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## Combination of 3D Fluorescence/PARAFAC and UV–Vis Absorption for the Characterization of Agricultural Soils from Morocco

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51 Such optical spectrometric characteristics should make it possible the characterization of the WEOM in these  
52 soils, and the discrimination of fluorescence signals originating from emitting organic pollutants persisting in the  
53 soil.

## 54 **Soil Sampling and Analysis**

### 55 **Soils classification and samples preparation**

56 The investigated soils are classified into four main types according to two previous studies [9, 10]:

57 - Vertisol, commonly labeled Tirs, (52%): deep and clayey soil, with a low permeability, characterized by a high  
58 water retention and considered of good productivity.

59 - Slightly evolved, commonly labeled Faid, (17%): deep soil, little to moderately permeable and characterized by  
60 a good permeability.

61 - Isohumic, commonly labeled Hamri, (16%): deep to moderately deep soil, with a sandy clay texture and  
62 characterized by a good permeability.

63 - Sandy / fersiallitic, commonly labeled R'mel, (13%): sandy in surface and coarse-textured soil, characterized  
64 by a high permeability and a low water retention capacity.

65 These soils are neutral to moderately basic [10].

66 Thirty representative soil samples were collected in the province of Sidi Bennour in non-cultivated and non-  
67 treated areas neither with pesticides nor with fertilizers. The samples were taken at soil depths ranging from 5 to  
68 20 cm, stored in plastic bags and then kept cold.

69 For each soil type, the samples were mixed and shared into several sub-samples of 20g. Avoiding the use of  
70 organic solvents, each sub-sample was mixed with 40 ml of water, as an extractor medium, under 1 hour stirring  
71 to improve the dissolution of the organic material. The extraction was made at room temperature (at about 20-  
72 24°C), protected from light. The water-soil mixture was then stored in dark bottle in the fridge for 24 to 48 h to  
73 decant; the final extracts showed pH values in the range 7.5–8.0. As no significant fluorescence intensity  
74 difference has been observed between the spectra of filtered through 0.45µm size pores and non-filtered  
75 supernatants, the measurements were recorded directly on top-supernatants without filtration. These top-  
76 supernatants were transferred into a quartz cell of 1 cm optical path for absorption and fluorescence analyses.

### 77 **UV-Vis. absorption and fluorescence measurements**

78 The absorption spectra were measured on the top-supernatants using a JASCO UV/VIS/ NIR V-570  
79 spectrophotometer; the scans covered the spectral range 200-800 nm.

80 For each type of soil, the average values of the absorbance ratios  $\frac{E_2}{E_3} = \frac{A_{250}}{A_{365}}$  and  $\frac{E_4}{E_6} = \frac{A_{465}}{A_{665}}$ , the slopes  
81  $S_{275-295}$  and  $S_{350-400}$  as well as slopes ratio  $S_R = \frac{S_{275-295}}{S_{350-400}}$  were calculated.  $A_{250}$ ,  $A_{365}$ ,  $A_{465}$  and  $A_{665}$  denote  
82 respectively the measured absorbances at 250, 365, 465 and 665 nm wavelengths. The spectral slopes over the  
83 intervals 275-295 nm and 350-400 nm were calculated using a linear regression of  $\ln(a_\lambda)$ , where  $a_\lambda$  is the  
84 absorption coefficient calculated in  $m^{-1}$  according to the equation  $a_\lambda = \frac{2.303 \times A(\lambda)}{l}$ , with  $A(\lambda)$  and  $l$  represent the  
85 measured absorbance and the quartz cell thickness (i.e. 0.01 m) [11].

86 The total fluorescence spectra, or excitation-emission matrices also called TEEMs matrices, were measured  
87 using a Shimadzu RF-5301PC spectrofluorimeter controlled by LabSolution software. Continuous fluorescence  
88 scans were collected between 250 and 700 nm, varying the excitation wavelength from 220 to 550 nm at a 5 nm  
89 step. The excitation and emission slits were set at 3 nm (exc.) / 3 nm (em.) or 5 nm (exc.) / 5 nm (em.) depending  
90 on the sample.

