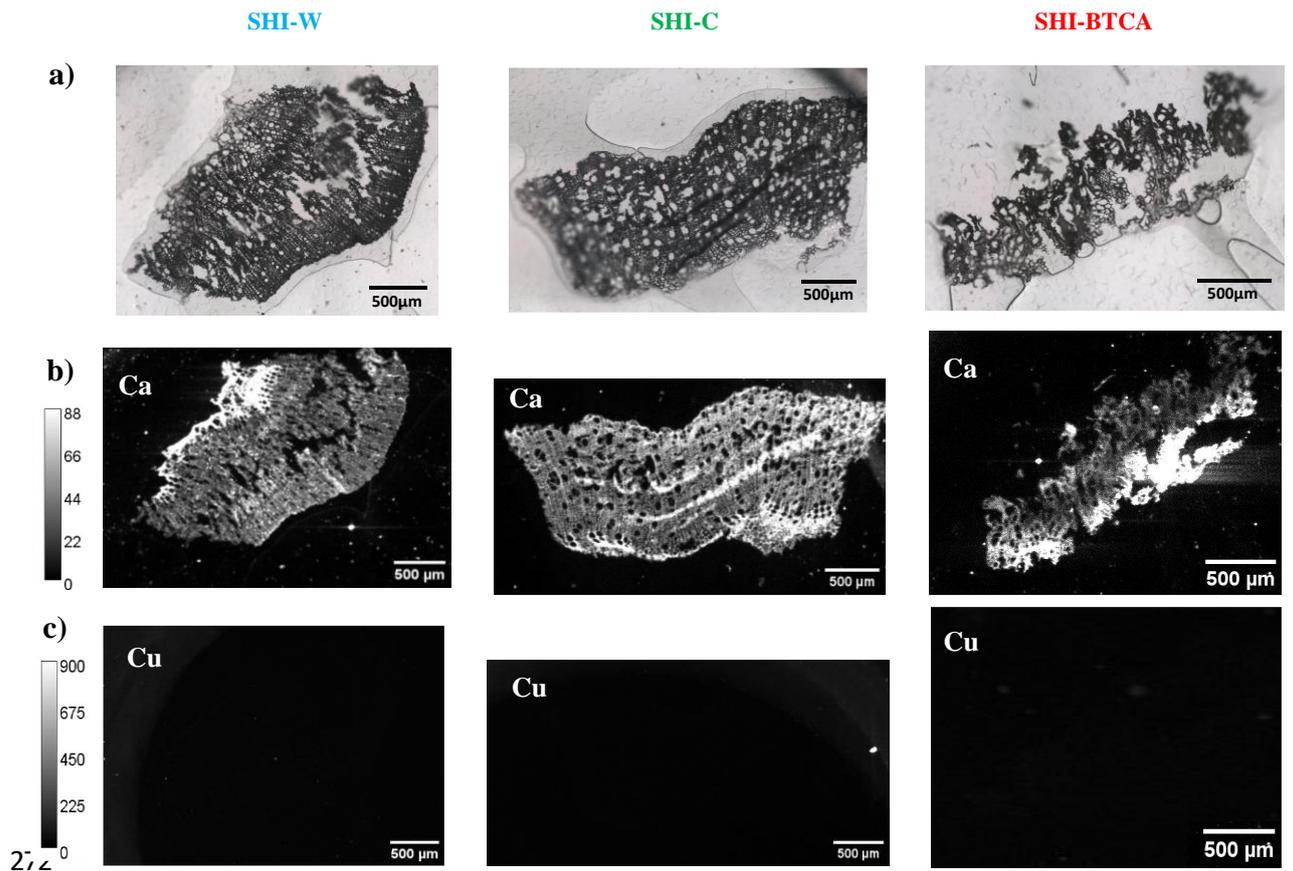
252 For SHI-CCu, the copper is distributed in all the surface of the shives due to the partial  
 253 removal of the hemicelluloses during sample activation (Mongiovi et al. 2021). Indeed,  
 254 the treatment with sodium carbonate induced in the cell wall some micro- and nano-  
 255 porosities into which the aqueous solution can diffuse. So, for this sample, the mechanism  
 256 of adsorption of the copper is completely different than that for SHI-BTCA.



257

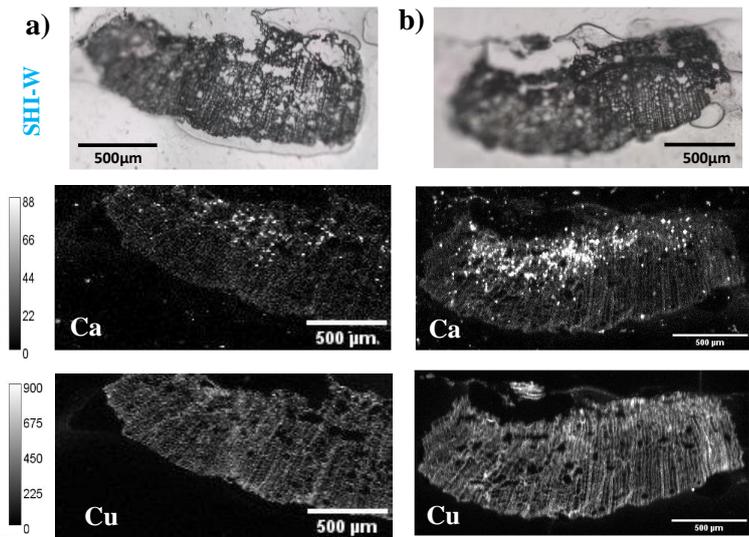
258 **Fig. 4** 3D K-edge subtraction tomography images for SHI-WCu, SHI-CCu and SHI-BTCA Cu shives  
 259 after copper adsorption: a) above Cu K-edge and b) after application of the subtraction method.

