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1 **Enrofloxacin and copper plant uptake by *Phragmites australis***
2 **from a liquid digestate: single *versus* combined application**

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17

18 **Abstract**

19 Application of anaerobically digested sludges and manure (digestates) to agricultural
20 fields reduces the need for fertilizer application, but might also pose environmental risks due
21 to the introduction of various pollutants –including metal trace elements and pharmaceuticals–
22 to amended soils. The simultaneous presence of different contaminants can affect plant
23 uptake, altering the performance of phytoremediation. This work aims to investigate and

24 compare the single and combined effects of Enrofloxacin (Enro) and Cu on their plant uptake
25 from digestate solutions. Plant uptake experiments were carried out by exposing, for 5 days,
26 *Phragmites australis* to three different concentrations of a liquid digestate doped with Cu
27 and/or Enro. Contaminant concentrations were afterward determined in both plant tissues and
28 digestate solutions. Contaminant speciation in solution and Cu speciation in plant roots were
29 also assessed. Cu plant uptake (mostly in plant roots, Cu concentration varied between 55 and
30 254 $\mu\text{g.g}^{-1}$) was observed, especially at low concentration of digestate. At high digestate
31 concentration, Cu uptake decreased due to the formation of Cu-DOC complexes rendering Cu
32 less bioavailable. The presence of the antibiotic slightly further reduced Cu plant uptake due
33 to the formation of Cu-Enro complexes. Plant roots also accumulated Enro, an accumulation
34 also higher for the lowest concentration of digestate (an increase of 40%), and that slightly
35 increased in the presence of Cu (up to 226 $\mu\text{g.g}^{-1}$). However, proportion of free Enro in
36 solution decreased (up to 70%) in the presence of Cu due to complexation. Cu speciation in
37 plant roots confirmed the implication of the complexation in the uptake of Cu and Enro when
38 they are simultaneously present in solution. Thus, the presence of amendments (such as
39 digestates) increasing the DOC content, in addition to the interactions between contaminants,
40 should be taken into account for field crop soils as well as for phytoremediation technologies.

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42

43 **Keywords:** plant uptake; metal; anaerobic digestion; biosolid; antibiotic; complex

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48 **1. Introduction**

49 Sludges generated during treatment processes in wastewater treatment plants
50 (WWTPs), as well as manure and wastewater resulting from animal excrements in farms, may
51 contain a plethora of organic and inorganic substances such as metal trace elements and
52 pharmaceuticals (Risberg et al., 2017; Verlicchi and Zambello, 2015). As they are rich in
53 nutrients and organic matter, their application to agricultural fields is commonly used as a soil
54 amendment (Frost and Ketchum Jr., 2000; Sabourin et al., 2012; Verlicchi and Zambello,
55 2015). However, this practice may also pose environmental risks by introducing various
56 pollutants -including metal trace elements and pharmaceuticals- to amended soils (Risberg et
57 al., 2017; Verlicchi and Zambello, 2015).

58 In most cases sludges and manure must be first stabilized to decrease their risk of
59 contaminant introduction in the environment, and anaerobic digestion is one of the most
60 widely used stabilization technologies, producing methane as biogas, which can be used as an
61 energy source. Thus, there is growing interest devoted to optimize anaerobic treatment to deal
62 with the high cost of sludge and animal waste management while offering alternative energy
63 sources (Braguglia et al., 2015; Neumann et al., 2016). However, there is a lack of
64 information about these treated sludges and manure by anaerobic digestion (digestates), their
65 potential environmental impacts or benefits connected to agronomic reuse or disposal, and the
66 behavior of contaminants in these digestates.

67 Among pharmaceuticals, fluoroquinolones are antibiotics frequently detected in
68 animal manure and sludges from WWTPs all around the world, and their disposal lead to
69 increasing concern due to their occurrence in soils and waters, their accumulation in different
70 plants, their ecotoxicity and the spread of antibiotic resistant genes and bacteria in the

71 environment (Kemper, 2008; Pico and Andreu, 2007; Prosser and Sibley, 2015; Wu et al.,
72 2015). Enrofloxacin (Enro) is a fluoroquinolone widely used in veterinary medicine,
73 worldwide detected in different environmental matrices. This antibiotic, as well as its main
74 metabolite ciprofloxacin can accumulate in plants (Carvalho et al., 2012; Leal et al., 2012; Li
75 et al., 2016; Migliore et al., 2003; Pan and Chu, 2017; Verlicchi and Zambello, 2015; Ho et al.
76 2014). It is known to interact with metals such as Cu which is become ubiquitous and present
77 in wastewater and soils due to industrial and agricultural practices as fungicides, fertilizers,
78 manure and sludge application (Ali et al., 2002; Wang et al., 2017). The formation of metal-
79 Enro complexes (Ftouni et al., 2012; Saraiva et al., 2010) modifies the speciation of both
80 contaminants and thus may greatly affect their behavior in the environment. For example, an
81 increased bioaccumulation and enhanced toxicity of Cd to earthworms were evidenced in the
82 presence of Enro (Li et al., 2016), and the presence of Cu modified the bioconcentration of
83 Enro in zebrafish (Zhao et al., 2018). Moreover, the sorption properties of Cu and Enro were
84 modified in the presence of each other due to the formation of a Cu-Enro ternary complex at
85 the soil surface (Graouer-Bacart et al., 2013 and 2015). An influence of the simultaneous
86 presence of Cu and Enro on their respective plant uptake can therefore be expected. Only few
87 studies deal with the simultaneous presence of inorganic and organic contaminants on their
88 plant uptake. For example, the herbicides 2,4-D and MCPA decreased the uptake of Cu by
89 wheat stems, due to the formation of complexes (Kobylecka et al. 2008). Degryse et al. (2006)
90 observed an increase of metal uptake by plants when aqueous metal-ligand complexes were
91 present, although it depended on the ligand employed. On the contrary, the presence of copper
92 reduced the accumulation of sulfadiazine in wheat seedlings (Xu et al., 2017). Also, Chen et
93 al. (2004) evidenced that the presence of 2,4-dichlorophenol applied to soil planted with
94 ryegrass increased the water soluble amounts of Cu and Zn. They underlined the importance
95 to consider, in further studies, the behavior of metals when simultaneously present with

96 organic contaminants. Studying the combined effect of pharmaceuticals and metals on plant
97 uptake appears therefore as a paramount concern, and to our knowledge there is no
98 information concerning the combined effect of Cu and Enro on plant uptake, which is the
99 topic of the present study.

100 *Phragmites australis* (common reed), a wetland macrophyte of broad geographical
101 distribution is known to highly accumulate Cu, being also widely used in constructed
102 wetlands (Ali et al., 2002; Ye et al., 2003). Moreover, the potential of *Phragmites australis*
103 for the removal of Enro from wastewater was demonstrated through plant uptake and/or
104 degradation (Carvalho et al., 2012).

105 In this study, *Phragmites australis* was selected due to its tolerance and high
106 accumulation of both Cu and Enro (i) as plant model for studying the influence of the
107 presence of both contaminants on their respective uptake and (ii) as phytoremediation plant to
108 assess if the simultaneous presence of both contaminants (as it is the case in the real
109 environment where contaminants are present in cocktail) could affect their respective removal
110 and alter the performance of phytoremediation, given that they are known to interact together
111 by complex formation. This study was carried out in hydroponic conditions since solutions
112 are more homogeneous and chemically better-defined than soils. The single and combined
113 effects of Enro and Cu on their plant uptake from digestate solutions were investigated and
114 compared to study (i) the influence of the simultaneous presence of both contaminants and (ii)
115 the influence of the digestate concentration. Experiments at molecular scale using X-ray
116 Absorption Near Edge Structure (XANES) were performed to precise the role of the
117 complexation in plant uptake of both contaminants. The results obtained will enable a better
118 knowledge of pharmaceuticals and metals ecodynamic in field crop soils and
119 phytoremediation technologies taking into account their interaction.

120

121 **2. Materials and methods**

122 *2.1. Materials and reagents*

123 All chemicals were of the highest available purity and used without further
124 purification. Enrofloxacin (Fig. S.1) as analytical solid standard (minimum purity 90 %) was
125 purchased from Sigma-Aldrich – USA. Copper (II) chloride dehydrate with minimum purity
126 of 99 % was purchased from Merck, Darmstadt – Germany. Hydrochloric acid ($\geq 37\%$), nitric
127 acid ($\geq 65\%$) and hydrogen peroxide solutions (34.5-36.5%) were purchased from Sigma-
128 Aldrich – USA.

129 A digestate obtained from a French farm (Champagne-Ardenne, France) was hygienized at 70
130 °C during 1h. It was centrifuged at 2500 r.p.m during 10 min to separate the liquid fraction of
131 the digestate for plant uptake experiments. For plant uptake experiments, three concentrations
132 of the liquid part of the digestate were considered after dilution in deionized water
133 (conductivity $< 0.1 \mu\text{S cm}^{-1}$) of the supernatant by a factor of 20, 50, and 100 (named d20,
134 d50, and d100, respectively). These three tested digestate solutions were characterized for pH
135 and Dissolved Organic Carbon (DOC) contents according to French and International
136 standard methods, following NF EN ISO 10523 and NF EN 1484, respectively (Table S1)
137 (AFNOR, NF EN ISO 10523, 2012; AFNOR, NF EN 1484, 1997).

138 *Phragmites australis* was collected in the Lima river estuary in Viana do Castelo located
139 about 80 km north of Porto (Portugal) in October 2016 with sediment attach to its
140 underground tissues. The sediments were manually removed from the roots at site and plant
141 roots were washed with local water. At the laboratory all plants were washed with deionized
142 water.

143 *2.2. Plant uptake experiments*

144 Plant uptake experiments were carried out in hydroponic solutions for each liquid
145 digestate concentration (d20, d50, and d100), and three systems were assembled: (i) plants in
146 presence of added Cu, (ii) plants in presence of added Enro, and (iii) plants in presence of Cu
147 and Enro simultaneously added. Plants of similar size were selected for each system (three
148 plants by flask), and placed in vessels (glass flasks) containing the liquid digestate spiked with
149 one contaminant (Cu or Enro) or both of them. The tested concentrations were 1.6×10^{-5}
150 mol.L^{-1} (5.7 mg.L^{-1}) and $1.5 \times 10^{-4} \text{ mol.L}^{-1}$ (9.5 mg.L^{-1}) for Enro and Cu, respectively. Vessels
151 were wrapped in aluminium foil to prevent the exposure of roots and digestate solutions to
152 light and to avoid Enro photodegradation. Flasks were kept in an open indoor environment,
153 subject to indoor environmental temperature variations (19-22°C) and natural day/night light
154 exposure for 5 days. Each system was prepared in triplicate, and control samples without
155 plants were also considered for each digestate concentration and each system (Enro and/or
156 Cu) to check the absence of degradation or retention onto vessels during experiments.
157 At the end of experiments, plants were removed from the flasks and roots and rhizomes were
158 washed with deionized water. The plants were then left to dry at room temperature. Roots and
159 rhizomes, as well as stems and leaves were pooled together for each replicate for further
160 analysis of contaminants in belowground and aboveground tissues, respectively. The digestate
161 solutions and plant tissues were kept at -20°C before analysis for Cu, Enro and ciprofloxacin
162 (Cipro, main metabolite of Enro) analysis.

163 *2.3. Copper analysis in digestate samples and in plant tissues*

164 The Cu amounts taken up by the plants in the lower (roots and rhizomes) and upper
165 parts (stems and leaves), as well as the contents of Cu remaining in the digestate, were
166 measured using atomic absorption spectroscopy (AAS) (Perkin Elmer, AAnalyst 200) after a

167 high pressure digestion in a microwave apparatus (ETHOS 1, Milestone Inc. (Shelton, CT,
168 USA)) with concentrated nitric acid and hydrogen peroxide.

169 To quantify the Cu in the different parts of the plants, 0.5 g of different parts of the
170 plant were weighed into a microwave Teflon vial and 1 mL of nitric acid and 5 mL of
171 hydrogen peroxide were added. For digestate samples, the same procedure was used being 2
172 mL of each sample put in microwave Teflon vessels.

173 The total running time for the microwave digestion was 25 minutes: 5 minutes at 250
174 W, 5 min at 400 W and 5 min at 500 W and 10 min at 0 W. After that, vessels were left to
175 cold down at room temperature and samples were transferred for tubes with 15 mL of
176 deionized water, which were kept refrigerated (at 4 °C) until AAS analysis, following a
177 procedure validated before in the laboratory (Almeida et al., 2004). Quantification was carried
178 out by external calibration with aqueous Cu standards.

