

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**

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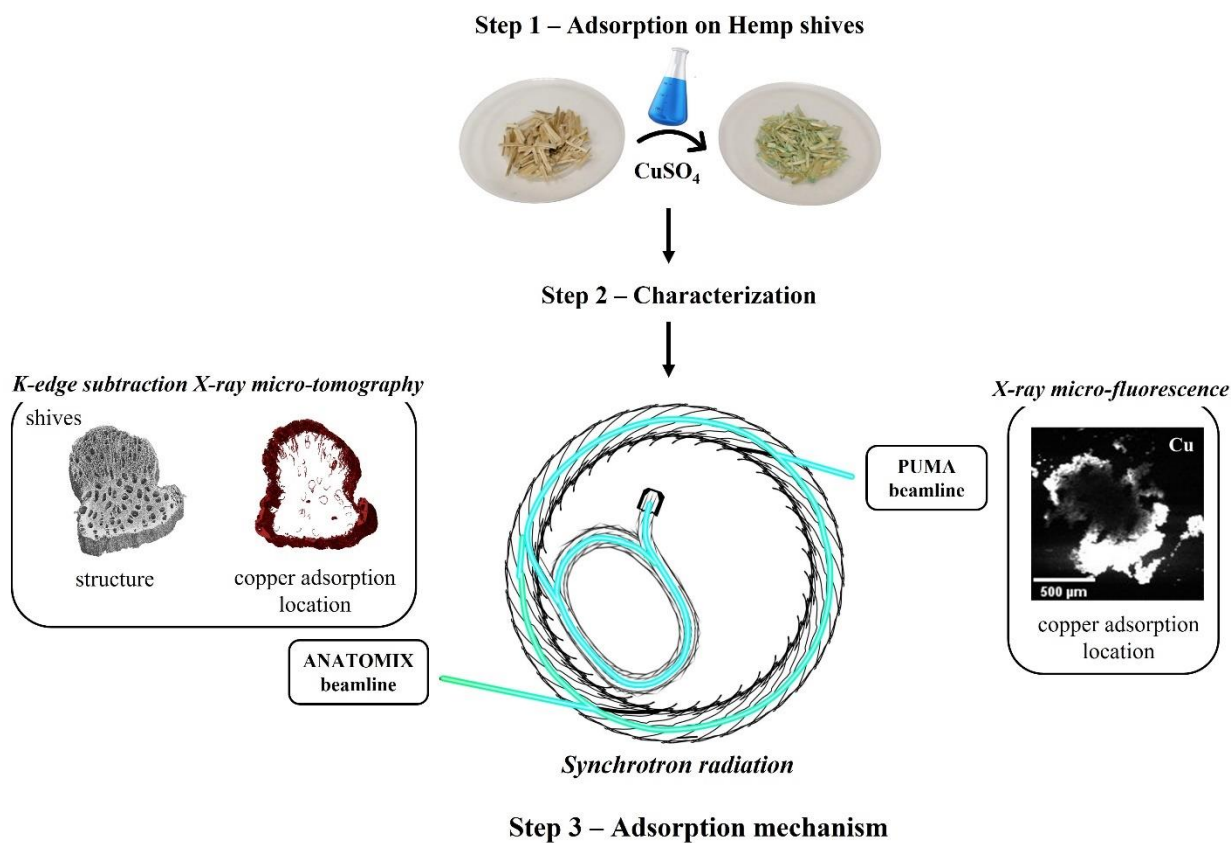
38 **Abstract** In the literature, hemp shives have been proposed as adsorbents capable of
39 effectively complexing metals in aqueous solution. However, studies on the localization of metals
40 in these multicell type lignocellulosic materials are scarce and further description of adsorption
41 sites 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.