260 To determine the spatial distribution of copper in hemp samples after adsorption,  $\mu$ -XRF  
 261 maps were also acquired. Elemental maps were extracted from the fluorescence spectra  
 262 collected in each pixel. The abundance of each element is represented by a grayscale: the  
 263 higher the intensity of the fluorescence signal, the brighter the image, and the more  
 264 concentrated the element. For a given element, the same intensity scale was chosen on  
 265 all maps in order to be able to compare them quantitatively. On the other hand, the  
 266 grayscales chosen are not identical for the different elements because of the very  
 267 different abundances. It is therefore not possible to directly compare the abundance of  
 268 one element to that of another from these images. The XRF map of the shives before  
 269 adsorption shows the presence of calcium, distributed over the entire sample, with  
 270 accumulation in the pith area (Figure 5). No copper signal is visible on these maps,  
 271 indicating its absence or the presence of a very low concentration.



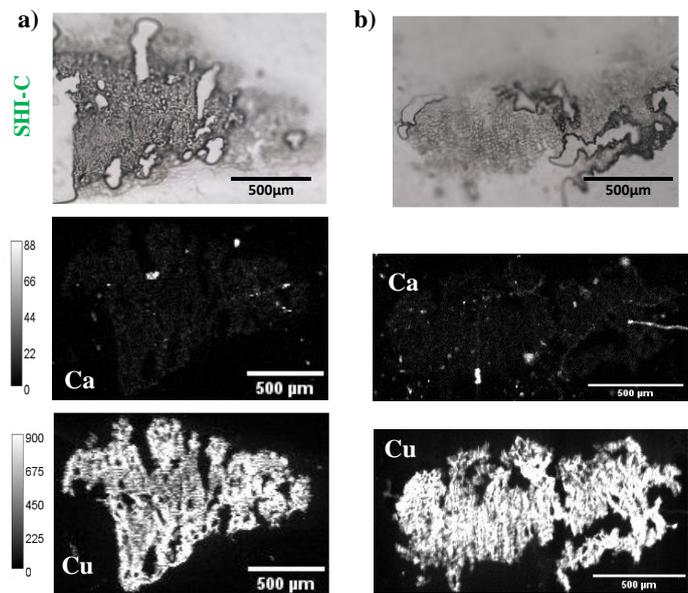
273 **Fig. 5** Comparison of transverse sections of SHI-W, SHI-C and SHI-BTCA samples before copper  
 274 adsorption obtained by a) optical microscopy, b) synchrotron-based XRF mapping of Ca, and c)  
 275 synchrotron-based XRF mapping of Cu. For a given element, the grey scale of the XRF images is the  
 276 same for the three samples.

277 After adsorption, all samples show the presence of the copper fluorescence signal. The  
 278 images of Figures 6, 7 and 8 show the maps of Cu and Ca recorded for transverse sections  
 279 at different depths for, respectively, SHI-WCu, SHI-CCu and SHI-BTCA-Cu shives. A  
 280 comparison of these maps reveals the following: (i) The fluorescence intensity of Cu is  
 281 lower in SHI-WCu than in SHI-CCu and SHI-BTCA-Cu, in agreement with published results  
 282 on the removal of Cu present in contaminated solutions (Mongiovi et al. 2022b); (ii) In the  
 283 SHI-WCu and SHI-CCu samples, Cu is distributed over all the free surface, internal and  
 284 external, with no obvious preferential accumulation areas; These images are therefore in  
 285 agreement with the results of micro-tomography and with the hypothesis of a mechanism  
 286 dominated by diffusion; (iii) For SHI-BTCA-Cu, a different copper distribution is observed  
 287 depending on the depth of the cross-section observed. In particular, for the most  
 288 superficial or external section (Figure 8a), Cu seems to be distributed over the entire  
 289 surface while, for the inner section (Figure 8b), the metal is rather located at the periphery  
 290 of the sample; this observation is in agreement with the results of micro-tomography,  
 291 confirming the hypothesis of phenomena such as ion exchange or microprecipitation.  
 292 Supplementary data also presents maps obtained on other transverse and longitudinal  
 293 sections which confirm the observations described above, such as the lower amounts of  
 294 adsorbed Cu for SHI-WCu than for SHI-CCu and SHI-BTCA-Cu.