91 The fluorescence matrix representation adopted in this work is the 2D one where the wavelengths  $\lambda_{\text{emission}}$  and  
92  $\lambda_{\text{excitation}}$  appear explicitly on the X and Y axes, and the fluorescence intensity is coded by colors ranging from  
93 blue to red in rising order of values. The fluorescence maxima correspond to peaks, or foci, identified by their  
94 wavelengths coordinates  $\lambda_{\text{exc}}/\lambda_{\text{em}}$ . Each TEEM requires the concatenation of an average of 57 conventional  
95 fluorescence spectra (excitation from 220 to 500 nm at a 5 nm step). LabSolution package crossed with Origin  
96 data analysis and graphing software, allowed the representation and manipulation of the measured TEEMs  
97 matrices.

## 99 PARAFAC chemometric analysis

100 The PARAFAC "Parallel Factor Analysis" algorithm is a tool which consists in resolving the measured  
 101 fluorescence matrices into individual sub-matrices corresponding to the fluorophores coexisting in the samples  
 102 analyzed (supernatants). This chemometric analysis makes it possible to separate the different spectral  
 103 contributions associated with the different types of fluorophores in these samples. This means that the positions  
 104  $\lambda_{exc}/\lambda_{em}$  of the various underlying signals in a TEEM fluorescence matrix will be determined with a minimal  
 105 error; the PARAFAC algorithm is considered by several authors to be a mathematical chromatography. Water  
 106 and soil total fluorescence analysis is the analytical method where PARAFAC is being used the most [12 - 14].  
 107 The algorithm has been largely elucidated by various authors [15, 16].

### 108 PARAFAC algorithm principle

109 The algorithm consists in the decomposition of the of the experimental TEEMs data cube, measured on all  
 110 samples, into a set of trilinear and residual terms as following.

111 The fluorescence intensity of the sample  $i$  for the excitation and emission wavelength pair  $(k, j)$  is given by the  
 112 equation:

$$X_{i,j,k} = \sum_{f=1}^F a_{i,f} b_{j,f} c_{k,f} + \epsilon_{i,j,k}$$

113 with  $i = 1, 2, \dots, I$ ;  $j = 1, 2, \dots, J$  and  $k = 1, 2, \dots, K$ .  $I$ ,  $J$  and  $K$  designate respectively the numbers of samples,  
 114 emission wavelengths and excitation wavelengths.

115  $f = 1, 2, \dots, F$ .  $F$  is the number of components in the model i.e. the presumed number of the fluorophores  
 116 coexisting in the samples analyzed.

117  $X_{i,j,k}$  represents the fluorescence intensity of sample  $i$  for the pair of excitation and emission wavelengths  $(k, j)$ .

118  $a_{i,f}$  represents the fluorescence factor (the concentration times the fluorescence quantum yield); each PARAFAC  
 119 component  $f$  has  $I$  values of  $a$  (scores).

120  $b_{j,f}$  represents the intensity of the emission spectrum of the fluorophore  $f$  at the emission wavelength  $j$ .

121  $c_{k,f}$  represents the value of the absorption spectrum of the fluorophore  $f$  at the excitation wavelength  $K$   
 122 (proportional to the specific absorption coefficient).

123  $\epsilon_{i,j,k}$  is the residual representing the variability not accounted for by the model, and which the calculation  
 124 searches to minimize. This term includes instrumental noises and other non-modellable variations.

125 In this work, the number of components (fluorophores) was varied between 2 and 4. This choice of a reduced  
 126 number of components is inspired by the measured TEEMs matrices showing a single fluorescence peak, large  
 127 and extended from 370 to 550 nm. The "split-half analysis" method integrated into the algorithm was chosen  
 128 during the model validation step. The version of the PARAFAC algorithm used is the online available one [17];  
 129 the graphics program used to create the matrices is Matlab.

130 The successful decomposition of a multi-way data system using the PARAFAC algorithm relies on assumptions  
 131 about variability, tri-linearity and additivity [15, 16]. In this work, the fluorescence spectra were measured on  
 132 samples revealing absorbances at 254 nm lower than 0.5 as can be observed on figure 1; no apparent internal  
 133 filter effect was observed. The experimental matrices were corrected from all of the instrumental response, the  
 134 internal filter effect and the Raman scattering [16 - 18].