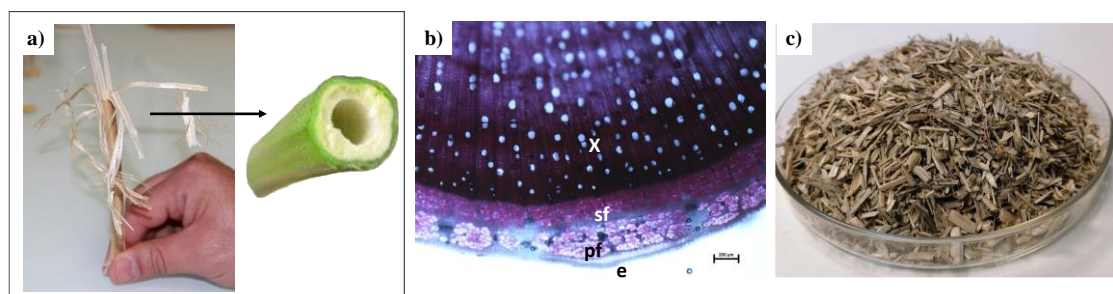
114 In this work, the location of copper adsorbed on the hemp shives was studied thanks to a
115 combination of two synchrotron techniques, namely K-edge subtraction X-ray micro-
116 tomography (KES tomography) and X-ray micro-fluorescence imaging, to better
117 understand mechanisms involved during 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 N₂. 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)**

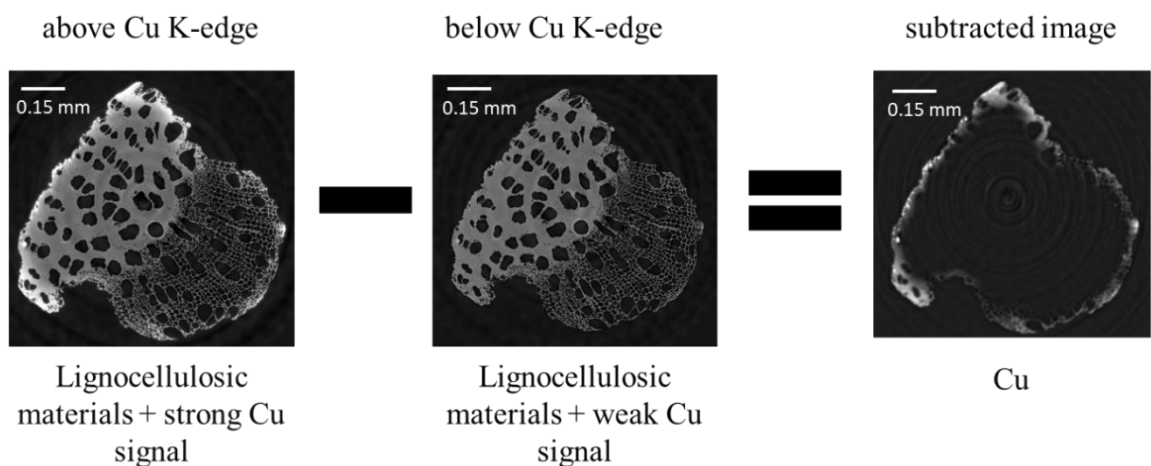
159 μ -XRF analysis was performed at the PUMA beamline of Synchrotron SOLEIL. A Si(111)
160 double crystal monochromator (DCM) was used to monochromatize the beam and a
161 Kirkpatrick-Baez (KB) mirror system focalized the X-rays on the surface of the samples in
162 a diameter of 5 μm (vertical) and 10 μm (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 μm x 5 μm 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 μm 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 mm² 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 μm) 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 μm and a field of view (FOV) of the detector of (1.3 mm)². 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 μm ; 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).