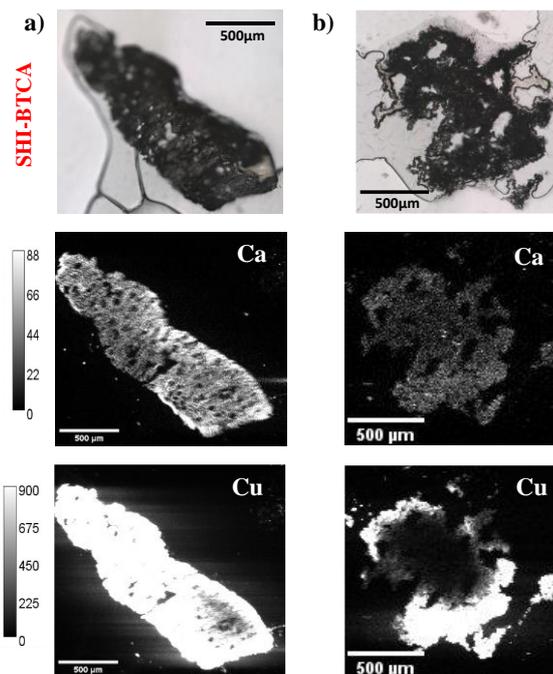
295

296 **Fig. 6** Optical microscope images (top) and X-ray fluorescence maps of Ca (middle) and Cu  
 297 (bottom) for two SHI-WCu transverse sections. The sections are obtained at two different depths  
 298 of the same hemp shives a superficial cross-section (a) and an internal cross-section (b). For a given  
 299 element, the gray scale of the XRF maps (in number of normalized shots) is the same for all samples  
 300 and is identical to the previous figure.



301

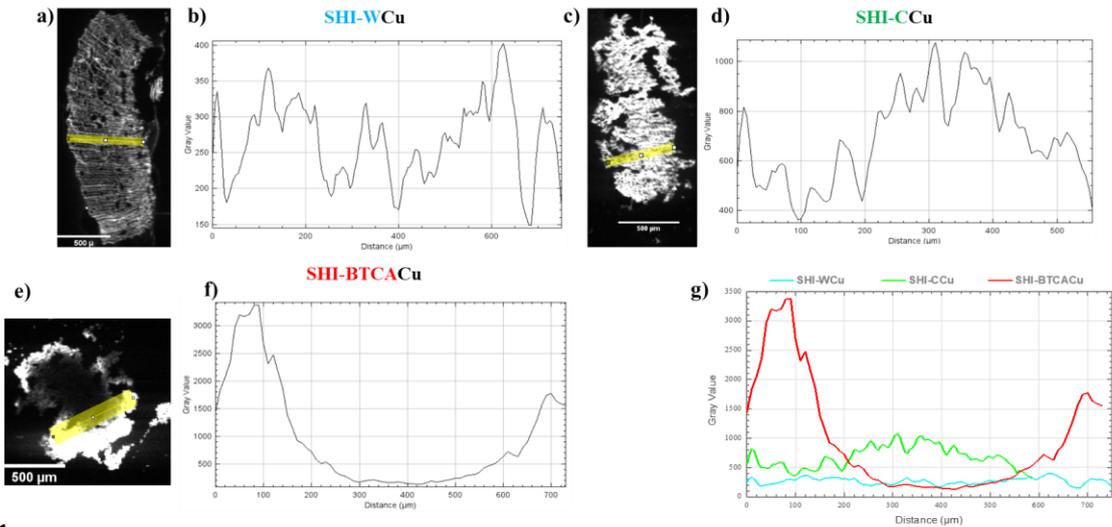
302 **Fig. 7** Optical microscope images (top), X-ray fluorescence maps of Ca (middle), and Cu (bottom)  
 303 of two SHI-CCu transverse sections. The sections are obtained at two different depths of the same  
 304 hemp shive, a superficial cross-section (a) and an internal cross-section (b). For a given element,  
 305 the gray scale of the XRF maps (in number of normalized counts) is the same for all samples and is  
 306 identical to the previous figures.



307

308 **Fig. 8** Optical microscope images (top), X-ray fluorescence maps of Ca (middle), and Cu (bottom)  
 309 for the two SHI-BTCaCu transverse sections. The sections are obtained at two different depths of  
 310 the same hemp shive, a superficial cross-section (a) and an internal cross-section (b). For a given  
 311 element, the gray scale of the XRF maps (in number of normalized counts) is the same for all  
 312 samples and is identical to the previous figures.