179 *2.4. Enrofloxacin and ciprofloxacin analysis in plant tissues*

180 The extraction method was adapted from the article of Gago-Ferrero et al. (2015).
181 Briefly, ground plant tissues were ultrasonic extracted with a methanol/MilliQ water solution.

182 After addition of 100 µL of dimethyl sulfoxide, the extract was evaporated at 50°C.
183 Afterwards, 900 µL of MilliQ water were added in the tube which was then vortexed for 1
184 min and centrifuged for 10 min at 13540 g. Finally, the supernatant was transferred to a liquid
185 chromatography (LC) vial and analyzed by LC coupled to tandem mass spectrometry (LC-
186 MS/MS). Each sample was extracted twice and the result was the mean of the two extractions.
187 Details are provided in Supplementary material.

188 *2.5. Enrofloxacin and ciprofloxacin analysis in digestate samples*

189 To quantify Enro and Cipro concentrations in the digestate samples, the samples were
190 previously pre-concentrated using solid-phase extraction (SPE) and analyzed by High

191 Performance Liquid Chromatography (HPLC) adapting a methodology previously optimized
192 for determination of Enro in wastewaters (Cavenati et al., 2012). Details are provided in
193 Supplementary material.

194

195

196 2.6. Statistical analyses

197 Statistical analyses were performed using Origin[®] 2017 (OriginLab Corporation) by
198 performing one-way analysis of variance (ANOVA) to test the difference significance among
199 the results according to the Turkey test at significance level 5% ($P < 0.05$). Values are reported
200 as mean \pm standard deviation (SD) of three replications. Bars with different letters were
201 significantly different at $P < 0.05$. The experimental factors considered were the concentrations
202 in solution and the amounts taken up by *Phragmites australis* for each contaminant under the
203 different tested conditions (single presence, in presence of the other contaminant, and
204 different digestate concentrations).

205

206 2.7. Speciation of both contaminants in solution

207 The concentrations of Cu and Enro when singly or simultaneously present were
208 calculated using the computer program HySS (Alderighi et al., 1999) enabling the plotting of
209 the speciation diagrams for Cu(II), Enro and for the Cu(II)-Enro system, using the protonation
210 constants of Enro and the stability constants of Cu(II)-Enro complexes previously calculated
211 (Saraiva et al., 2010; Graouer-Bacart et al., 2013). Given the pH range of the studied digestate
212 solutions (between 8.0 and 8.4), the possible formation of Cu hydroxylated species was
213 considered using the corresponding constants of Cu(OH)^+ ($\log \beta = -7.9$), Cu(OH)_2^0 ($\log \beta = -$
214 16.8), Cu(OH)_3^- ($\log \beta = -27.5$), Cu(OH)_4^{2-} ($\log \beta = -40.4$), $\text{Cu}_2(\text{OH})^{3+}$ ($\log \beta = -5.8$),

215 $\text{Cu}_2(\text{OH})_2^{2+}$ ($\log \beta = -11.0$), $\text{Cu}_3(\text{OH})_4^{2+}$ ($\log \beta = -22.5$), and $\text{Cu}(\text{OH})_{2(s)}$ ($\log K_{sp} = 9.04$) from
216 the constant database IUPAC.

217 The interactions between Cu(II) and DOC were evaluated using simple organic
218 molecules as DOC models. Dissolved organic matter is constituted by about 50% of humic
219 substances and Cu is bound to humic substances via two kinds of binding site: (i) low-affinity
220 type sites or carboxylic-type sites and (ii) high-affinity type sites or phenolic-type sites (Croué
221 et al., 2003). Thus, we selected salicylic acid (Sac) as organic molecule, which is often
222 considered and used as a simple model of DOC (Cabaniss and Shuman, 1988; Cathalifaud et
223 al., 1997; Hapeman and al., 1998), and possesses the two types of site (carboxylic and
224 phenolic). In addition, we selected glycine (Gly) since Croué et al. (2003) underlined the
225 importance of nitrogen-type sites (amino similar to those of amino acids) in the metal binding
226 by DOC. The proportion of these two selected DOC mimics was guided by the elemental
227 composition of the humic substance model proposed by Schulten (1999) constituted by 305
228 Carbon for 16 Nitrogen. Thus, to respect this C/N ratio, we considered that 90% of DOC was
229 provided by Sac, and 10% by Gly. The protonation constants of Sac ($\log \beta_1 = 13.60$; $\log \beta_2 =$
230 16.40) and Gly ($\log \beta_1 = 9.60$; $\log \beta_2 = 11.90$) and the stability constants of Cu(II)-Sac ($\log \beta_1$
231 $= 10.80$) and Cu(II)-Gly ($\log \beta_1 = 8.50$; $\log \beta_2 = 14.60$) complexes were obtained from the
232 constant database IUPAC, and the speciation diagrams for the Cu(II)-Sac-Gly, and Cu(II)-
233 Sac-Gly-Enro systems were plotted using the HySS program at the three tested digestate
234 concentrations: d20 (DOC = 167.7 mg.L^{-1}), d50 (DOC = 61.1 mg.L^{-1}), and d100 (DOC = 32.2
235 mg.L^{-1}) (Table S1). The concentrations of Sac and Gly were calculated for each tested
236 digestate concentration with respect to the corresponding DOC content (Table S1). The
237 proportion and the concentration of the different Enro and Cu species were determined with
238 respect to the pH value of the three tested digestate solutions: d20 (pH = 8.4), d50 (pH = 8.2),
239 and d100 (pH = 8.0) (Table S1).

240 2.8. X-ray Absorption Near Edge Structure (XANES) analyses of root samples

241 The speciation of Cu in roots was compared after uptake in *Phragmites australis*
242 from the digestate solution at the medium concentration (d50), both in absence (“named
243 Root_{Cu}”) and in presence of Enro (named “Root_{Cu+Enro}”), using X-ray Absorption
244 Spectroscopy (XAS). Both root samples were grounded and prepared as pellets which were
245 then embedded in Kapton for XAS analyses.

246 Cu K-edge XANES spectra were recorded at the BM30B (FAME) beamline of the European
247 Synchrotron Radiation Facility (ESRF, Grenoble, France). The storage ring was operating in
248 multi-bunches mode at 6 GeV with a 200 mA current. The beam energy was selected using a
249 Si(220) N₂ cryo-cooled double-crystal monochromator with an experimental resolution close
250 to the one theoretically predicted (namely ~ 0.5 eV). The beam spot on the sample was
251 approximately 300×110 mm² (H×V, FWHM). Because of the low Cu concentrations, sample
252 spectra were recorded in fluorescence mode with a 30-element solid state Ge detector
253 (Canberra) in a He cryostat. The temperature was kept at 15K during data collection and
254 samples were moved at each scan to prevent sample damage. The energy was calibrated with
255 a Cu foil. Data were collected from 8830 to 8970 eV using 5 eV steps of 2s, from 8970 to
256 9038 eV using 0.5 eV steps of 2s, and from 9038 to 9320 eV with a k-step of 0.05 Å⁻¹ and 2s
257 per step. For each sample three scans were averaged and XANES spectra were background-
258 corrected by a linear regression through the pre-edge region and a polynomial one through the
259 post-edge region and normalized to the edge-jump. Standard spectra were also recorded (in
260 transmission mode) for comparison and included the Cu(Enro)₂(H₂O)₂ solid complex
261 prepared as previously reported (Ftouni et al., 2012).

262 XANES spectra normalization and analysis were accomplished by using Athena software
263 from the IFEFFIT software (0.8.056 version) (Ravel and Newville, 2005). Spectra used for
264 LCF were the Cu(Enr)₂(H₂O)₂ solid complex (named “complex Cu-Enro”) and Root_{Cu}. The

265 variable weights were not constrained to be between 0 and 1 and weights were not forced to
266 sum to 1.

267 **3. Results and discussion**

268 3.1. Copper plant uptake

269 In Fig. 1 are reported the concentrations of Cu in the digestate solution at the end of
270 the experiments at the three tested digestate concentrations. In presence of plant (Fig. 1b), a
271 decrease of Cu in solution was observed compared to the controls without plants (Fig. 1a)
272 suggesting plant uptake, especially at low concentration of digestate. A higher digestate
273 concentration led to a higher pH value and a higher DOC content (Table S1) inducing a
274 decrease of Cu availability in solution due to Cu precipitation and/or complexation with DOC.
275 The presence of Enro in the digestate did not modify the Cu remaining concentrations in
276 solution (Fig. 1). Nevertheless, even if the presence of Enro did not modify the total
277 concentration of Cu in solution, it can modify its speciation in solution and thus its
278 bioavailability.

279 In Fig. 2 are reported the corresponding amounts of Cu measured in plant tissues at the
280 end of the experiments. The total amounts of Cu taken up by *Phragmites australis* were
281 comprised between 55.3 (d20) and 253.9 $\mu\text{g}\cdot\text{g}^{-1}$ (d100), primarily in the underground part of
282 the plants with less than 6% of the total amount transported to the aerial parts. This latter
283 observation is in accordance with previous works reporting that most of Cu absorbed by
284 *Phragmites australis* was present in roots with a limited translocation to shoots (Ali et al.,
285 2002; Ye et al., 2003). The influence of the digestate concentration is clearly evidenced in
286 Fig. 2a indicating a significant decrease of Cu uptake with increasing concentration of
287 digestate: it is decreased by 45% from d100 to d50 and by 73% from d100 to d20. This is in
288 accordance with the decrease of Cu availability in solution mentioned above (Fig. 1) due to

289 Cu precipitation and/or complexation with DOC. Otherwise one can notice a decrease of Cu
290 uptake in roots in presence of the antibiotic which was significant at high and low
291 concentration of digestate (d20 and d100). For example, Cu uptake decreased from 253.9
292 $\mu\text{g.g}^{-1}$ to 206.8 $\mu\text{g.g}^{-1}$ at the low digestate concentration. As indicated above, the amounts of
293 translocated Cu were very low and no difference was noticed in absence and in presence of
294 the antibiotic regarding the amounts transported to the plant aboveground parts.

295 The bioconcentration factors (BCFs) calculated as the total Cu concentration in plant
296 tissues to that in the digestate solution at the end of the experiment were reported in Fig.3a for
297 the different tested conditions. This factor was significantly decreased from 49.2 L.kg^{-1} at low
298 digestate concentration to 7.1 L.kg^{-1} at high digestate concentration. However, the presence of
299 Enro did not modify this evolution. These BCF values were more than 15 times lower than the
300 ones reported by Ali et al. (2002) also measured in hydroponic conditions with the same
301 introduced Cu concentration, but from a nutrient solution and after 15 experiment days. In the
302 present study, the lower values could be attributed to the high pH value (between 8.0 and 8.4,
303 Table S.1) and/or the DOC content of the digestate which may greatly reduce the metal
304 bioavailability, and therefore plant uptake.

305 3.2. Enrofloxacin plant uptake

306 The concentrations of Enro in the digestate solutions are reported in Fig. 4 for the
307 controls (without plant) and plant uptake experiments. In addition, the concentrations of
308 ciprofloxacin, Enro main metabolite, are reported in light grey. The controls (Fig. 4a)
309 indicated a loss of Enro in solution which could be due to its degradation despite the
310 precautions taken (aluminium foil). However, as the amounts of detected Cipro were
311 negligible (between 4.0 and 8.3% of the Enro concentration), we may formulate the
312 hypothesis of the presence of non-identified degradation products, or more likely antibiotic

313 adsorption on suspended particulate matter (such as organic matter particles). This loss of
314 Enro was higher when the digestate concentration was high, suggesting Enro adsorption on
315 suspended particles (in higher amount at high digestate concentration) rather than degradation
316 (Lin et al., 2018). Furthermore, the antibiotic loss in solution was lower in the presence of Cu
317 especially at high digestate concentration (Fig. 4a), which may indicate Cu complexation with
318 Enro in solution and therefore competition with sorption onto suspended particles.

319 In presence of plants (Fig. 4b), Enro concentrations in solution were lower compared
320 to the controls suggesting antibiotic plant uptake, as confirmed by the measures in plant
321 tissues reported in Fig 5. As in the controls, the concentrations of Cipro measured in the
322 digestate solutions were very low and represented between 3.6 and 8.7% of the ones of Enro.
323 In general, no significant influence of the digestate concentration was noted on Enro
324 concentrations in solution. But one can notice that the presence of Cu allowed to maintain
325 more Enro in solution with increasing digestate concentration (Fig 4a), which may be due to
326 complexation between the metal and the antibiotic.