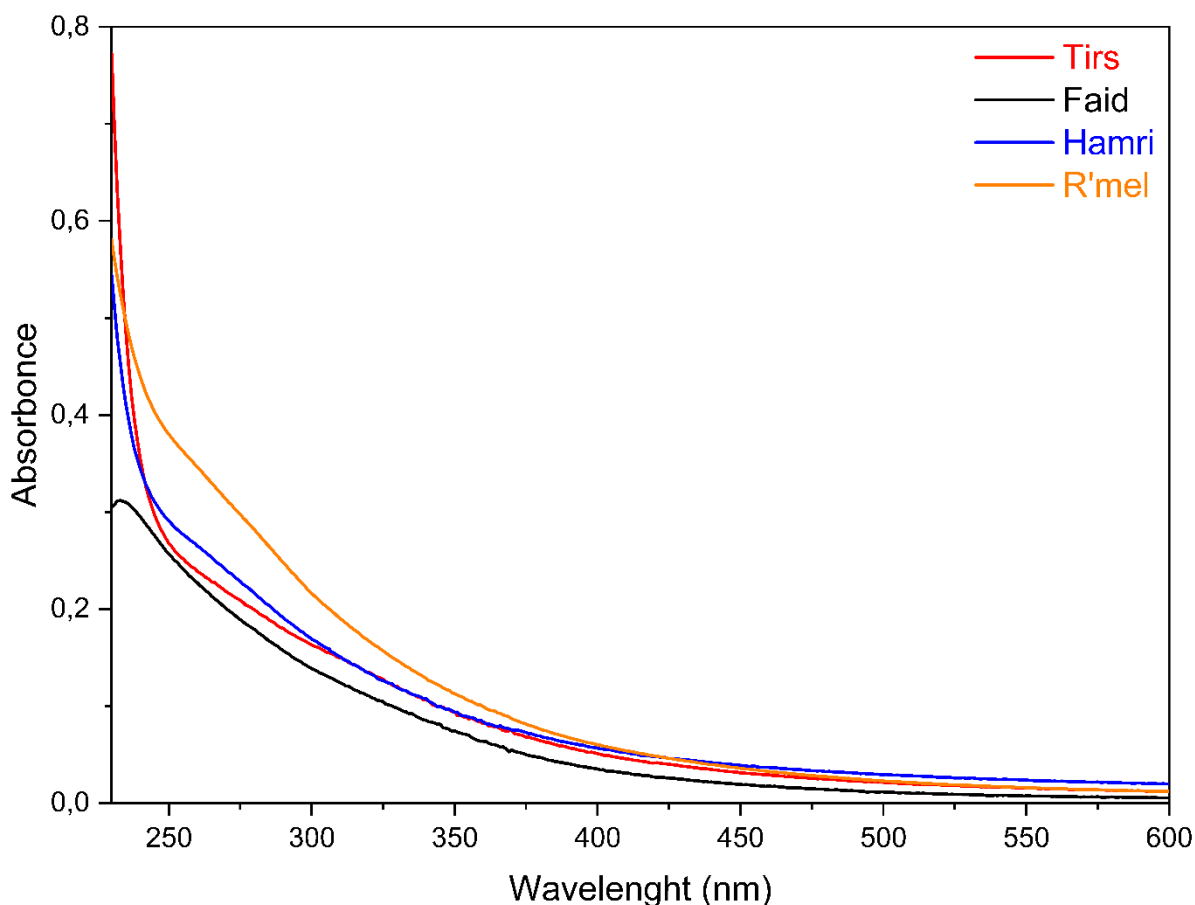
## 135 Results and discussion

### 136 UV-Visible Absorption: absorbance and slope ratios

137 In Table 1 are reported, for each type of soil, the average values of the absorbance ratios  $\frac{E_2}{E_3} = \frac{A_{250}}{A_{365}}$  and  $\frac{E_4}{E_6} =$   
 138  $\frac{A_{465}}{A_{665}}$ , the slopes  $S_{275-295}$  and  $S_{350-400}$  as well as the ratios of these slopes  $S_R = \frac{S_{275-295}}{S_{350-400}}$ .

139  $E_4/E_6$  ratio is generally related to the degree of condensation and aromaticity of the organic material in the soil  
140 [19, 20]; this ratio varies inversely with respect to the degree of condensation / aromaticity. The values found in  
141 the case of the four soil types studied, ranging between 3.26 and 4.82, suggest that these soils contain organic  
142 materials in close humification stages. The highest value, 4.82, corresponding to the soil Faïd is indicative of an  
143 organic material with an aliphatic prevalence of this soil compared to the other ones [20]. This result is also  
144 confirmed by the values obtained for  $E_2/E_3$  absorbance ratios varying from 3.77 to 4.33, similar to those  
145 reported for other soils [21].  $E_2/E_3$  ratio is associated with the average weight and molecular size of the organic  
146 material contained in the soil [22], and reflects the relative contents of humic acids (HAs) and fulvic acids (FAs).  
147 In the present case the values found for  $E_2/E_3$  ratios, all greater than 3.5, indicate organic materials with FAs  
148 contents greater than those of HAs [23, 24].