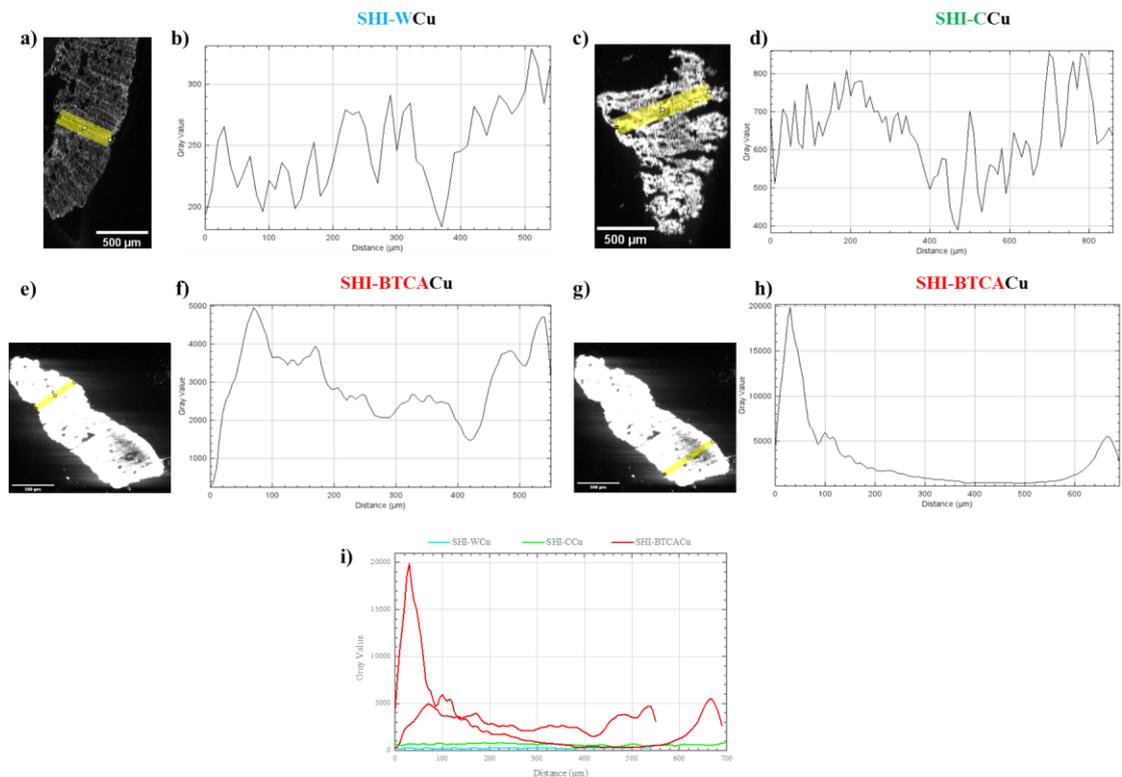
313 The data analysis made it possible to carry out a statistical study of the distribution of  
 314 fluorescence intensities in the XRF maps and thus of the distribution of copper. Two types  
 315 of analysis were performed: the first is the extraction of a Cu distribution profile along a  
 316 15-pixel-thick line passing through the sample in the radial direction, and the second is  
 317 the determination of the average copper value (in terms of fluorescence intensity) over  
 318 the entire surface of the sample. Figure 9 shows the Cu distribution profiles extracted  
 319 from  $\mu$ -XRF maps for internal transverse sections. In Figures 9b and 9d, it can be seen that,  
 320 for SHI-WCu and SHI-CCu samples, the signal is present throughout the area analyzed. The  
 321 metal appears to be distributed evenly in the washed sample, with intensities between  
 322 200 and 400 counts on the scale (Figure 9b). The SHI-C profile shows signals with higher  
 323 intensities (values between 600 and 1000 counts, as opposed to 200 to 400 for SHI-WCu)  
 324 except for the area around 100  $\mu$ m, where excessive degradation of the sample structure  
 325 led to lower Cu presence and therefore a decrease in the signal (Figure 9d). Concerning  
 326 the SHI-BTCaCu sample, it can be seen that, for the inner transverse section, the  
 327 distribution profile of copper is totally different from that in SHI-WCu and SHI-CCu,  
 328 characterized on the one hand by two areas of high intensity (between 1500 and 3000  
 329 counts) in the most external parts of the section and, on the other hand, the absence of  
 330 signal in the inner part, between 250 and 550  $\mu$ m (Figure 9f).



331

332 **Fig. 9** XRF fluorescence maps of the Cu element (a, c and e) and Cu distribution profiles (b, d and  
 333 f) extracted from internal sections of SHI-WCu, SHI-CCu and SHI-BTCAcCu shives, and comparison  
 334 of the profile intensity of the three materials (g). The location of the profile is shown in yellow on  
 335 the fluorescence maps.

336 For the external transverse sections of SHI-WCu and SHI-CCu samples (Figure 10), the  
 337 profiles plotted do not differ significantly from those of the internal sections (Figure 10b  
 338 and Figure 10d, respectively). For the SHI-BTCAcCu sample, two different areas were  
 339 analyzed. The first shows that copper is distributed throughout the analyzed area (Figure  
 340 10f) while, for the second, the Cu distribution profile shows a pattern similar to that of  
 341 the inner section, with external peaks of very high intensity (up to 20000 counts) and a  
 342 central zone with a very low signal (Figure 10h).