327 The corresponding amounts of Enro (and Cipro) taken up by plants are reported in Fig.
328 5. As for Cu, the antibiotic was accumulated in roots and was not transported to the aerial
329 parts of *Phragmites australis* (less than 5% in the aboveground parts). A decrease of Enro
330 taken up by plants with increasing digestate concentration, and an increase of antibiotic
331 accumulation in *Phragmites australis* in presence of Cu can be noticed, although not
332 significantly, which may be related with compounds speciation (see section 3.3.). Up to 226
333 $\mu\text{g}\cdot\text{g}^{-1}$ of Enro were taken up in roots in presence of Cu at low digestate concentration. The
334 amounts of Cipro measured in plant tissues were negligible, less than 1.9 and 0.1 $\mu\text{g}\cdot\text{g}^{-1}$ in the
335 underground and aboveground parts, respectively.

336 The BCFs of Enro, calculated as the total Enro concentration in plant tissues to that in
337 the digestate solution at the end of the experiment were reported in Fig.3b. The obtained
338 values, comprised between 277.6 and 741.6 L.kg⁻¹, were much higher than the values
339 measured for Cu. No significant influence on BCF values, neither of the digestate
340 concentration, nor of the Cu presence, was observed. Thus, great amounts of Enro may be
341 taken up by *Phragmites australis* even in presence of high Cu amounts and in presence of
342 digestate, meaning that these plants can still remain efficient for the removal of
343 fluoroquinolones in a context of multi-contamination as it is the case in the environment.

344 3.3. Plant uptake *versus* speciation

345 As contaminant bioaccumulation in plants is strongly dependent on the contaminant
346 bioavailability rather than on the total contaminant concentration (Dumbrava et al., 2013),
347 uptake was then related to the contaminant speciation to investigate the possibility of
348 predicting contaminant uptake when singly or simultaneously present. According to Trapp
349 (2004), the establishment of empirical relations for selected problems is needed and an arising
350 question is to know whether complexes play a role. It seems thus very important to bring
351 knowledge in this sense. Thus, to try explaining plant uptake results, the speciation in solution
352 of both contaminants when singly or simultaneously present was determined to assess their
353 bioavailability.

354 3.3.1. Cu(II), Enro, and Cu(II)-Enro systems

355 The distribution curves in solution of both contaminants were plotted considering their
356 single and simultaneous presence to precise the proportions of the different species present in
357 solution at the digestate pH (Fig. 6).

358 Concerning Cu singly added, Fig. 6a and Table 1 show that Cu was mainly present as
359 precipitated Cu(OH)_{2(s)} (> 99.8%) at the studied pH values with negligible amounts of species

360 available in solution (Cu^{2+} , $\text{Cu}_2(\text{OH})_2^{2+}$ and $\text{Cu}(\text{OH})^+$). Thus, Cu had a very low availability in
361 the digestate solutions, explaining the low measured BCF values. The proportion of
362 precipitated Cu in the form of $\text{Cu}(\text{OH})_{2(s)}$ decreased in the presence of Enro, being more
363 important than the dilution of the digestate (from 96.9% to 93.6% with decreasing digestate
364 concentration) (Fig. 6c, Table 1a-b). More Cu was available in solution in the presence of
365 Enro due to the formation of complexes between the metal and the antibiotic (CuEnr,
366 CuEnrH_1 and CuEnr_2H_2), and the proportion of free Cu^{2+} was negligible ($< 0.1\%$) (Table
367 1b). Thus, the presence of the antibiotic increased the availability of Cu in solution, but all Cu
368 present in solution was primarily complexed with Enro, mainly as CuEnrH_1 , a decrease of Cu
369 uptake by *Phragmites australis* being observed in the presence of Enro (Fig. 2a). Thus, the
370 presence of Cu-Enro complexes decreased Cu uptake. Moreover, we can note a decrease of
371 the proportion of Cu-Enro complexes in solution, and therefore of the total concentration of
372 Cu available in solution, due to the precipitation of Cu with increasing digestate concentration
373 as a result of the increase of the pH value (Fig. 6c, Table 1b). The decreasing uptake of Cu
374 observed with increasing digestate concentration (Fig. 2) was coherent with this evolution of
375 the speciation: the lower the total concentration of Cu in solution, the lower the metal uptake.
376 To bring a better insight of the relationship between plant uptake and Cu speciation, the
377 correlations between the amounts taken up by *Phragmites australis* and the concentrations of
378 the different species present in solution were considered (Figs. 7 and 8), even if these results
379 should be taken with caution due to the low number of data points. In the case of Cu singly
380 added (Fig. 7), as expected, the amounts of Cu taken up were positively correlated ($r > 0.99$) to
381 the different Cu species available in solution. When Cu was simultaneously present with Enro
382 in solution (Fig. 8), these correlations were still relevant but the values of the correlation
383 coefficient were lower ($r = 0.94392$ at maximum). Positive correlations with the
384 concentrations of the positively charged Cu-Enro complexes in solution, CuEnr and CuEnrH_1

385 were observed, with a slightly higher value of r obtained for CuEnrH_1 (0.98888) than for Cu
386 species. But the concentrations of the neutral species CuEnr_2H_2 were not correlated to the
387 amounts of Cu taken up by plants (data not shown). Logically, as in the case of Cu singly
388 added, Cu uptake was negatively correlated with the concentration of precipitated Cu in the
389 form of $\text{Cu}(\text{OH})_{2(s)}$ ($r = -0.97874$). Globally, Cu uptake was rather well correlated to the
390 concentrations of Cu cationic species present in solution (including complexes with Enro)
391 (Fig. 8, $r = 0.98607$). Therefore, even if the presence of Cu-Enro complexes decreased the
392 amounts of Cu taken up by *Phragmites australis*, these complexed species would be however
393 absorbed by roots since the correlations were improved taking the contribution of their
394 concentrations into account.

395 When Enro was singly added (Fig. 6b, Table 1c), it was primarily present as the
396 negative species EnrH_1 and to a lesser extent as the zwitterionic form Enr, the ratio EnrH_1
397 $/\text{Enr}$ increasing with increasing digestate concentration (Fig. 6b, Table 1c). Thus, the
398 antibiotic uptake decreased with increasing proportion of its negative species. This negative
399 correlation between Enro uptake by *Phragmites australis* and the concentration in solution of
400 EnrH_1 is evidenced in Fig. 9a ($r = -0.96962$; $R^2 = 0.94016$), in addition to the positive
401 correlation between Enro uptake and the concentration of the zwitterionic species Enr ($r =$
402 0.97360) (Fig. 9a-b). The proportion of free Enro (as $\text{Enr} + \text{EnrH}_1$ species) decreased in the
403 presence of Cu due to complexation (Fig. 6d, Table 1): from 100% to about 30% for d100 and
404 60% for d20 (Table 1). Indeed, in the studied pH range, Enro was also present as complexes
405 with Cu, mainly CuEnrH_1 (positive species) and CuEnr_2H_2 (neutral species), but also to a
406 lesser extent CuEnr and CuEnr_2 (positive species). The percentages of the different species
407 reported in Table 1 indicate that the concentrations in solution of free Enro ($\text{Enr} + \text{EnrH}_1$)
408 increased with increasing digestate concentration, whereas the concentrations of complexed
409 Enro species ($\text{CuEnrH}_1 + \text{CuEnr}_2\text{H}_2$) decreased, as did Enro plant uptake (Fig 5). This forces

410 one to ask whether the presence of Cu-Enr complexes would support Enr uptake, particularly
411 because Enr uptake increased in the presence of Cu (Fig. 5a). This hypothesis was checked by
412 looking at the correlations between Enro uptake and the concentrations of the different species
413 in solution (Fig. 9c-f). The amounts of Enro taken up by *Phragmites australis* were negatively
414 correlated with the concentrations of the negative species EnrH_{-1} ($r = -0.99866$), not correlated
415 with the concentrations of the neutral species $\text{CuEnr}_2\text{H}_{-2}$ (data not shown), but positively
416 correlated with the concentrations of the positively charged complexes CuEnrH_{-1} ($r =$
417 0.99972), CuEnr ($r = 0.99201$), and CuEnr_2 ($r = 0.99521$).

418 3.3.2. Cu(II)-Sac-Gly and Cu(II)-Sac-Gly-Enro systems

419 As mentioned in section 3.1., a higher digestate concentration led to a higher DOC
420 content (Table S1) which can induce a decrease of Cu bioavailability in solution due to
421 complexation with DOC. Thus, in this second part, the distribution curves in solution of Cu
422 and Enro (when singly added and in presence of each other) were plotted considering Sac and
423 Gly as DOC mimics to assess the importance of Cu(II)-DOC interactions at the three tested
424 digestate concentrations and thus for a better insight of the speciation (Figs. 10 and 11).

425 When Cu was singly added, the presence of DOC (as Sac and Gly) limited its
426 precipitation as $\text{Cu(OH)}_{2(s)}$ due to Cu complexation with DOC (as Cu-Sac and Cu-Gly
427 complexes) (Fig. 10a-c): the proportion of precipitated Cu decreased from 53% (d100) to 0%
428 (d20) with increasing digestate concentration, whereas the proportions of Cu complexed with
429 DOC (primarily as Cu-Gly complexes) increased from 47% (d100) to 100% (d20). As in
430 absence of DOC, the proportion of Cu^{2+} (as well as Cu(OH)^+ and $\text{Cu}_2(\text{OH})_2^{2+}$) remained
431 negligible and decreased with increasing digestate concentrations (Table 2a). The presence of
432 Enro did not affect the amounts of Cu^{2+} , Cu(OH)^+ and $\text{Cu}_2(\text{OH})_2^{2+}$ (except at d20), but slightly
433 further decreased the proportion of precipitated $\text{Cu(OH)}_{2(s)}$ due to the formation of additional

434 Cu complexes with Enro (CuEnrH_1 , CuEnr_2H_2 , CuEnr , and CuEnr_2) (Fig. 10d-f, Table 2b).
435 The proportion of Cu-Enro complexes decreased with increasing digestate concentration,
436 from 6.2% (d100) to 0.2% (d20), but the presence of Enro did not significantly modify the
437 proportion of Cu involved in complexes with DOC (Table 2b). By comparing Tables 1a-b and
438 2a-b, it can be seen that the proportions of Cu-Enro are similar in absence and in presence of
439 DOC for d50 and d100 and only differed at high DOC content (for d20), decreasing from
440 3.1% in absence of DOC to 0.2% in presence of DOC. The main difference is in the
441 proportion of precipitated Cu which is 96.9% of $\text{Cu}(\text{OH})_{2(s)}$ and 0.01% of Cu^{2+} in absence of
442 DOC, but 0% of $\text{Cu}(\text{OH})_{2(s)}$, insignificant amount of Cu^{2+} , and close to 100% of Cu-DOC
443 complexes in presence of DOC.

444 The correlations between the amounts taken up by *Phragmites australis* and the
445 concentrations of the different Cu species present in solution are reported in Figs. 12 and 13,
446 even if these results should be taken with caution due to the low number of data points. In
447 presence of Sac and Gly, the amounts of Cu taken up remained positively correlated with the
448 concentrations of the cationic species Cu^{2+} , $\text{Cu}(\text{OH})^+$ and $\text{Cu}_2(\text{OH})_2^{2+}$, but the negative
449 correlation ($r = -0.98297$) observed with the concentration of the neutral complex CuGly_2 can
450 be emphasized (Figs.12 and 13). The concentrations of the cationic species CuGly and of the
451 neutral species CuSac were not correlated to the amounts of Cu taken up by plants (data not
452 shown). In presence of DOC and Enro (Fig. 13), the amounts of Cu taken up by plants
453 remained positively correlated ($r > 0.98$) to the concentrations of the positively charged
454 complexes CuEnrH_1 and CuEnr , as in absence of DOC.

455 When Enro was simultaneously added with Cu, the presence of DOC (as Sac and Gly)
456 at low and medium content (d100 and d50) did not affect the speciation of Enro, but the
457 proportion of Cu-Enro complexes greatly decreased at high DOC content (d20), from 38% in
458 absence of DOC to 3% in presence of 162.7 mg.L^{-1} DOC (d20), whereas the proportion of

459 free Enro (as Enr and EnrH₁) logically increased from 61% to 97% (Table 2d). Even in
460 presence of DOC, the amounts of Enro taken up by *Phragmites australis* remained positively
461 correlated to the concentrations of the positively charged complexes CuEnrH₁ ($r = 0.96158$),
462 CuEnr ($r = 1.00000$), and CuEnr₂ ($r = 0.99967$) (Fig.14).