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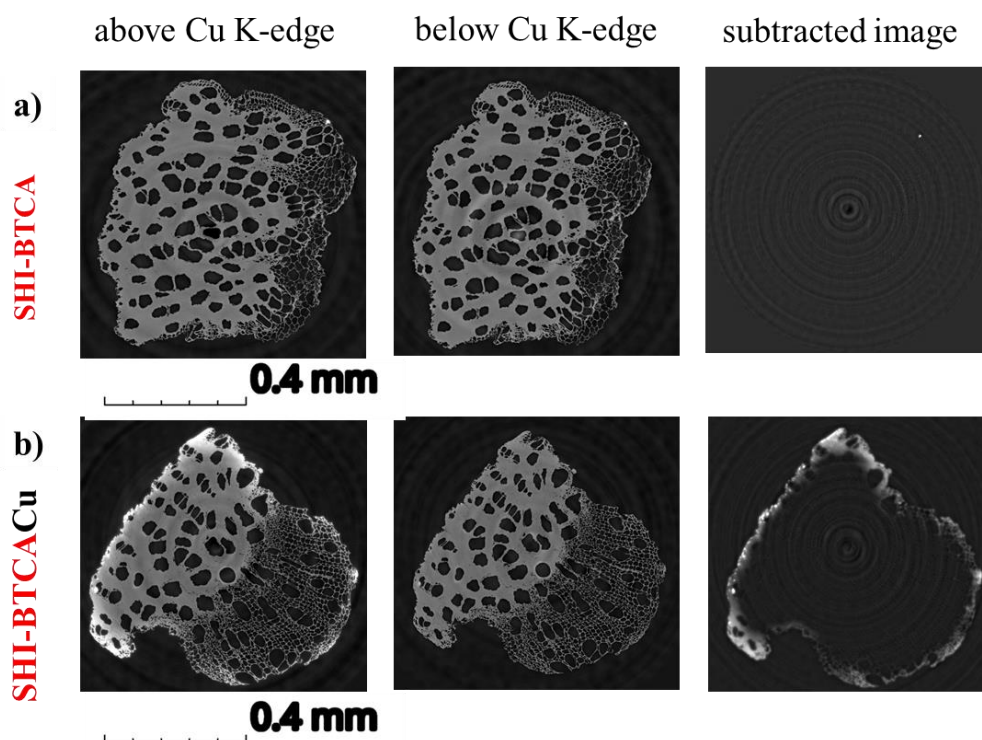
223 **Fig. 2** K-edge subtraction micro-tomography virtual cross-sections of shives taken at photon
 224 energies above (left) and below Cu K-edge (middle) and resulting image after application of the
 225 subtraction method (right).

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.

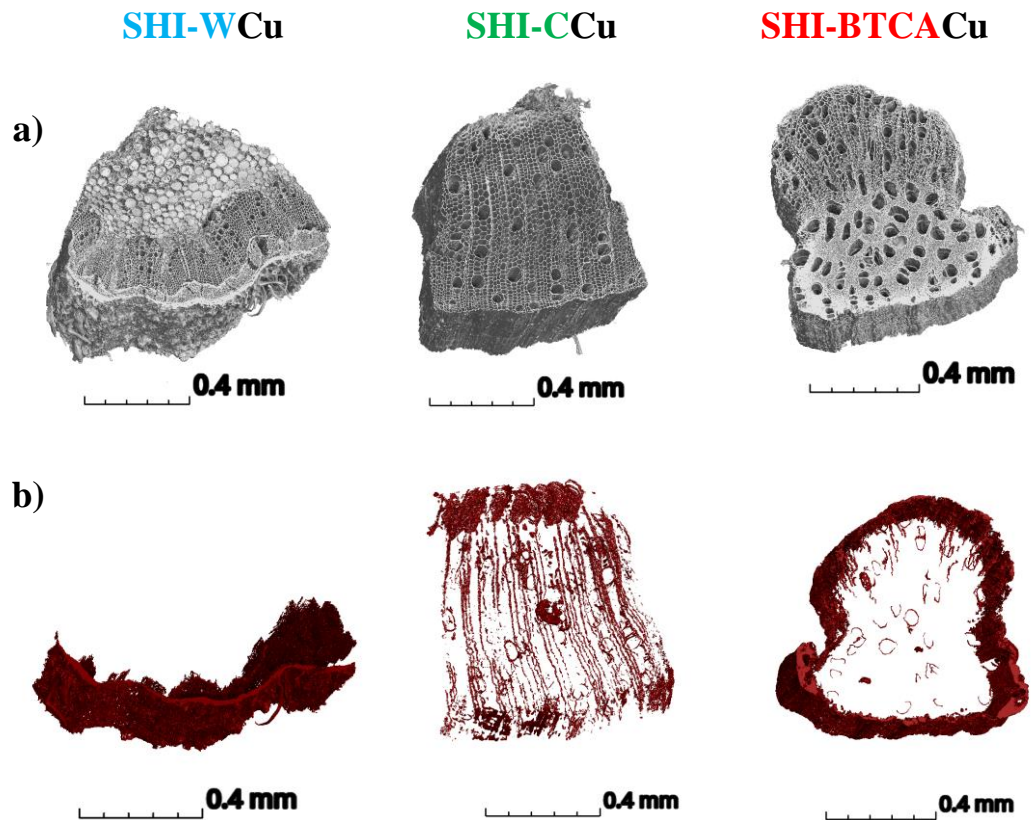


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242 **Fig. 3** Micro-tomography virtual cross-sections (slices) taken at X-ray photon energies above
 243 (left) and below the Cu K-edge (middle) and subtracted image (right) for the SHI-BTCA sample
 244 before (a) and after (b) copper adsorption.