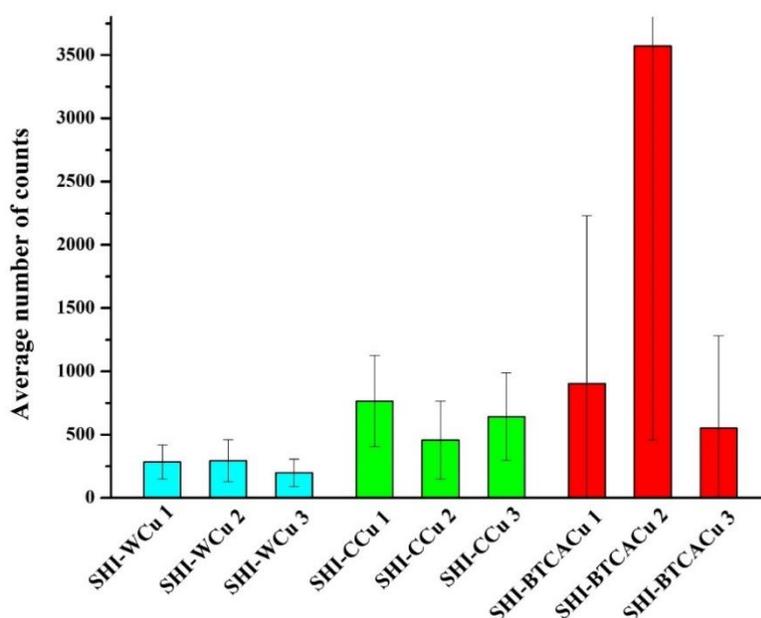
343

344 **Fig. 10** XRF maps of Cu (a, c, e, g) and Cu distribution profiles (b, d, f, h) extracted from superficial  
345 sections of SHI-WCu, SHI-CCu and SHI-BTCACu shives, and comparison of the profile intensity of  
346 the three materials (i). The location of the profiles is shown in yellow on the fluorescence maps.

347 Finally, Figure 11 shows the average Cu intensity values calculated by analyzing the area  
348 of three transverse sections of each sample. For SHI-WCu and SHI-CCu, Cu distribution on  
349 the sur-face is more homogeneous and comparable from one section to another, since  
350 the values are similar with lower standard deviations, compared to SHI-BTCA. This  
351 distribution is in accordance with a diffusion mechanism. In addition, if we compare SHI-  
352 WCu and SHI-CCu samples, the amounts of Cu present in the transverse sections are  
353 greater for SHI-CCu. It can therefore be concluded that the distribution of copper on the  
354 surface depends on the structure of the sample and therefore on the treatment  
355 undergone (to be related to the different degradation and chemical composition for the  
356 two samples).

357 For SHI-BTCACu, there is a large variation in both mean copper values and standard  
358 deviations. This result can be explained if we consider that the distribution of copper  
359 depends on the area of contact with the adsorbent during adsorption experiments. This  
360 observation is consistent with a grafting of non-homogeneous BTCA ligands over the  
361 entire surface, and consequently, the existence of preferential areas of interaction be-  
362 tween Cu and these functions.

363



364

365 **Fig. 11** Average copper fluorescence intensity values (number of counts) for SHI-WCu, SHI-CCu  
366 and SHI-BTCACu samples obtained by analyzing three different transverse sections.

## 367 Conclusion

368 In this work, the spatial distribution of the copper complexed by three hemp shives has  
369 been determined by a combination of K-edge subtraction micro-tomography and X-ray  
370 micro-fluorescence imaging. These two synchrotron techniques are complementary as  
371 tomography provides 3D information but is less sensitive to elemental concentrations,

372 while X-ray fluorescence imaging allows for a lower detection limit but only provides 2D  
373 information. Both techniques confirmed that the Cu distribution is totally different for the  
374 three samples. For SHI-W and SHI-C, Cu is present both in the internal and external surface  
375 of the adsorbent while for SHI-BTCA, it is mainly localized at the surface where carboxylic  
376 groups are present. The observations corroborate a diffusion mechanism for SHI-W and  
377 SHI-C and a chemisorption mechanism for SHI-BTCA during the solid-liquid adsorption  
378 experiments involving hemp adsorbents and copper present in monocontaminated  
379 solutions. To continue this work, we will use the same techniques to study the interactions  
380 between the same materials and metals present in polycontaminated solutions.

381

## 382 **Acknowledgements**

383 Grégorio Crini thanks Eurochanvre (Arc-les-Gray, France) for the gift of the materials. Chiara  
384 Mongiovi thanks the Région Bourgogne Franche-Comté for awarding her a research grant. The  
385 authors acknowledge Synchrotron SOLEIL for providing beamtime (proposal 20220289).  
386 ANATOMIX is an Equipment of Excellence (EQUIPEX) funded by the Investments for the Future  
387 program of the French National Research Agency (ANR), project NanoimagesX, grant no. ANR-11-  
388 EQPX-0031.

## 389 **Funding**

390 The authors thank the *Région Bourgogne Franche-Comté* and *Université de Franche-Comté*  
391 (FINEAU project 2020-2024) for financial support. This work has also been supported by the EIPHI  
392 Graduate School (contract ANR-17-EURE-0002).