463 Finally, an increase of the ratio Enro/Cu in plant tissues with increasing digestate
464 concentration (from about 1 to 3) was observed (Fig. 3c), whereas in solution the
465 concentrations of Cu-Enro complexes decreased and the concentrations of free Enro
466 increased. At the highest digestate concentration an important part of the antibiotic would be
467 taken up as free Enro which would explain the decrease of Enro uptake, an uptake that would
468 be favored in the presence of Cu.

469 To sum up, the decreasing bioavailability of Cu with increasing digestate
470 concentration was not due to Cu precipitation but to the formation of Cu-DOC complexes
471 limiting Cu uptake. The presence of Enro decreased Cu uptake which was favored as free Cu
472 rather than complexed Cu. On the contrary, the presence of Cu increased Enro uptake due to
473 Cu-Enro complexation. Thus, the lower amounts of Cu-Enro complexes at high digestate
474 concentration led to a decrease of Enro uptake with increasing digestate concentration.

475

476 3.4. XANES analyses

477 The uptake of Cu by *Phragmites australis* was studied at molecular scale by XANES
478 at Cu K-edge to compare the speciation of the metal in roots when singly added and
479 simultaneously present with Enro. The normalized K-edge XANES spectra of both root
480 samples are reported in Fig. 15 and are superimposed with the spectrum of the complex
481 Cu(Enro)₂(H₂O)₂ (Ftouni et al., 2012) taken as reference.

482 The XANES spectra of Root_{Cu} and $\text{Root}_{\text{Cu+Enro}}$ and their corresponding first
483 derivatives were slightly different, and by comparing their characteristic shapes with the ones
484 of the standard complex, it can be observed that the sample $\text{Root}_{\text{Cu+Enro}}$ was intermediate
485 between the one of Root_{Cu} and the one of the complex reference spectrum. Linear
486 Combination Fitting (LCF) of the $\text{Root}_{\text{Cu+Enro}}$ spectrum was performed using as components
487 the spectra of Root_{Cu} and of the complex Cu-Enro (Fig. 15). The experimental data were very
488 well fitted with contributions of 78.2 and 22.5% for Root_{Cu} and for the complex, respectively
489 (R-factor = 0.000183, sum of components = 100.7%). Thus, these analyses at molecular scale
490 confirmed the implication of the complexation in the uptake of Cu and Enro when they are
491 simultaneously present in solution. Even if the uptake of Cu was less favored in presence of
492 Enro, a non-negligible part of the metal was taken up by *Phragmites australis* as Cu-Enro
493 complex, even if the major part of Cu.

494

495 Conclusions

496 The presence of digestate increased both the pH value and the DOC content of the
497 medium, and decreased plant uptake of Cu and Enro. The increase of digestate concentration
498 limited Cu precipitation but decreased Cu plant uptake and bioavailability due to the
499 formation of Cu-DOC complexes. In addition, the presence of Enro led to the formation of
500 Cu-Enro complexes which decreased Cu plant uptake. Globally, the formation of Cu
501 complexes (primarily with DOC, and to a lesser extent with Enro at high DOC content) was
502 not favorable to Cu plant uptake, decreasing its bioavailability. However, a part of Cu can be
503 taken up as Cu-Enro complexes in roots as evidenced by XANES. The increase of DOC
504 content at high digestate concentration limited the formation of Cu-Enro complexes in favor
505 of Cu-DOC complexes which led to a decrease of Enro plant uptake. Thus, the presence of Cu

506 favored the antibiotic plant uptake which could be taken up both as free Enro (mainly in the
507 zwitterionic form) and as Cu-Enro complexes (as positively charged complexes).

508 The speciation data in absence of Sac and Gly indicated that in absence of DOC or at
509 low DOC content (such as natural waters) and at high pH values, the precipitation of Cu
510 limited its plant uptake. The presence of Enro decreased the proportion of precipitated Cu as
511 $\text{Cu}(\text{OH})_{2(s)}$ due to the formation of Cu-Enro complexes but the presence of these complexes
512 decreased the metal bioavailability, and Cu plant uptake was favored in the form of free Cu
513 rather than in the form of complexes.

514 Although in some cases the differences in term of amounts were not significant, there
515 was a modification of the chemical forms of both contaminants (different speciation). In a
516 scenario of multi-contamination, as it can be found in the environment, namely in soils
517 amended with digestate containing different types of contaminants, the modification of the
518 chemical forms of both contaminants (different speciation), can have different impacts onto
519 the plants and modify their uptake behavior in the long term. Thus, the interactions between
520 contaminants should be taken into account for field crop soils as well as for phytoremediation
521 technologies. Globally great amounts of Enro may be taken up by *Phragmites australis* even
522 in presence of high Cu amounts and in presence of digestate. Thus, these plants can still
523 remain efficient for the removal of fluoroquinolones in a context of multi-contamination as it
524 is the case in the environment. However, it is not the case for Cu for which the
525 phytoremediation performances could be significantly decreased at high DOC content and/or
526 high pH values.

527 In further research, efforts should be made on trying to better characterize the DOC
528 present in the liquid fraction of digestates from different origins for a better insight of their
529 speciation and thus bioavailability.

530

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537

538 Conflict of interest

539 All authors declare no conflict of interest.

540

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Table 1: Proportions (%) in the digestate solutions of the different species of both contaminants obtained by speciation diagrams: Cu when singly (a) or simultaneously added with Enr (b), and Enr when singly (c) or simultaneously added with Cu (d).

(a)

<i>Cu (%) when singly added</i>	d20	d50	d100
Cu(OH)_{2(s)}	99.93	99.93	99.80
Cu²⁺	0.01	0.03	0.07
Cu(OH)⁺	0.04	0.06	0.09
Cu₂(OH)₂²⁺	< 0.01	< 0.01	<0.01

(b)

<i>Cu (%) when added in presence of Enr</i>	d20	d50	d100
Cu(OH)_{2(s)}	96.90	95.30	93.60
Cu²⁺	0.01	0.03	0.07
Cu(OH)⁺	0.04	0.06	0.09
Cu₂(OH)₂²⁺	< 0.01	< 0.01	<0.01
CuEnr	0.05	0.14	0.3
CuEnrH₋₁	1.96	3.30	4.80
CuEnr₂H₋₂	1.05	1.18	1.05

(c)

<i>Enr (%) when singly added</i>	d20	d50	d100
EnrH	0.10	0.23	0.51
Enr	17.25	24.80	34.29
EnrH₋₁	82.60	74.95	65.19

(d)

<i>Enr (%) when added in presence of Cu</i>	d20	d50	d100
EnrH	0.06	0.11	0.16
Enr	10.80	11.47	10.80
EnrH₋₁	50.50	34.50	21.00
CuEnr	0.48	1.20	2.90
CuEnrH₋₁	18.30	30.6	44.7
CuEnr₂H₋₂	19.60	22.10	19.80
CuEnr₂	0.06	0.18	0.38

For sake of clarity, charges were omitted for species involving Enr. The species with their respective charge are as below:

(EnrH)⁺
 (Enr)^{+/-}
 (EnrH₋₁)⁻
 (CuEnr)²⁺
 (CuEnrH₋₁)⁺
 (CuEnr₂H₋₂)⁰
 (CuEnr₂)²⁺

Table 2: Proportions (%) in the digestate solutions, obtained by speciation diagrams, of the different species of both contaminants in presence of Sac and Gly as DOC simple models: Cu when singly (a) or simultaneously added with Enr (b), and Enr when singly (c) or simultaneously added with Cu (d).

(a)

<i>Cu (%) when singly added, in presence of DOC</i>	d20	d50	d100
Cu(OH)_{2(S)}	-	19.30	52.95
Cu²⁺	-	< 0.03	0.07
Cu(OH)⁺	< 0.002	0.06	0.09
Cu₂(OH)₂²⁺	-	-	< 0.002
CuSac	0.32	4.73	3.94
CuGly	3.34	20.20	20.39
CuGly₂	96.33	55.65	22.55

(b)

<i>Cu (%) when added in presence of Enr, in presence of DOC</i>	d20	d50	d100
Cu(OH)_{2(S)}	-	14.80	46.73
Cu²⁺	-	0.03	0.07
Cu(OH)⁺	< 0.002	0.06	0.09
Cu₂(OH)₂²⁺	-	-	< 0.002
CuEnr	0.003	0.13	0.31
CuEnrH₋₁	0.12	3.22	4.83
CuEnr₂H₋₂	0.10	1.18	1.05
CuEnr₂	-	0.01	0.02
CuSac	0.32	4.73	3.94
CuGly	3.33	20.20	20.39
CuGly₂	96.13	55.60	22.55

(c)

<i>Enr (%) when singly added, in presence of DOC</i>	d20	d50	d100
EnrH	0.10	0.23	0.51
Enr	17.25	24.80	34.29
EnrH₋₁	82.60	74.95	65.19

(d)

<i>Enr (%) when added in presence of Cu, in presence of DOC</i>	d20	d50	d100
EnrH	0.10	0.11	0.16
Enr	16.70	11.48	10.84
EnrH₋₁	80.19	34.70	20.62
CuEnr	0.03	1.23	2.93
CuEnrH₋₁	1.10	30.20	45.30
CuEnr₂H₋₂	1.87	22.14	19.75
CuEnr₂	0.006	0.17	0.39

For sake of clarity, charges were omitted for species involving Enr, Sac, and Gly. The species with their respective charge are as below:

(EnrH)⁺
 (Enr)^{+/-}
 (EnrH₋₁)⁻
 (CuEnr)²⁺
 (CuEnrH₋₁)⁺
 (CuEnr₂H₋₂)⁰
 (CuEnr₂)²⁺
 (CuGly)⁺
 (CuGly₂)⁰
 (CuSac)⁰

(a) Controls without plant

(b) With plant

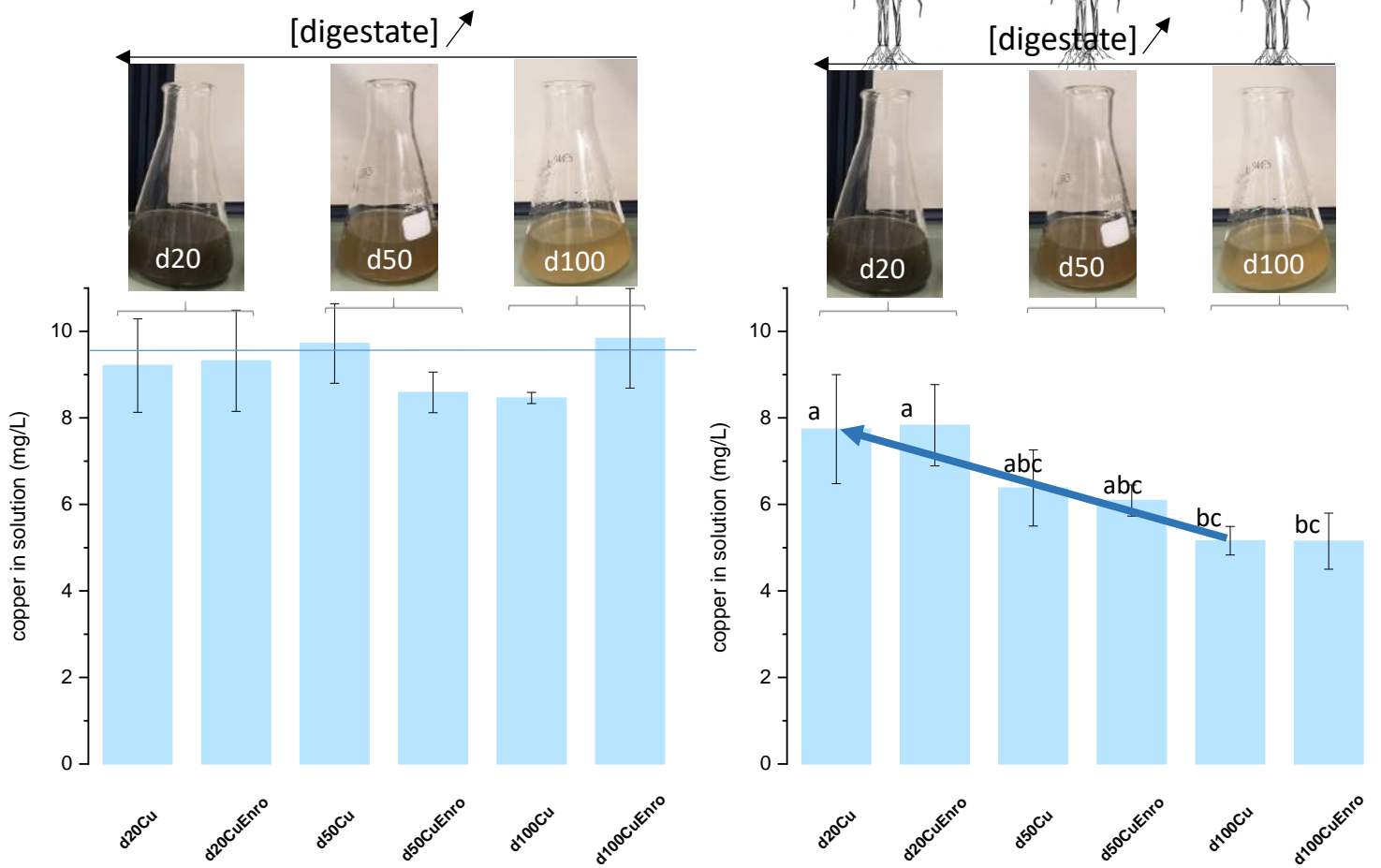


Figure 1: Copper concentrations in solution in controls without plants (a) and in samples containing *Phragmites australis* (b), in the cases of single Cu application (left bars) and combined application of Cu and Enro (right bars) at the three tested digestate concentrations (digestate diluted by a factor of 20, 50, and 100, named d20, d50, and d100, respectively). Results are presented as mean \pm SD (n=3); different letters indicate a significant difference at $P < 0.05$. The blue line corresponds to the added concentration of Cu.