149 Concerning the slopes  $S_{275-295}$  and  $S_{350-400}$ , the average values found for all soil types range respectively in the  
150 intervals  $0.0101-0.0141 \text{ nm}^{-1}$  and  $0.0122-0.0152 \text{ nm}^{-1}$ , and are consistent with those reported for other soils  
151 [11, 25]. Moreover, the obtained  $S_R$  slope ratios, all lower than 1, are reasonably characteristic of terrigenous  
152 organic materials generally characterized by  $S_{275-295}$  lower than  $S_{350-400}$ . The difference, of  
153 about 20%, observed in  $S_R$  between Tirs and R'mel may be probably related to a significant difference in  
154 chromophoric DOM (CDOM) in these soils which are respectively the darker and the lighter ones. Furthermore,  
155 as slopes and slope ratios are commonly related to the average molecular weight of the organic matter, the  
156 similarity of these ratios reveals that the four soil kinds contain organic materials with similar molecular weights  
157 [11]. Subsequently, these soils are very likely in similar humification levels as indicated before by  $E_2/E_3$  and  
158  $E_4/E_6$  absorbance ratios.



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**Fig. 1** UV-Vis. Absorption spectra measured on top-supernatants of water-soil mixtures.

165 **Table 1** Averaged spectroscopic parameters calculated from absorption and fluorescence spectra\*

Sol	Tirs	Faid	Hamri	R'mel
$E_2/E_3$	$3.85 \pm 0.05$	$4.33 \pm 0.18$	$3.88 \pm 0.03$	$3.77 \pm 0.09$
$E_4/E_6$	$3.51 \pm 0.11$	$4.82 \pm 0.54$	$3.26 \pm 0.24$	$3.37 \pm 0.18$
$S_{275-295}(\text{nm}^{-1})$	$0.0101 \pm 0.0011$	$0.0141 \pm 0.0009$	$0.0124 \pm 0.0001$	$0.0126 \pm 0.0001$
$S_{350-400}(\text{nm}^{-1})$	$0.0122 \pm 0.0005$	$0.0152 \pm 0.0009$	$0.0133 \pm 0.0001$	$0.0128 \pm 0.0002$
$S_R$	$0.8279 \pm 0.0450$	$0.9276 \pm 0.0047$	$0.9323 \pm 0.0007$	$0.9844 \pm 0.0331$
FI	$1.56 \pm 0.01$	$1.52 \pm 0.03$	$1.61 \pm 0.01$	$1.68 \pm 0.04$
HIX	$9.05 \pm 0.77$	$9.26 \pm 0.66$	$12.25 \pm 0.83$	$11.8 \pm 0.60$

166

167 \* More details on the shown parameters are in the text.

168 **Fluorescence: FI and HIX indices**

169 The fluorescence index  $FI = \frac{I_{fl}(\lambda_{em}=450 \text{ nm})}{I_{fl}(\lambda_{em}=500 \text{ nm})}$ , calculated as the fluorescence intensities ratio at the excitation  
 170 wavelength  $\lambda_{exc.} = 370 \text{ nm}$ , provides information on the origin of the soil organic material; it highlights in  
 171 particular the relative importance of the terrigenous material with respect to the microbial biomass one [26].