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).

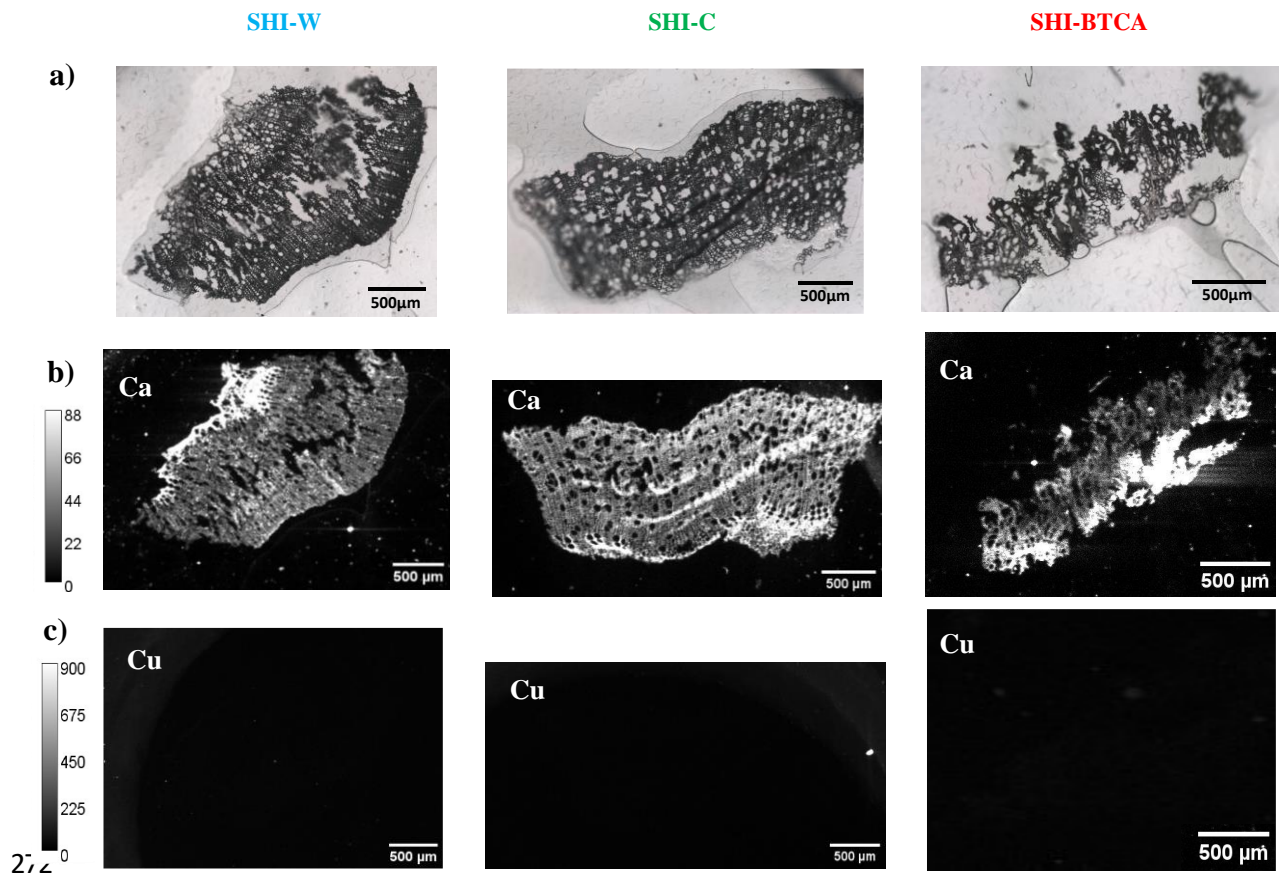
252 For SHI-CCu, the copper is distributed in all the surface of the shives due to the partial
 253 removal of the hemicelluloses during sample activation (Mongiovi et al. 2021). Indeed,
 254 the treatment with sodium carbonate induced in the cell wall some micro- and nano-
 255 porosities into which 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.