## 393 **Author contributions**

394 CM, XG, JG, WA, MC, JP, TW, SD, LB, CR, SC, KM: Conducting experiments, analysing and  
395 interpreting data, data curation, software. JB, VP and GC: Validation. CM, XG, CR, JB, VP and GC:  
396 Conceptualization, methodology, validation, supervision. CM and GC: writing original draft, review  
397 and editing. GC: Resources, funding acquisition, project administration. All the authors have given  
398 approval to the final version of the manuscript.

## 399 **Declarations**

## 400 **Supplementary Information**

401 The online version contains supplementary material available at.

## 402 **Conflict of interest**

403 The authors declare that they have no known competing financial interests or personal  
404 relationships that could have appeared to influence the work reported in this paper.

## 405 **Informed consent**

406 All the authors have provided their consent for the submission of the presented work.

## 407 **References**

- 408 Ahmed E, Zeitoun A, Hamad G, Zeitoun MAM, Taha A, Korma SA, Esatbeyoglu T (2022)  
409 Lignocellulosic biomasses from agricultural wastes improved the quality and physicochemical  
410 properties of frying oils. *Foods* 11:3149. <https://doi.org/10.3390/foods11193149>
- 411 Akköz Y, Coşkun R (2023) Preparation of highly effective bio-adsorbent from hemp fiber for  
412 removal of malachite green oxalate (MGO). *Cellulose* 30:4511-4525. <https://doi.org/10.1007/s10570-023-05167-y>
- 414 Asberry HB, Kuo CY, Gung CH, Conte ED, Suen SY (2014) Characterization of water bamboo husk  
415 biosorbents and their application in heavy metal ion trapping. *Microchem J* 113:59-63.  
416 <https://doi.org/10.1016/j.microc.2013.11.011>
- 417 Basnet P, Gyawali D, Ghimire KN, Paudyal H (2022) An assessment of the lignocellulose-based  
418 biosorbents in removing Cr(VI) from contaminated water: A critical review. *Results Chem* 4:100406.  
419 <https://doi.org/10.1016/j.rechem.2022.100406>
- 420 Biziks V, Van den Bulcke J, Grinins J, Militz H, Andersons B, Andersone I, Dhaene J, Van Acker J  
421 (2016) Assessment of wood microstructural changes after one-stage thermo-hydro treatment  
422 (THT) by micro-X-ray computed tomography. *Holzforschung* 70:167-177.  
423 <https://doi.org/10.1515/hf-2014-0337>
- 424 Chandraghatgi R, Englezos T (2008) Sequestering of heavy metal ions from aqueous solutions by  
425 using a lignocellulosic material. *Int J Environ Pollut* 32:509-518.  
426 <https://doi.org/10.1504/IJEP.2008.018414>
- 427 Crini G, Bradu C, Cosentino C, Staelens JN, Martel B, Fourmentin M, Loiacono S, Chanet G, Torri G,  
428 Morin-Crini N (2021) Simultaneous removal of inorganic and organic pollutants from  
429 polycontaminated wastewaters on modified hemp-based felts. *Rev Chim* 72:25-43.  
430 <https://doi.org/10.37358/Rev.Chim.1949>
- 431 Cruz-Lopes L, Macena M, Esteves B, Santos-Vieira I (2022) Lignocellulosic materials used as  
432 biosorbents for the capture of nickel (II) in aqueous solution. *Appl Sci* 12:933.  
433 <https://doi.org/10.3390/app12020933>
- 434 da Rocha Rodrigues EJ, Cucinelli Neto RP, Lopes Alves HD, Paciornik S, Santos de Sá D, Ghavami K,  
435 Pandoli OG (2023) An investigation of the fluid-holding cavities in a lignocellulosic-based bamboo  
436 matrix via a combined X-Ray microtomography and proton time-domain NMR approach. *Cellulose*  
437 30:4925-4940. <https://doi.org/10.1007/s10570-023-05197-6>
- 438 de Quadros Melo D, de Oliveira Sousa Neto V, de Freitas Barros FC, Cabral Raulino GS, Bastos Vidal  
439 C, do Nascimento RF (2016) Chemical modifications of lignocellulosic materials and their  
440 application for removal of cations and anions from aqueous solutions. *J Appl Polym Sci* 133:43286.  
441 <https://doi.org/10.1002/app.43286>