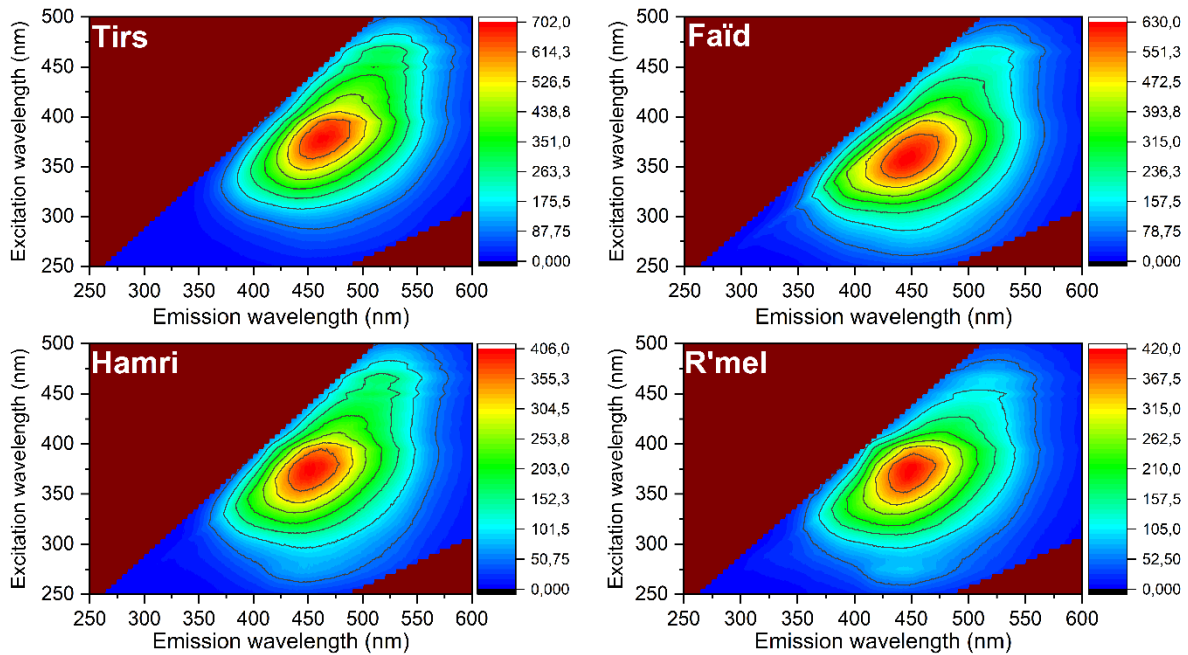
172 An FI index above 1.8 corresponds to a material with a microbial precursor, while an FI index close to 1.2 is  
 173 indicative of a material with a rather terrigenous precursor [27]. In the case of the investigated soils, the FI  
 174 indices range in the interval 1.52-1.68. This order of magnitude indicates organic materials from terrigenous  
 175 sources with a certain microbial biological component. A similar result was observed in soils from China [28].

176 The humification index HIX calculated as the ratio of fluorescence intensity areas  $HIX = \frac{\sum_{\lambda_{em}=435}^{\lambda_{em}=480} I_{fl}}{\sum_{\lambda_{em}=300}^{\lambda_{em}=345} I_{fl}}$ , while  
 177 excitation wavelength is set at 254 nm, is a parameter positively correlated with the humification / maturation  
 178 degree of the soil organic matter [26]. It is often taken as an indicator of DOM variation in agricultural soils [29].  
 179 In the present case, the HIX indices vary from 9 to 12. In comparison with the reference intervals given by  
 180 various authors [30, 31] the investigated soils can be considered as materials with an important humic character  
 181 as a consequence of an advanced maturation stage.

182 **Total fluorescence and PARAFAC chemometrics**

183 **Experimental TEEMs**

184 All soil extracts in water, prepared as described above, were subjected to total fluorescence measurements. In  
 185 Figure 2 are presented four examples of the collected raw experimental TEEMs matrices. Likewise, these typical  
 186 matrices show a single large fluorescence peak extended, in emission, from 370 to 550 nm. This signal is  
 187 attributable to humic substances in the soil organic matter [32, 33]. However, no signal attributable to any  
 188 protein fraction has been observed.