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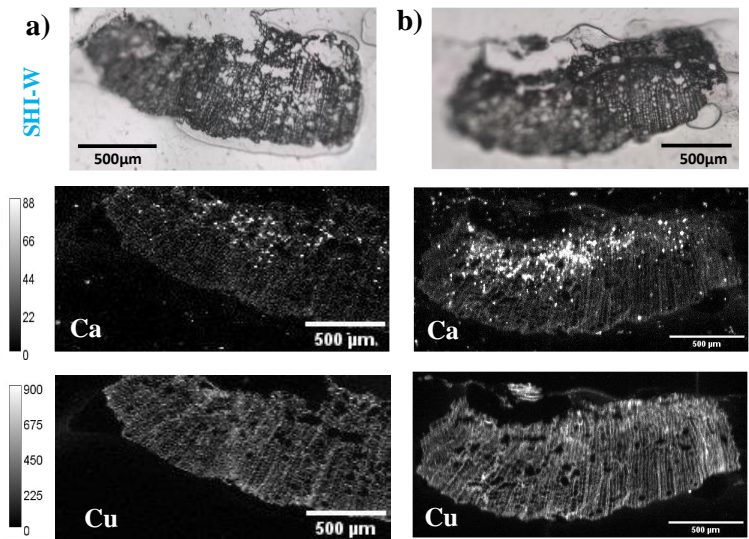
258 **Fig. 4** 3D K-edge subtraction tomography images for SHI-WCu, SHI-CCu and SHI-BTCA Cu shives
 259 after copper adsorption: a) above Cu K-edge and b) after application of the subtraction method.

260 To determine the spatial distribution of copper in hemp samples after adsorption, μ -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.



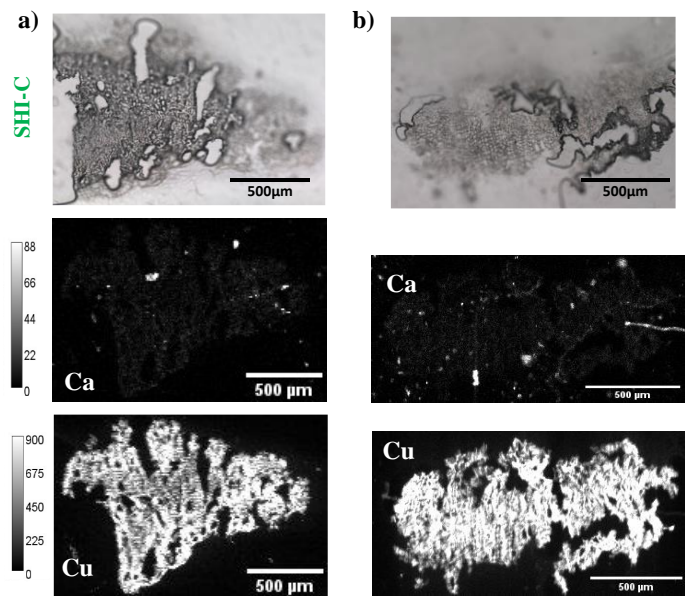
273 **Fig. 5** Comparison of transverse sections of SHI-W, SHI-C and SHI-BTCA samples before copper
 274 adsorption obtained by a) optical microscopy, b) synchrotron-based XRF mapping of Ca, and c)
 275 synchrotron-based XRF mapping of Cu. For a given element, the grey scale of the XRF images is the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