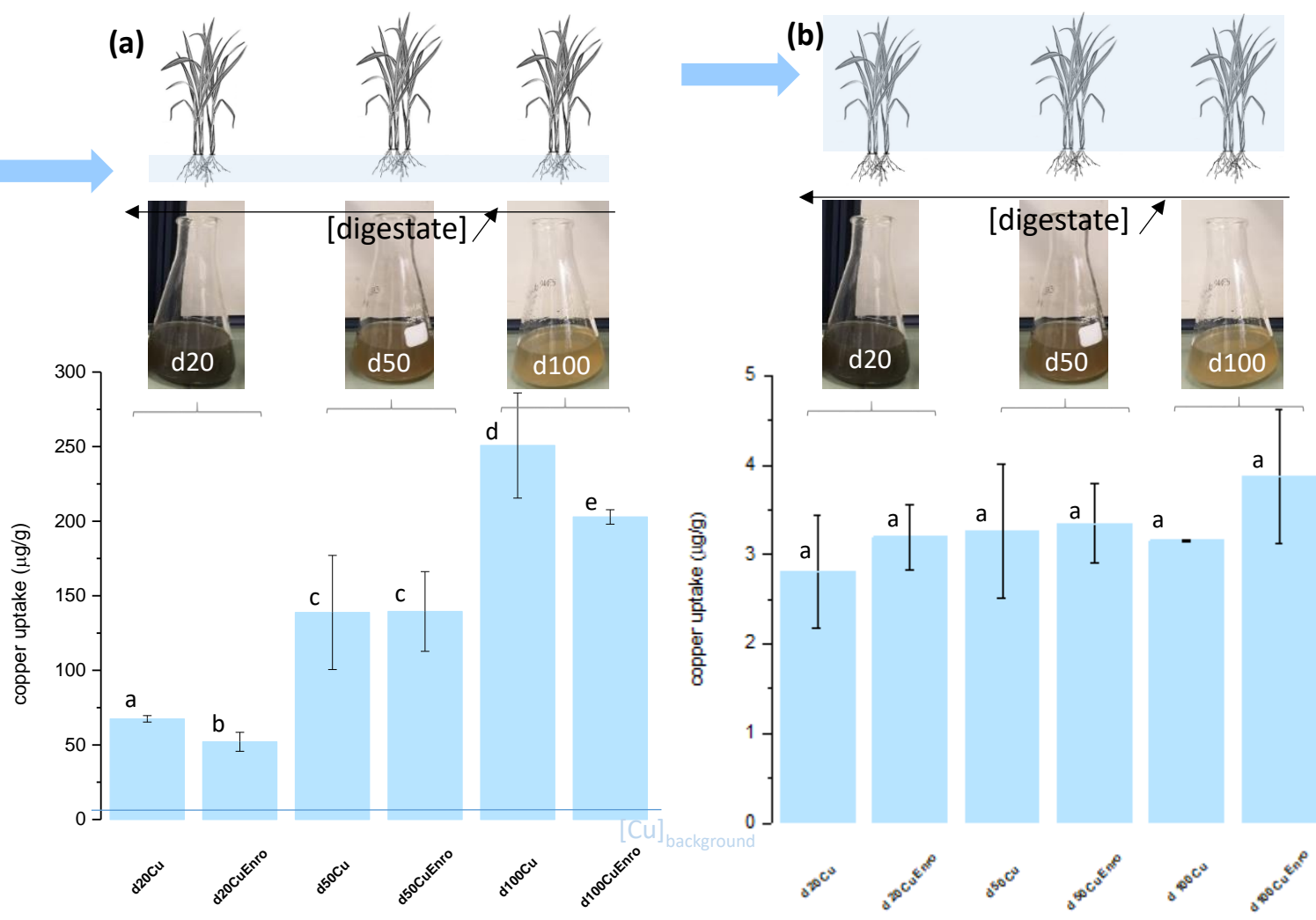


Figure 2: Amounts of Cu taken up by *Phragmites australis* in the underground (a) and aboveground (b) parts of the plants in the cases of single Cu application (left bars) and combined application of Cu and Enro (right bars) at the three tested digestate concentrations (digestate diluted by a factor of 20, 50, and 100, named d20, d50, and d100, respectively). Results are presented as mean \pm SD (n=3); different letters indicate a significant difference at P<0.05.

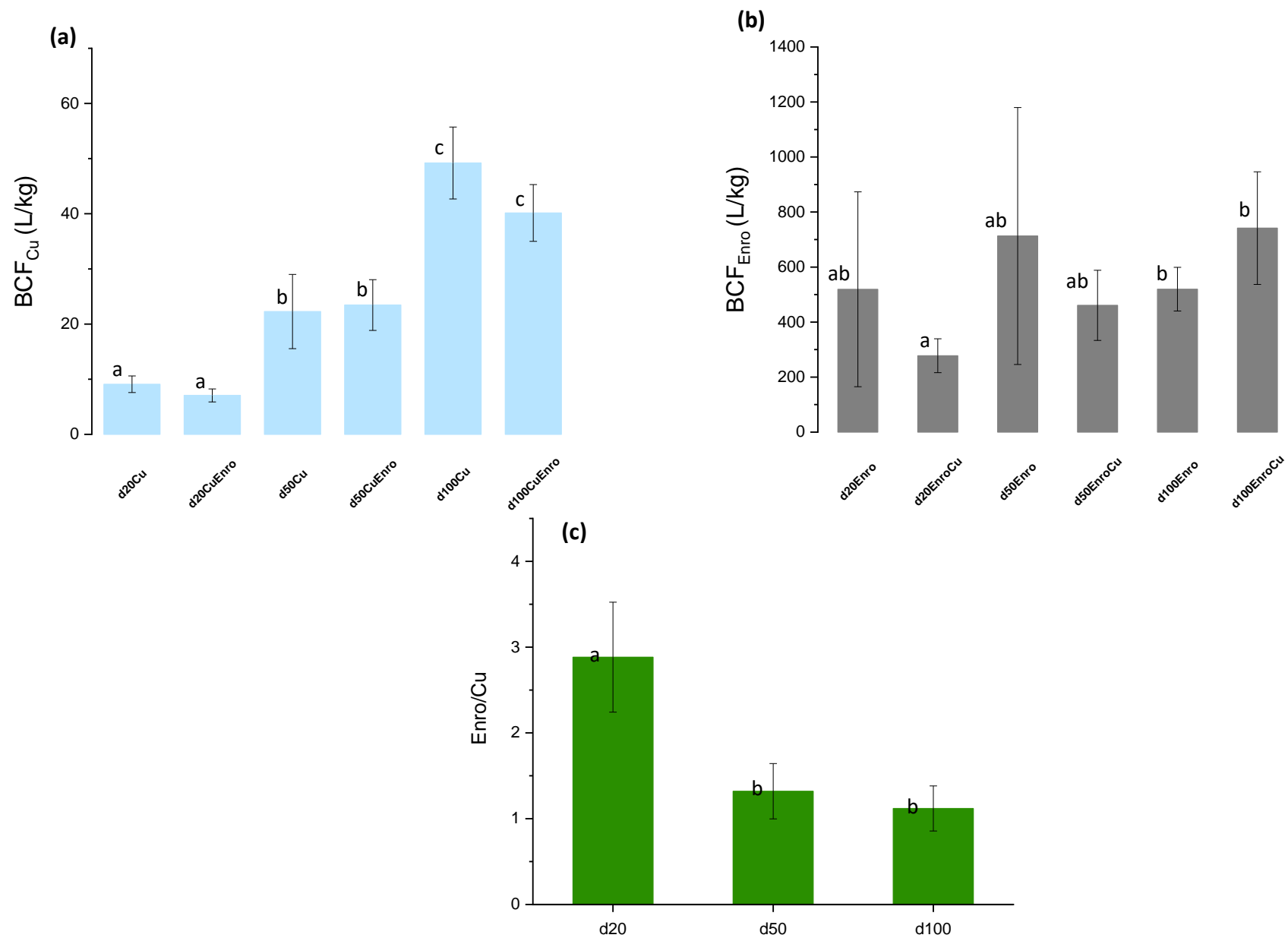


Figure 3: Bioconcentration factors (BCFs) of Cu (a) and Enro (b) in *Phragmites australis* when singly added (left bars) or simultaneously present (right bars) in the different digestate solutions, and values of the ratio of Enr to Cu concentrations taken up by *Phragmite australis* as a function of the digestate concentration (digestate diluted by a factor of 20, 50, and 100, named d20, d50, and d100, respectively). (c). Results are presented as mean \pm SD (n=3); different letters indicate a significant difference at $P < 0.05$. BCFs were calculated as the total contaminant concentration in plant tissues to that in the digestate solution at the end of the experiment.

(a) Controls without plant

(b) With plant

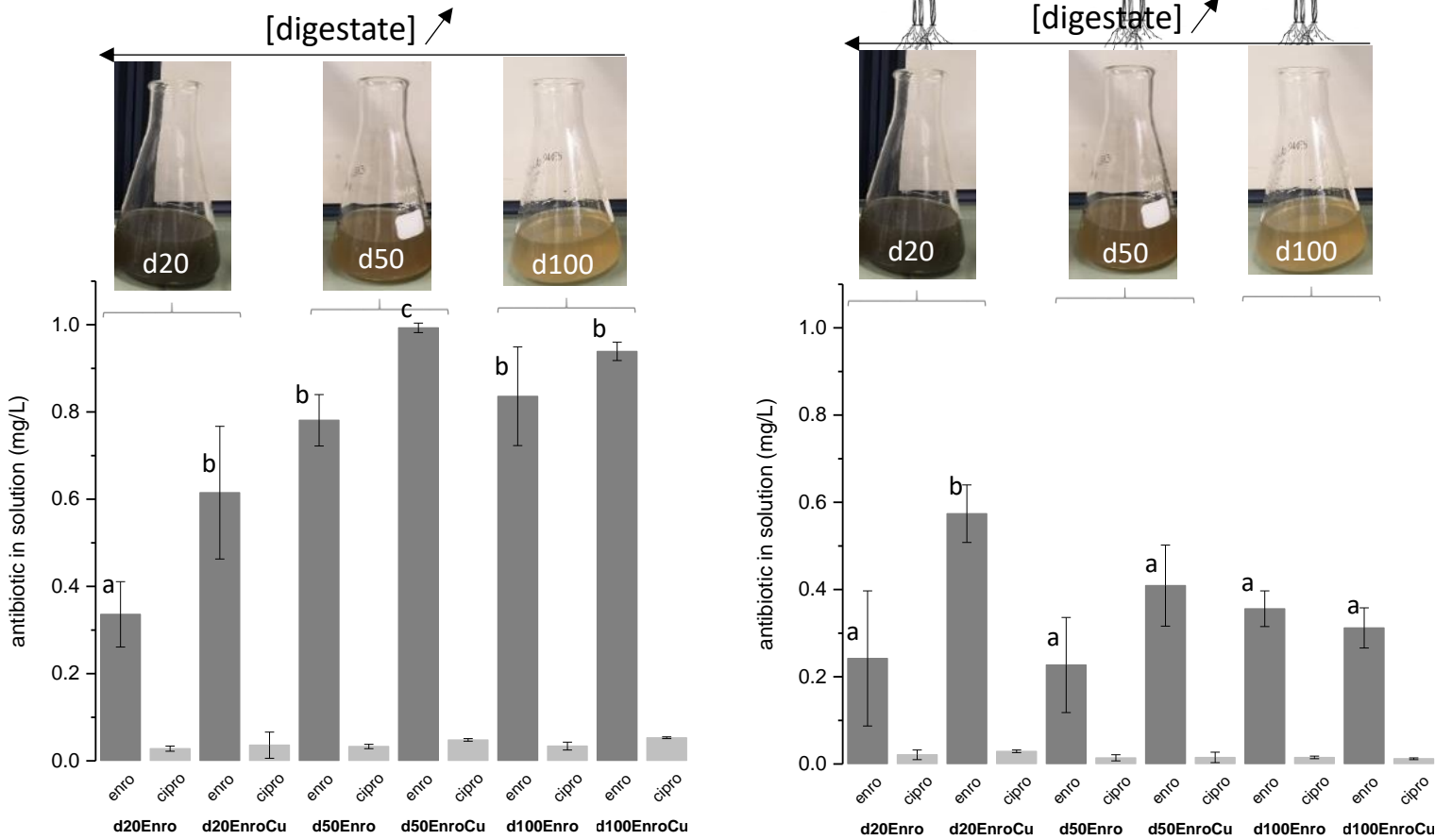


Figure 4: Enro and Cipro concentrations in solution in controls without plants (a) and in samples containing *Phragmites australis* (b), in the cases of single Enr application (left bars) and combined application of Cu and Enr (right bars) at the three tested digestate concentrations (digestate diluted by a factor of 20, 50, and 100, named d20, d50, and d100, respectively). Results are presented as mean \pm SD (n=3); different letters indicate a significant difference at P<0.05.