- 442 Desjardins K, Scheel M, Giorgetta JL, Weitkamp T, Menneglier C, Carcy A (2018) Design of indirect  
443 X-ray detectors for tomography on the ANATOMIX beamline. Proc. 10th Mechanical Engineering  
444 Design of Synchrotron Radiation Equipment and Instrumentation (MEDSI 2018), pp. 355-357.  
445 <https://doi.org/10.18429/JACoW-MEDSI2018-THPH09>
- 446 Furlan FL, Consolin N, Consolin MFB, Gonçalves MS, Valderrama P, Genena AK (2018) Use of  
447 agricultural and agroindustrial residues as alternative adsorbents of manganese and iron in  
448 aqueous solution. Rev. Ambiente Água 13:1-13. <https://doi.org/10.4136/ambi-agua.2181>
- 449 Halys V, Sevastyanova O, Pikus S, Dobelev G, Pasalskiy B, Gun'ko M, Kartel M (2020) Sugarcane  
450 bagasse and straw as low-cost lignocellulosic sorbents for the removal of dyes and metal ions from  
451 water. Cellulose 27:81-8197. <https://doi.org/10.1007/s10570-020-03339-8>
- 452 Jiang Y, Ansell MP, Jia X, Hussain A, Lawrence M (2017) Physical characterization of hemp shiv: cell  
453 wall structure and porosity. Academic J Civ Eng 35:22-28. <https://doi.org/10.26168/icbbm2017.1>
- 454 Jiang Y, Lawrence M, MP Ansell, Hussain A (2018) Cell wall microstructure, pore size distribution  
455 and absolute density of hemp shiv. R Soc Open Sci 5:171945. <https://doi.org/10.1098/rsos.171945>
- 456 Junior ACG, Strey L, Lindino CA, Nacke H, Schwantes D, Seidel EP (2012) Applicability of the Pinus  
457 bark (*Pinus elliottii*) for the adsorption of toxic heavy metals from aqueous solutions. Acta Sci  
458 Technol 34:79-87. <https://doi.org/10.4025/actascitechnol.v34i1.95>
- 459 Lee BG, Rowell RM (2004) Removal of heavy metal ions from aqueous solutions using  
460 lignocellulosic fibers. J Nat Fibers 1:97-108. [https://doi.org/10.1300/J395v01n01\\_07](https://doi.org/10.1300/J395v01n01_07)
- 461 Maceda A, Terrazas T (2022) Fluorescence microscopy methods for the analysis and  
462 characterization of lignin. Polymers. 14:961. <https://doi.org/10.3390/polym14050961>.
- 463 Müller D, Graetz J, Balles A, Stier S, Hanke R, Fella C (2021) A novel nano-tomography setup for  
464 material science and engineering applications, Cornell University.  
465 <https://doi.org/10.48550/arXiv.2102.06644>
- 466 Mergbi M, Galloni MG, Aboagye D, Elimian E, Su PD, Ikram BM, Nabgan W, Bedai J, Amor HB,  
467 Contreras S, Median F, Djellabi R (2023) Valorization of lignocellulosic biomass into sustainable  
468 materials for adsorption and photocatalytic applications in water and air remediation. Environ Sci  
469 Pollut Res 30:74544-74574. <https://doi.org/10.1007/s11356-023-27484-2>
- 470 Mirone A, Brun E, Gouillart E, Tafforeau P, Kieffer J (2014) The PyHST2 hybrid distributed code for  
471 high speed tomographic reconstruction with iterative reconstruction and a priori knowledge  
472 capabilities. Nucl Instrum Methods Phys Res B 324:41-48.  
473 <https://doi.org/10.1016/j.nimb.2013.09.030>
- 474 Mokgehle TM, Tavengwa NT (2021) Recent developments in materials used for the removal of  
475 metal ions from acid mine drainage. Appl Water Sci 11:42. <https://doi.org/10.1007/s13201-020-01350-9>
- 477 Mongiovi C, Crini G (2023) Copper recovery from aqueous solutions by hemp shives: Adsorption  
478 studies and modeling. Processes. 11:191. <https://doi.org/10.3390/pr11010191>
- 479 Mongiovi C, Lacalamita D, N Morin-Crini, Gabrion X, Ivanovska A, Sala F, Placet V, Rizzi V, Gubitosa  
480 J, Mesto E, Ribeiro ARL, Fini P, Vietro ND, Schingaro E, Kostić M, Cosentino C, Cosma P, Bradu C,  
481 Chanet G, Crini G (2021) Use of chènevotte, a valuable co-product of industrial hemp fiber, as  
482 adsorbent for pollutant removal. Part I: Chemical, microscopic, spectroscopic and  
483 thermogravimetric characterization of raw and modified samples. Molecules 26:4574.  
484 <https://doi.org/10.3390/molecules26154574>
- 485 Mongiovi C, Crini G, Gabrion X, Placet V, Blondeau-Patissier V, Krystianiak A, Durand S, Beaugrand  
486 J, Dorlando A, Rivard C, Gautier L, Ribeiro ARL, Lacalamita D, Martel B, Staelens JN, Ivanovska A,

487 Kostić M, Heintz O, Bradu C, Raschetti M, Morin-Crini N (2022a) Revealing the adsorption  
488 mechanism of copper on hemp-based materials through EDX, nano-CT, XPS, FTIR, Raman, and  
489 XANES characterization techniques. *Chem Eng J Adv* 10:100282.  
490 <https://doi.org/10.1016/j.cej.2022.100282>

491 Mongiovi C, Lacalamita D, Morin-Crini N, Gabrion X, Placet V, Ribeiro ARL, Ivanovska A, Kostić M,  
492 Bradu C, Staelens JN, Martel B, Raschetti M, Crini G (2022b) Use of chènevotte, a valuable co-  
493 product of industrial hemp fiber, as adsorbent for copper ions: Kinetic studies and modeling.  
494 *Arabian J Chem* 15:103742. <https://doi.org/10.1016/j.arabjc.2022.103742>