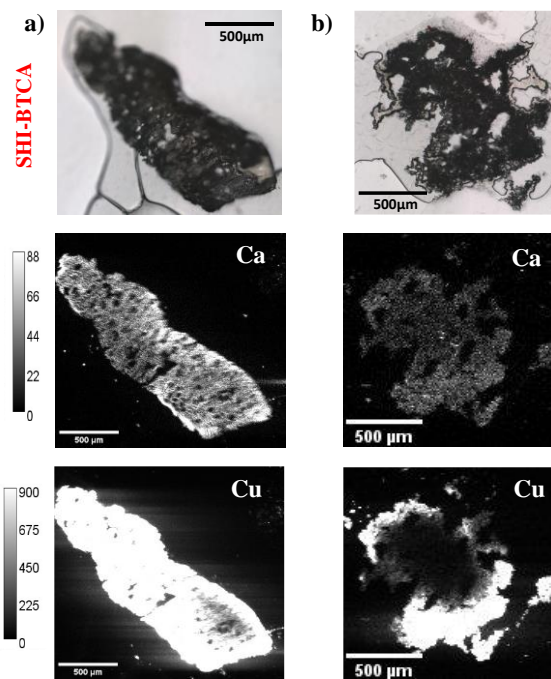
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296 **Fig. 6** Optical microscope images (top) and X-ray fluorescence maps of Ca (middle) and Cu
 297 (bottom) for two SHI-WCu transverse sections. The sections are obtained at two different depths
 298 of the same hemp shives a superficial cross-section (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

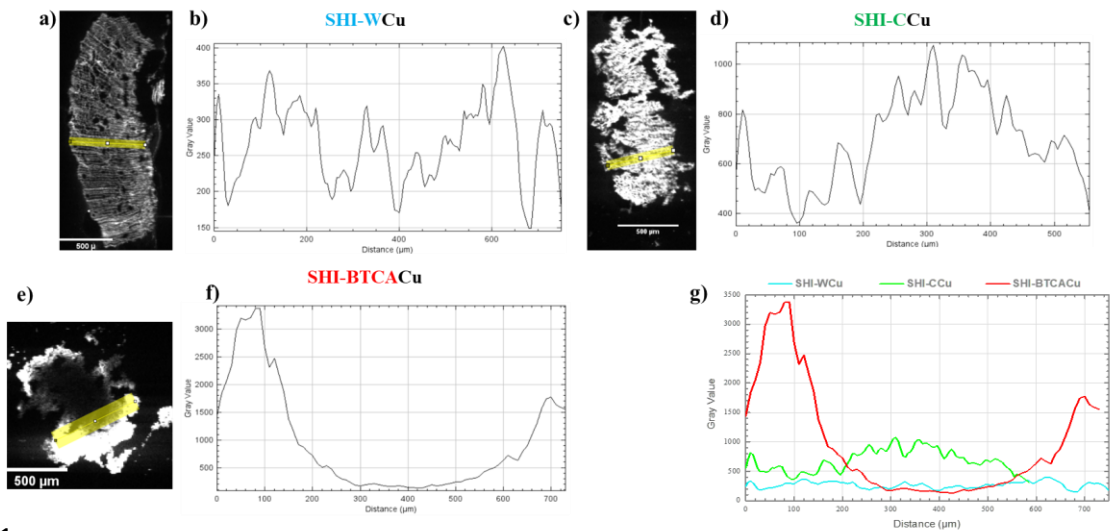
302 **Fig. 7** Optical microscope images (top), X-ray fluorescence maps of Ca (middle), and Cu (bottom)
 303 of two SHI-CCu transverse sections. The sections are obtained at two different depths of the same
 304 hemp shive, a superficial cross-section (a) and an internal cross-section (b). For a given 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-BTCA₂Cu 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.