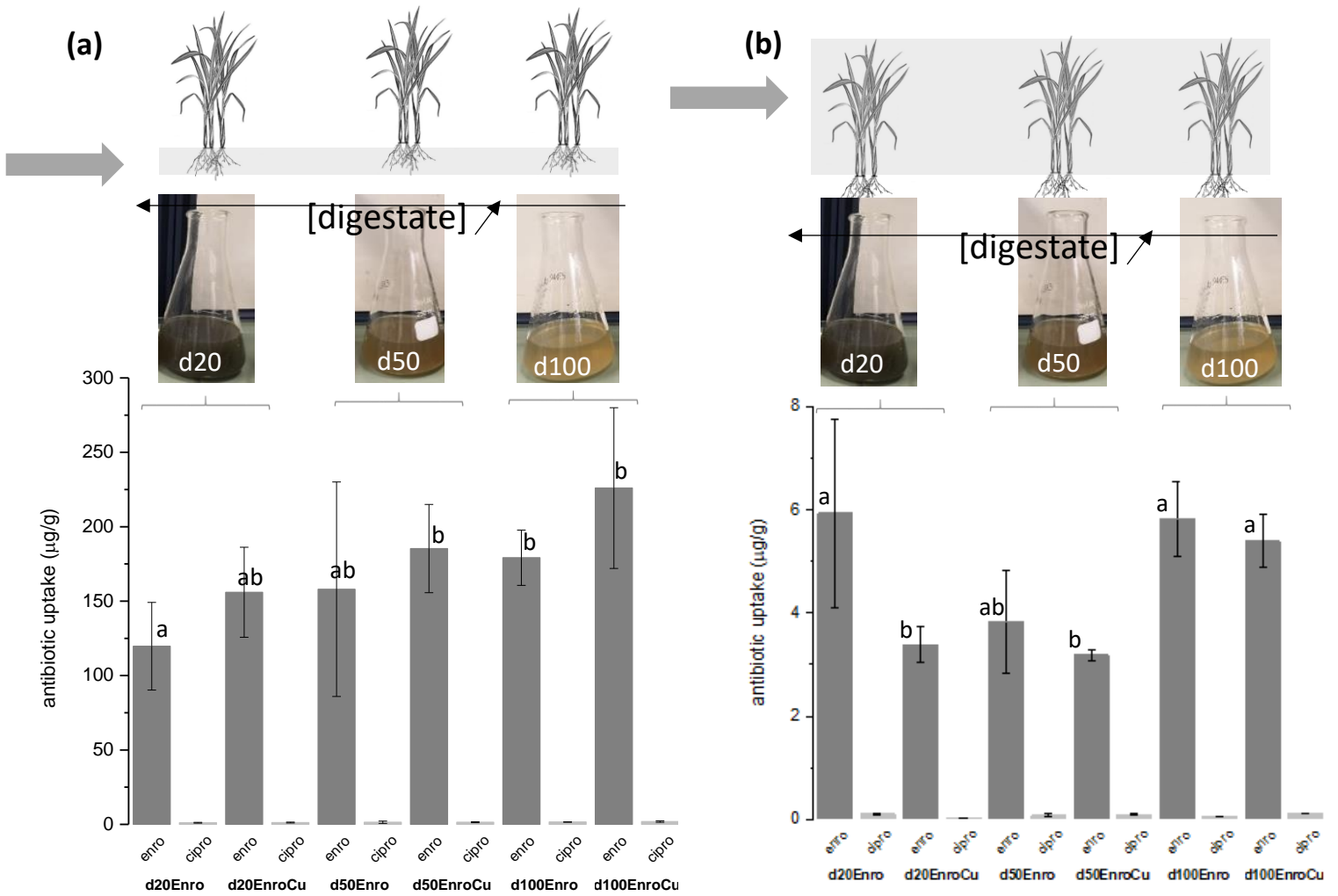


Figure 5: Amounts of Enro and Cipro taken up by *Phragmites australis* in the underground (a) and aboveground (b) parts of the plants in the cases of single Enr application (left bars) and combined application of Cu and Enr (right bars) at the three tested digestate concentrations (digestate diluted by a factor of 20, 50, and 100, named d20, d50, and d100, respectively). Results are presented as mean \pm SD (n=3); different letters indicate a significant difference at $P < 0.05$.

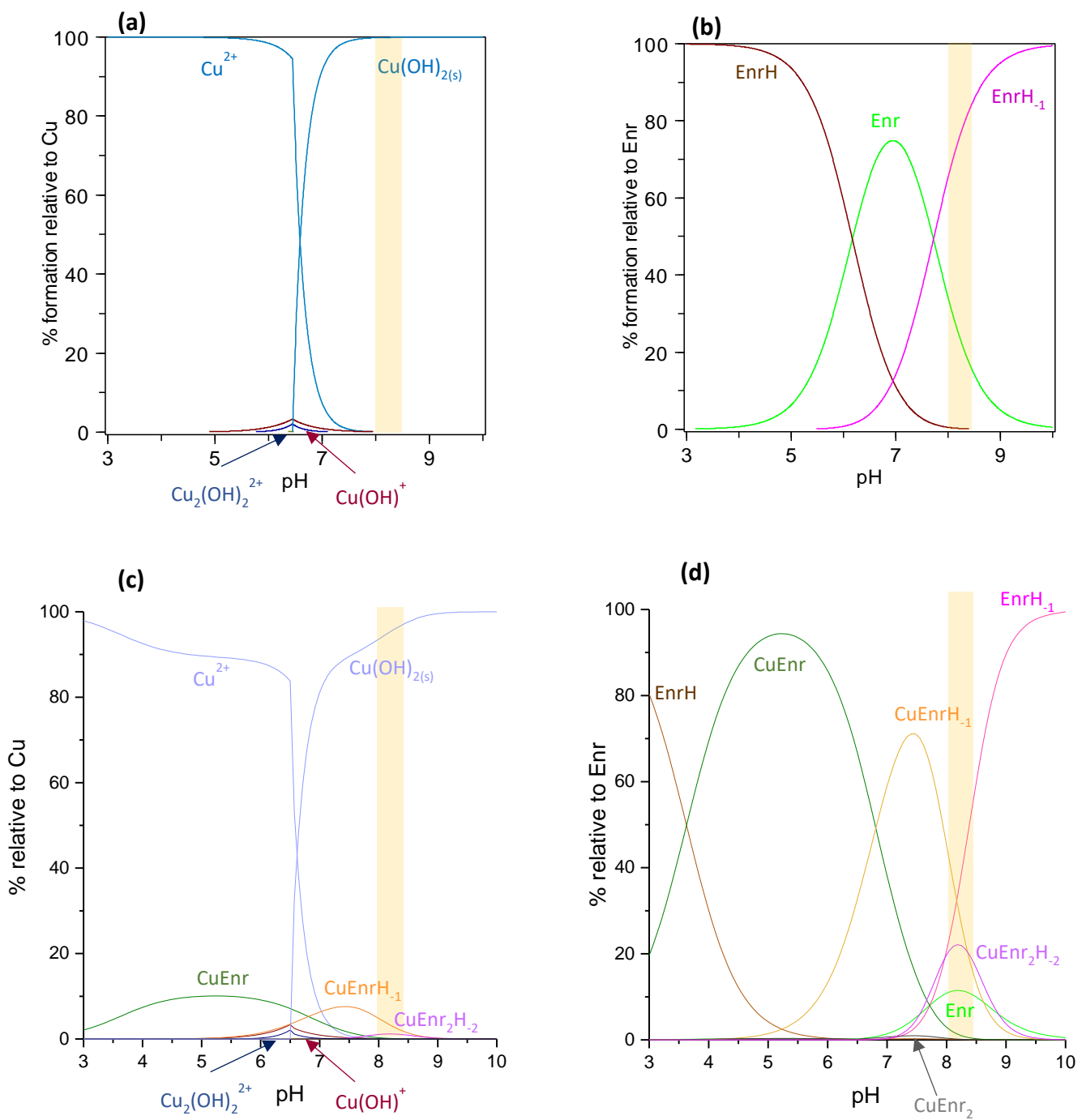


Figure 6: Solution speciation diagrams for Cu present alone (a) and in presence of Enro (c), and for Enro present alone (b) and in presence of Cu (d); $[\text{Cu}]_{\text{T}} = 1.6 \times 10^{-5} \text{ mol.L}^{-1}$ and $[\text{Enro}]_{\text{T}} = 1.6 \times 10^{-5} \text{ mol.L}^{-1}$. Enr corresponds to enrofloxacin in the zwitterionic form. The yellow bar corresponds to the pH range of the digestate solutions.

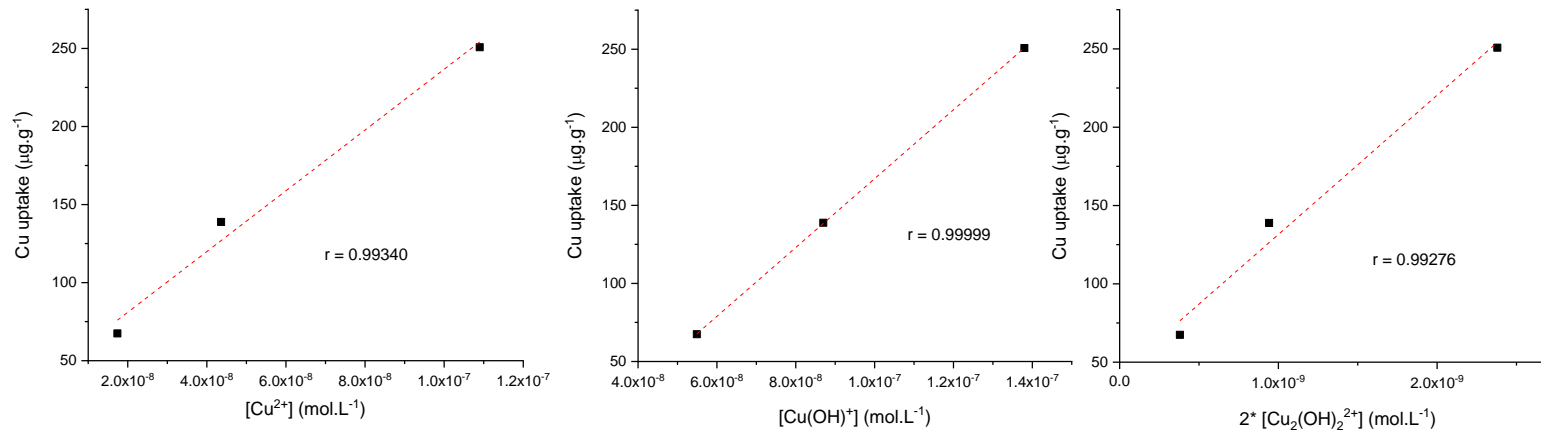


Figure 7: Cu uptake *versus* the concentration in solution of the different Cu species, when Cu is singly added.