495 Morin-Crini N, Loiacono S, Placet V, Torri G, Bradu C, Kostić M, Cosentino C, Chanet G, Martel B,  
496 Lichtfouse E, Crini G (2019) Hemp-based adsorbents for sequestration of metals: A review. *Environ*  
497 *Chem Lett* 17:393-408. <https://doi.org/10.1007/s10311-018-0812-x>

498 Morin-Crini N, Staelens JN, Loiacono S, Martel B, Chanet G, Crini G (2020) Simultaneous removal  
499 of Cd, Co, Cu, Mn, Ni and Zn from synthetic solutions on a hemp-based felt. III. Real discharge  
500 waters. *J Appl Polym Sci* 137:48823. <https://doi.org/10.1002/app.48823>

501 Pinsard L, Revol N, Pomikal H, De Luycker E, Ouagne P (2023) Production of long hemp fibers using  
502 the flax value chain. *Fibers*. 11: 38. <https://doi.org/10.3390/fib11050038>

503 Santander P, Butter B, Oyarce E, Yáñez M, Xiao LP, Sánchez J (2021) Lignin-based adsorbent  
504 materials for metal ion removal from wastewater: A review. *Ind Crops Prod* 167:113510.  
505 <https://doi.org/10.1016/j.indcrop.2021.113510>

506 Scheel M, Perrin J, Koch F, Daniel G, Giorgetta J, Cauchon G, King A, Yurgens V, Le Roux V, David C,  
507 Weitkamp T (2022) Current status of hard X-ray nano-tomography on the transmission microscope  
508 at the ANATOMIX beamline. *J Phys Conf Ser* 2380:012045. <https://doi.org/10.1088/1742-6596/2380/1/012045>

510 Sharma A, Anjana, Rana H, Goswami S (2022) A comprehensive review on the heavy metal removal  
511 for water remediation by the application of lignocellulosic biomass-derived nanocellulose. *J Polym*  
512 *Environ* 30:1-18. <https://doi.org/10.1007/s10924-021-02185-4>

513 Shen XP, Xie YJ, Wang QW, Yi X, Shamshina JL, Rogers RD (2019) Enhanced heavy metal adsorption  
514 ability of lignocellulosic hydrogel adsorbents by the structural support effect of lignin. *Cellulose*  
515 26:1005-4019. <https://doi.org/10.1007/s10570-019-02328-w>

516 Sjahro N, Yunus R, Abdullah LC, Rashid SA, Asis AJ, Akhlisah ZN (2021) Recent advances in the  
517 application of cellulose derivatives for removal of contaminants from aquatic environments.  
518 *Cellulose* 28:7521-7557. <https://doi.org/10.1007/s10570-021-03985-6>

519 Solé VA, Papillon E, Cotte M, Walter P, Susini J (2007) A multiplatform code for the analysis of  
520 energy-dispersive X-ray fluorescence spectra. *Spectrochim Acta Part B* 62:63-68.  
521 <https://doi.org/10.1016/j.sab.2006.12.002>

522 Sun Q, Xia YD, Klinger J, Seifert R, Kane J, Thompson V, Chen QS (2021) X-ray computed  
523 tomography-based porosity analysis: Algorithms and application for porous woody biomass.  
524 *Powder Technol* 388:496-504. <https://doi.org/10.1016/j.powtec.2021.05.006>

525 Thompson AC, Kirz J, Attwood DT, Gullikson EM, Howells MR, Kortright JB, YW Liu, Robinson AL,  
526 Under-wood JH, Kim KJ, Lindau I, Pianetta P, Winick H, Williams GP, Scofield JH. (2009) X-Ray Data  
527 Booklet. Lawrence Berkeley National Laboratory, University of California, Berkeley CA 94720

528 Weitkamp T, Scheel M, Perrin J, Daniel G, King A, Le Roux V, Giorgetta J, Carcy A, Langlois F,  
529 Desjardins K, Menneglier C, Cerato M, Engblom C, Caucon G, T Moreno, Rivard C, Y Gohon, Polack  
530 F (2022) Microtomography on the ANATOMIX beamline at Synchrotron SOLEIL. *J Phy Conf Ser*  
531 2380:012122. <https://doi.org/10.1088/1742-6596/2380/1/012122>

- 532 Wang D (2019) A critical review of cellulose-based nanomaterials for water purification in industrial  
533 processes. *Cellulose* 26:687-701. <https://doi/10.1007/s10570-018-2143-2>
- 534 Zeng H, Su YM, Gong X, Zheng LC, Zhang LJ, Meng PP, Zhou QY, Ren JJ (2023) Competitive  
535 adsorption behavior of typical heavy metal ions from acid mine drainage by  
536 multigroup-functionalization cellulose: qualitative and quantitative mechanism. *Environ Sci Pollut*  
537 *Res* 30:68191-68205. <https://doi.org/10.1007/s11356-023-27188-7>