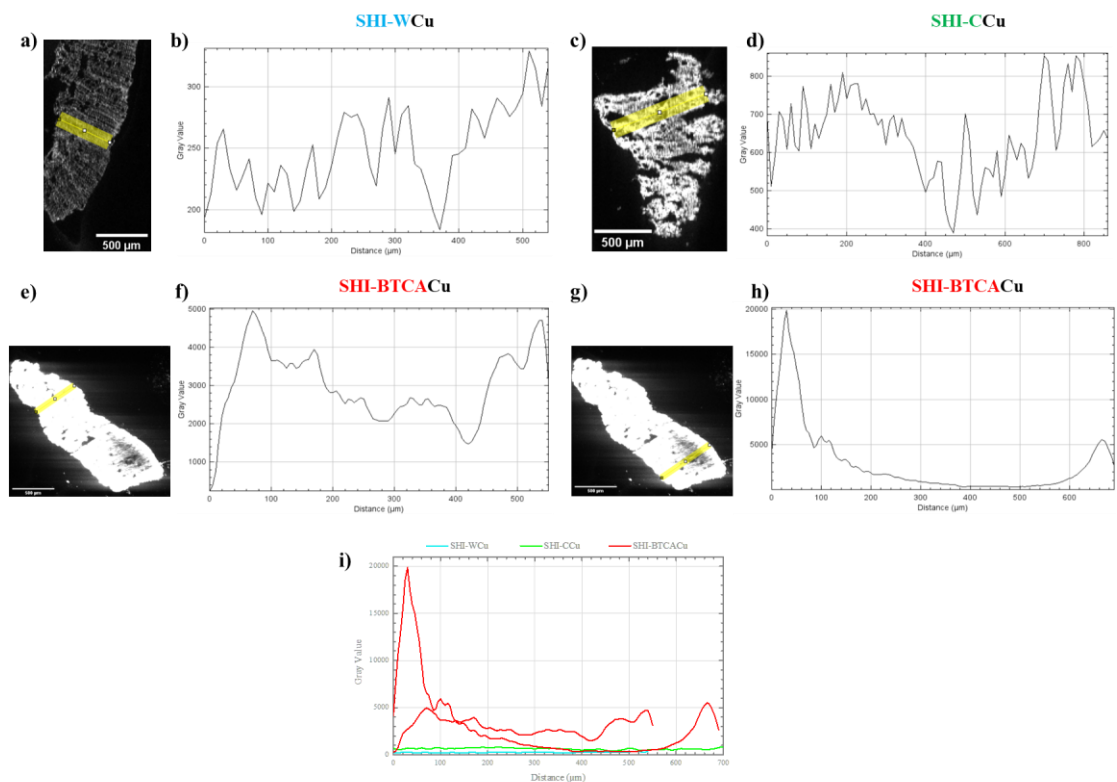
313 The data analysis made it possible to carry out a statistical study of the distribution of
 314 fluorescence intensities in the XRF maps and thus of the distribution of copper. Two types
 315 of analysis were performed: the first is the extraction of a Cu distribution profile along a
 316 15-pixel-thick 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 μ -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 μ 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-BTCA₂Cu 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 μ 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).



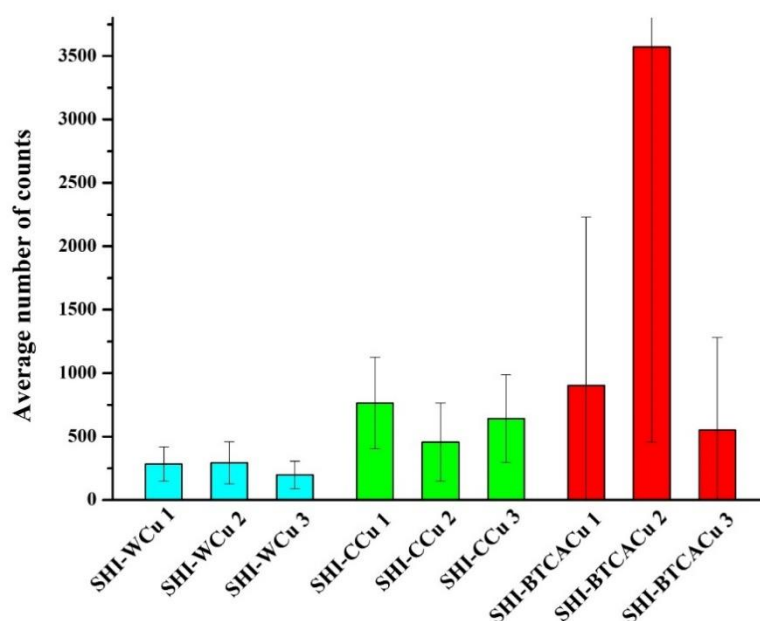
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344 **Fig. 10** XRF maps of Cu (a, c, e, g) and Cu distribution profiles (b, d, f, h) extracted from superficial
345 sections of SHI-WCu, SHI-CCu and SHI-BTCACu shives, and comparison of the profile intensity of
346 the three materials (i). The location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

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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.

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