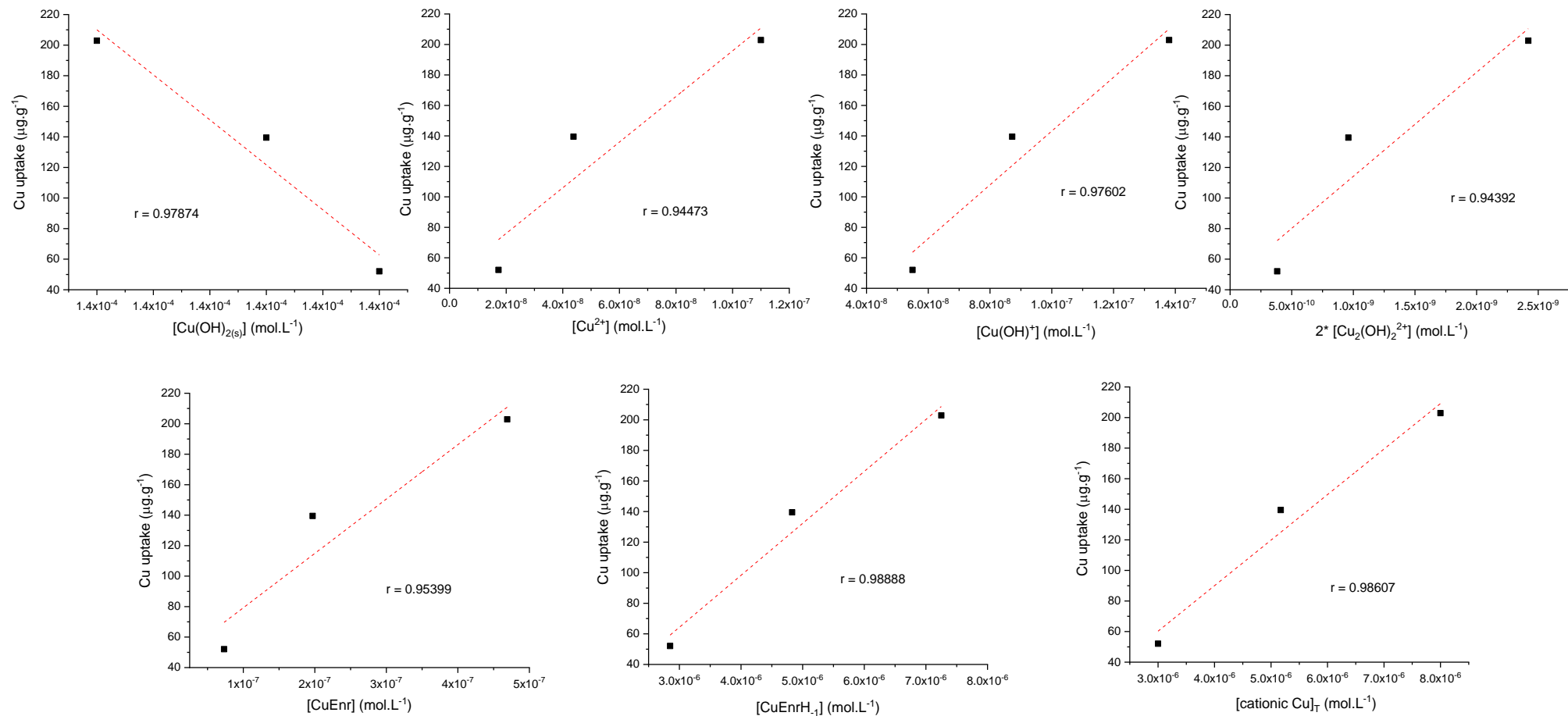


Figure 8: Cu uptake *versus* the concentration in solution of different Cu species, when Cu is simultaneously added with Enro.

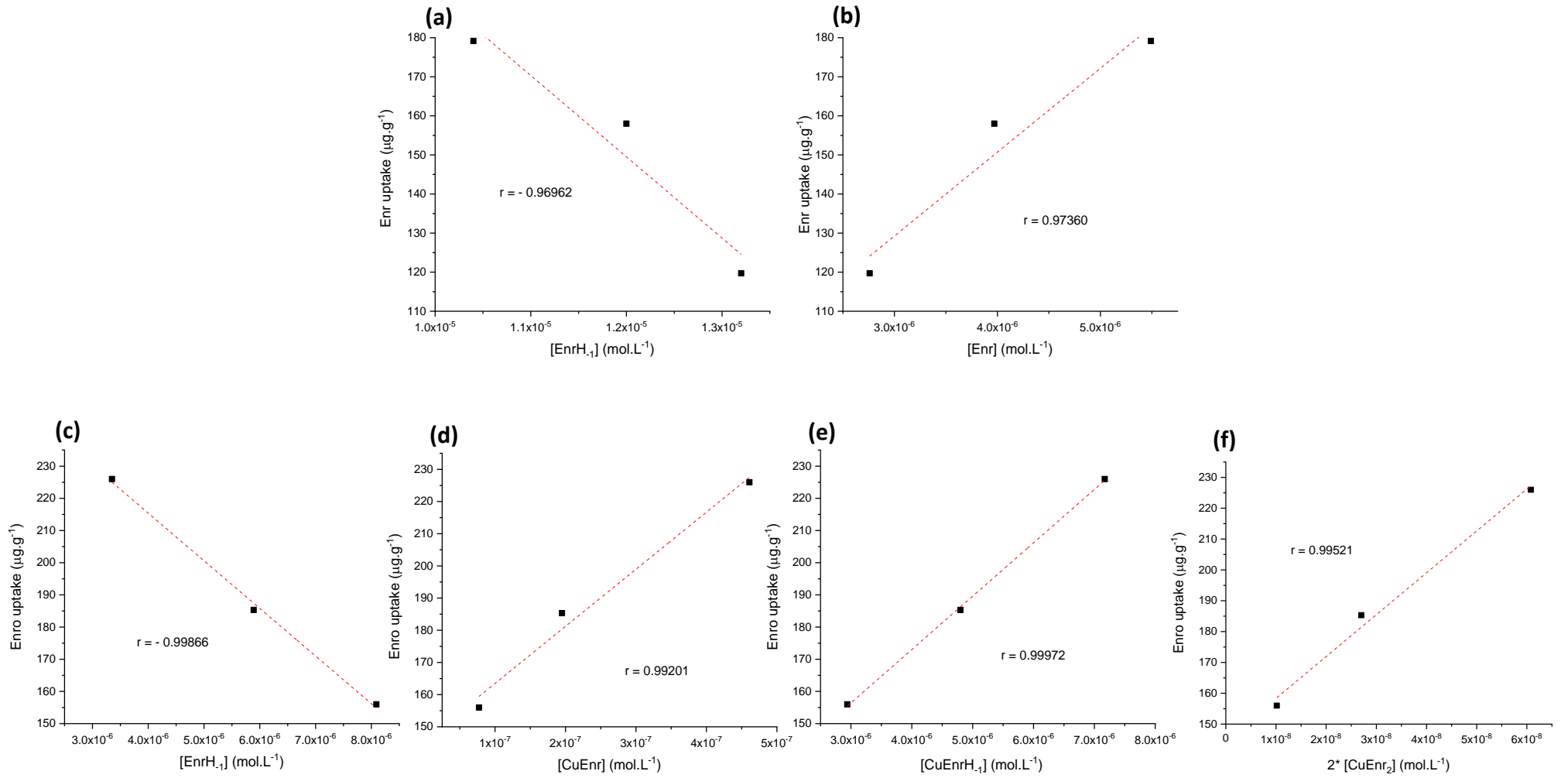


Figure 9: Enro uptake *versus* the concentration in solution of different Enro species, when Enro is singly added (a-b) and simultaneously added with Cu (c-f).

(c)

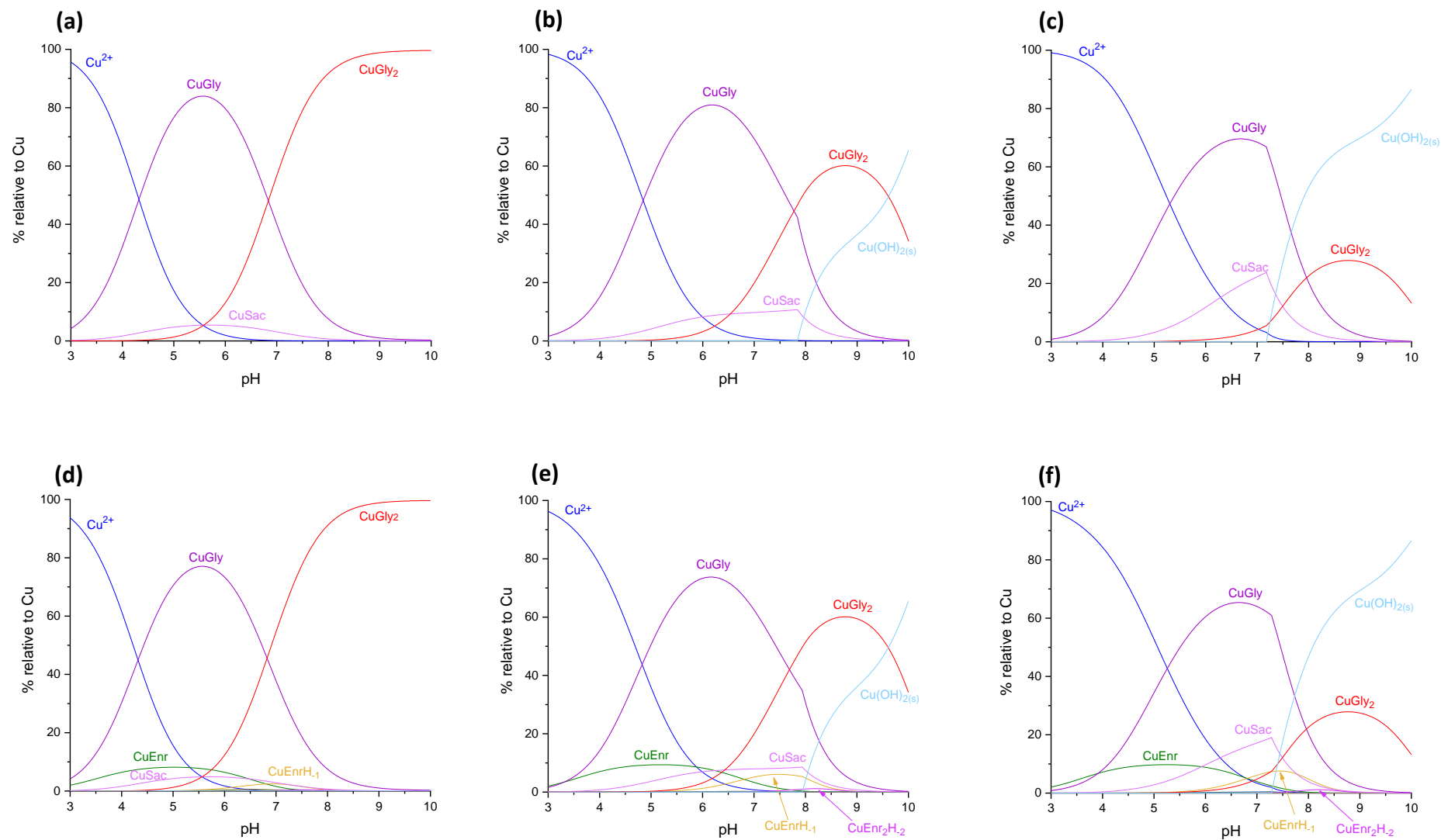


Figure 10: Solution speciation diagrams for Cu present alone at the three tested digestate concentrations d20 (a), d50 (b), and d100 (c) and for Cu in presence of Enro at the three tested digestate concentrations d20 (d), d50 (e), and d100 (f); $[Cu]_T = 1.6 \times 10^{-5} \text{ mol.L}^{-1}$ and $[Enro]_T = 1.6 \times 10^{-5} \text{ mol.L}^{-1}$, in presence of Sac and Gly as DOC simple models.