**Fig. 2** Examples of experimental total excitation emission matrices (TEEMs).

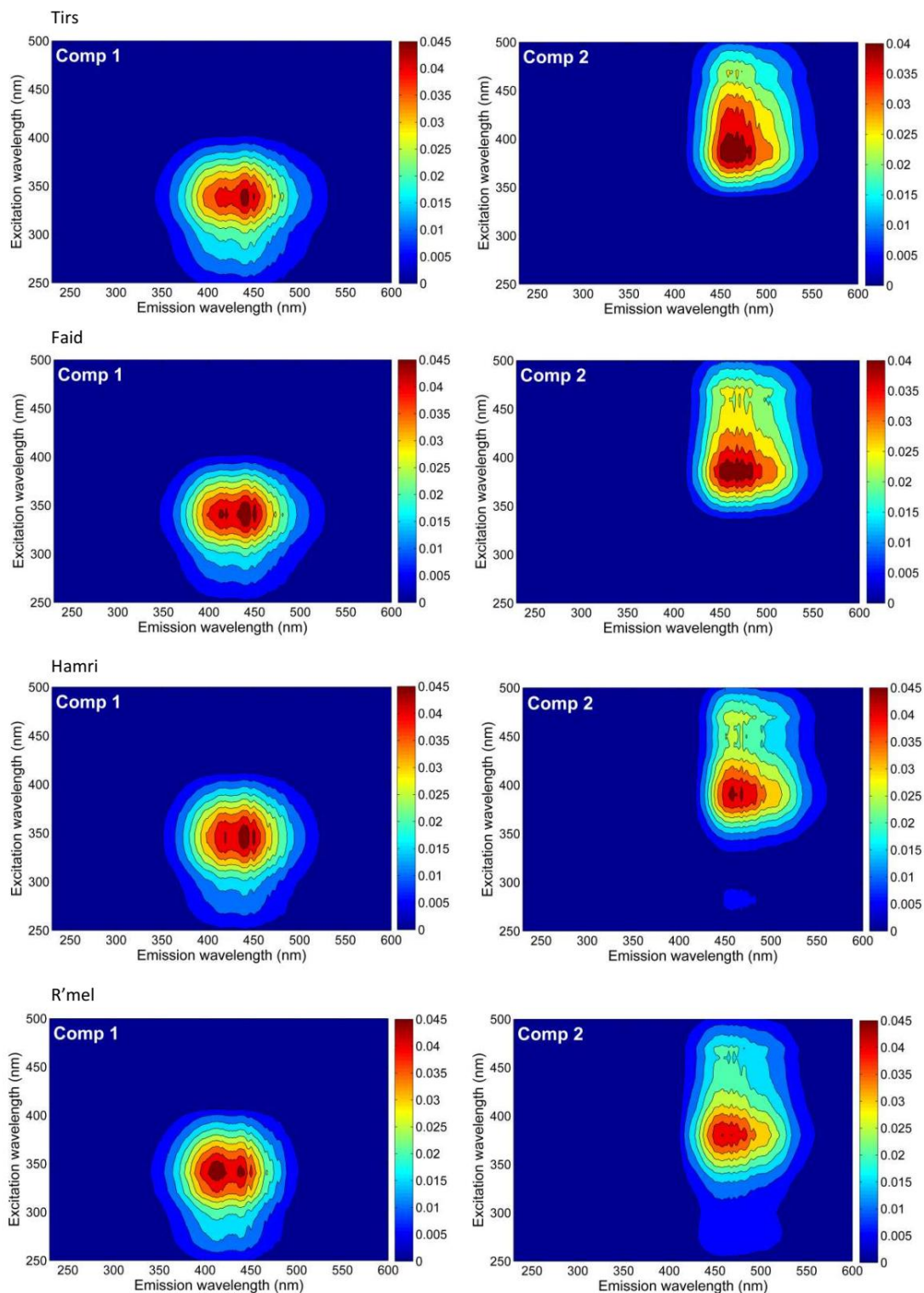
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### 191 PARAFAC Analysis

192 The PARAFAC chemometric analysis has made it possible to discriminate the fluorescent components  
 193 previously overlapping each other in the experimental TEEMs matrices; the procedure is described above. For  
 194 all soils, the validated model corresponds to a number of components equal to two,  $C_1$  and  $C_2$ . This model is  
 195 entirely consistent with the experimental TEEMs that did not reveal any fluorescence signal outside the spectral  
 196 emission range 370-550 nm. Figure 3 shows the individual matrices corresponding to the PARAFAC  
 197 components obtained for the four soil types. In all cases the component  $C_1$  appearing around 340exc./442em.  
 198 corresponds to the fulvic acid (FAs) fraction while the component  $C_2$  appearing around 385exc./465em.  
 199 corresponds to the humic acid (HAs) one of the soils WEOM. Indeed, these fractions are generally observed in  
 200 the respective intervals (320-350 nm) exc./ (400-450 nm) em. and (370-390 nm) exc./ (460-500 nm) em. [34,  
 201 35].

202 In all soils, the ordering of FAs fraction as component  $C_1$  and HAs fraction as component  $C_2$  is entirely consistent  
 203 with the obtained values of E2/E3 absorbance ratios which are greater than 3.5 indicating an organic material  
 204 with FAs prevalence. Hence, in the whole of the investigated soils, these findings reflect a dominance of the FAs  
 205 fraction with respect to the HAs one [36]. Furthermore, the absence of any PARAFAC component compatible  
 206 with a protein fraction, such as tyrosine or tryptophan, reveals highly humified organic materials as previously  
 207 highlighted by the HIX humification indices ranging between 9 and 12. The absence of any protein-type  
 208 fluorescence signal indicates the absence or, at least, the weakness of proteins microbiological activity in the soil  
 209 lands investigated; this is a consequence of the revealed advanced stage of humification. Probably, in the present  
 210 soils, the formation of humic substances may be governed by the lignin mechanism putting forward the  
 211 hypothesis of the transformation of HAs into FAs [37]. Soil humification means the process leading to the  
 212 increasing of the humic material content (namely FAs and HAs); an advanced humification stage may be  
 213 associated with an increase in FAs concentration with a decrease in HAs concentration [38]. The formation of  
 214 humic substances (HAs, FAs and Humins) is one of the least understood aspects of humus chemistry and one of  
 215 the most intriguing.



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**Fig. 3** Individual total excitation emission matrices of PARAFAC components. Comp1 and Comp2 designate respectively C1 and C2 components.

219

## 220 Conclusion

221 The present study focused on the WEOM in a set of thirty agricultural soil samples collected from the zone of  
 222 Doukkala in Morocco. The set has covered the four types of agricultural soil (Tirs, Faid, Hamri and R'mel),  
 223 while the analysis was performed on the soils extracts in water by means of UV-visible absorption and total  
 224 fluorescence combined to PARAFAC chemometrics.



225 E<sub>4</sub>/E<sub>6</sub> and E<sub>2</sub>/E<sub>3</sub> absorbance ratios, ranging respectively in the intervals 3.26 - 4.82 and 3.77- 4.33, revealed that  
226 all soils contain organic materials in similar humification stages, with a predominance of FAs fractions with  
227 respect to HAs ones. The average spectral slopes S<sub>275-295</sub> and S<sub>350-400</sub> varying respectively in the intervals  
228 0.0101-0.0141 nm<sup>-1</sup> and 0.0122 - 0.0152 nm<sup>-1</sup>, with slope ratios S<sub>R</sub> lower than 1, are characteristic of terrigenous  
229 organic materials. In addition, the FI fluorescence indices found between 1.52 and 1.68 revealed organic  
230 materials from terrigenous sources, having some microbial biological component. In parallel, HIX humification  
231 indices ranging from 9 to 12 indicated that the soils are in advanced humification stages.

232 3D fluorescence did not detect any protein fraction in the soils WEOM studied, highlighting thus poor fresh  
233 organic matter soils. The PARAFAC chemometric analysis, in full agreement with experiments, revealed only  
234 two fluorescent components in the humic material with FAs fraction classified as component 1 while HAs one  
235 classified as component 2.

236 Consequently, the four types of agricultural soils are compartments in similar humification stages, containing  
237 organic materials dominated by FAs fractions and very poorly provided in fresh organic matter.

238 Considering the issue of pesticide remains, the accomplished 3D fluorescence/PARAFAC characterization of the  
239 WEOM in the investigated soils will make it possible to discriminate additional fluorescence signals originating  
240 from any fluorescent contaminants persisting in the soil.

241 **Authors' contributions.** Hassan Ba-Haddaou: (Experimental investigations, first draft preparation and  
242 submission). Hicham Hassoun: (Sampling, material preparation and experimental investigations). Salim Foudeil:  
243 (Data collection, first draft preparation and submission). Saadia Ait Lyazidi: (Supervision, methodology, final  
244 draft reviewing and project administration). Mustapha Haddad: (Discussion, validation and funding acquisition).  
245 Matthieu Masson: (Discussion and validation). Marina Coquery: (Discussion and validation). Christelle  
246 Margoum: (Experimental logistics, discussion and validation).

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250 **Data Availability** Not applicable.

251 **Declarations**

252 **Conflicts of interest/Competing interests** The authors declare they have no conflicts of interest and no  
253 competing interests.

254 **Ethics Approval** Not applicable as the study does not include any use of animals and humans.

255 **Consent to Participate** All authors consent to participate in the research.

256 **Consent for Publication** All authors consent to participate in the publication of the research.

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