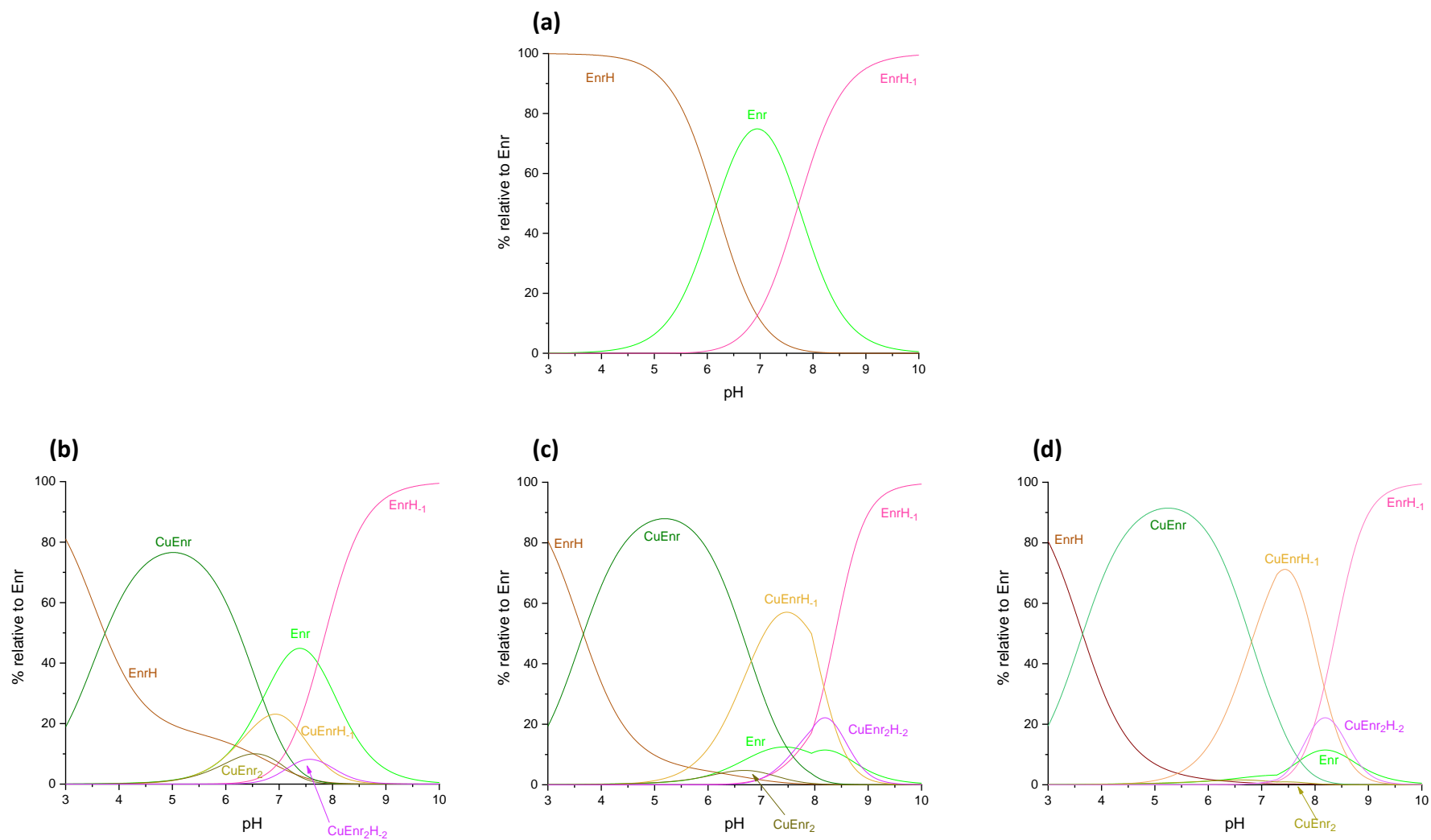


Figure 11: Solution speciation diagrams for Enro present alone (a), and for Enro in presence of Cu at the three tested digestate concentrations d20 (b), d50 (c), and d100 (d); $[Cu]_T = 1.6 \times 10^{-5} \text{ mol.L}^{-1}$ and $[Enro]_T = 1.6 \times 10^{-5} \text{ mol.L}^{-1}$, in presence of Sac and Gly as DOC simple models.

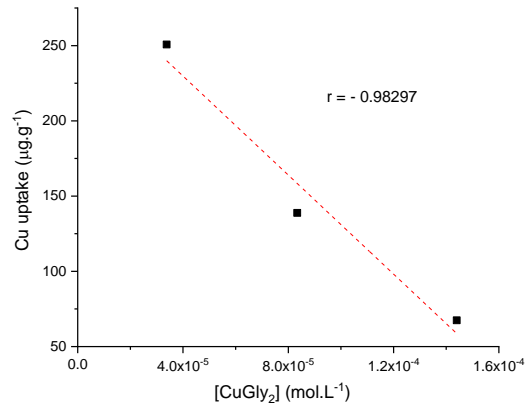
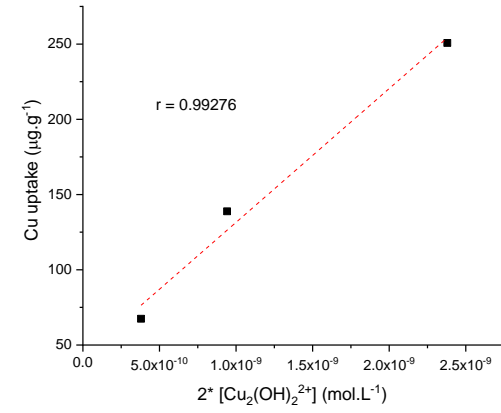
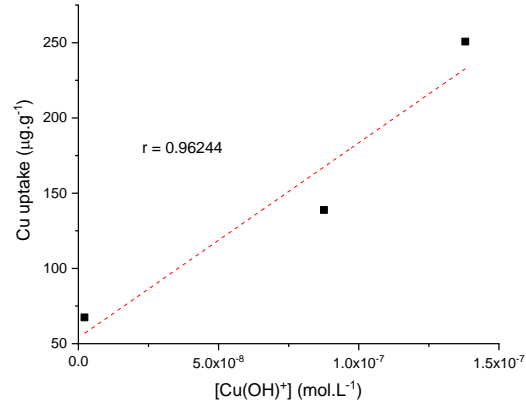
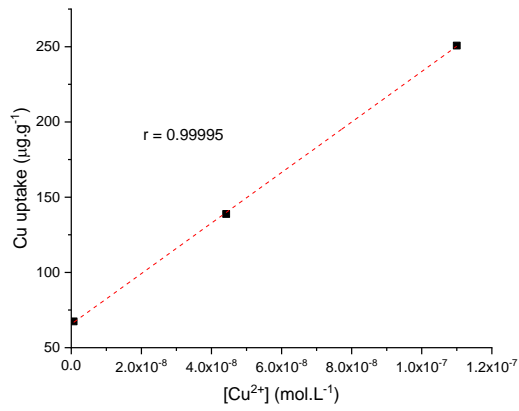


Figure 12: Cu uptake *versus* the concentration in solution of different Cu species, when Cu is singly added, in presence of Sac and Gly as DOC simple models.

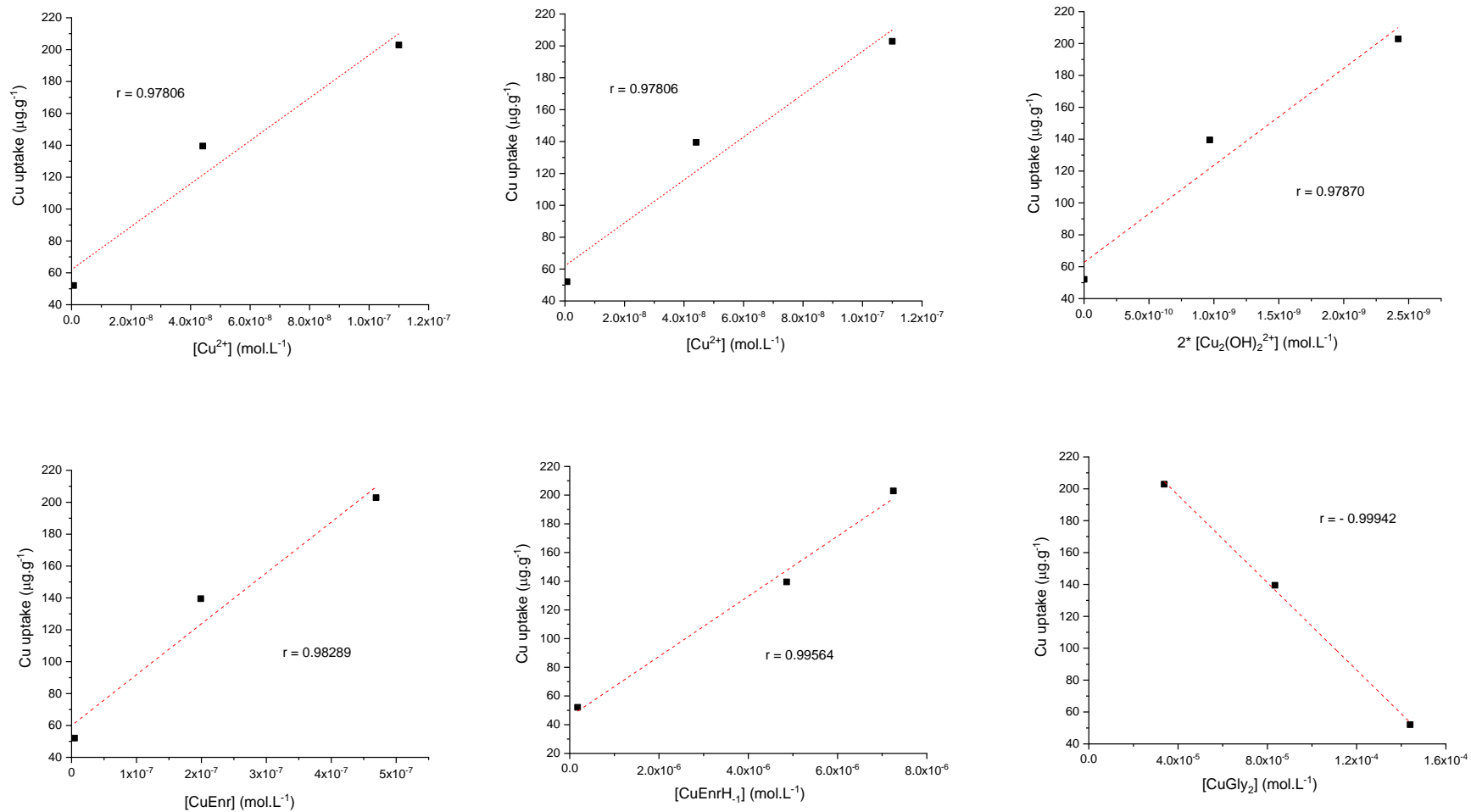


Figure 13: Cu uptake *versus* the concentration in solution of different Cu species, when Cu is simultaneously added with Enro, in presence of Sac and Gly as DOC simple models.

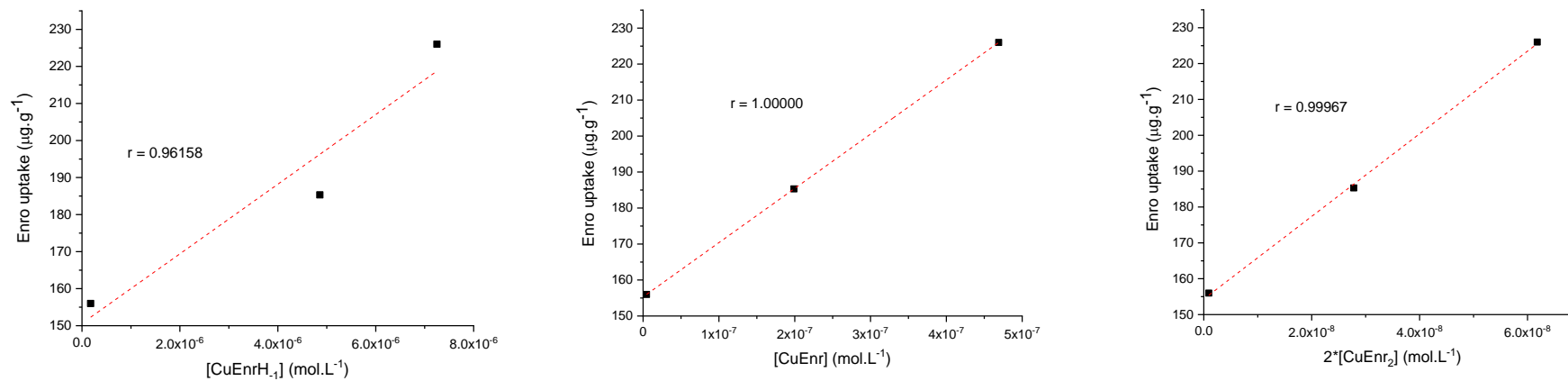


Figure 14: Enro uptake *versus* the concentration in solution of different Enro species, when Enro is simultaneously added with Cu, in presence of Sac and Gly as DOC simple models.

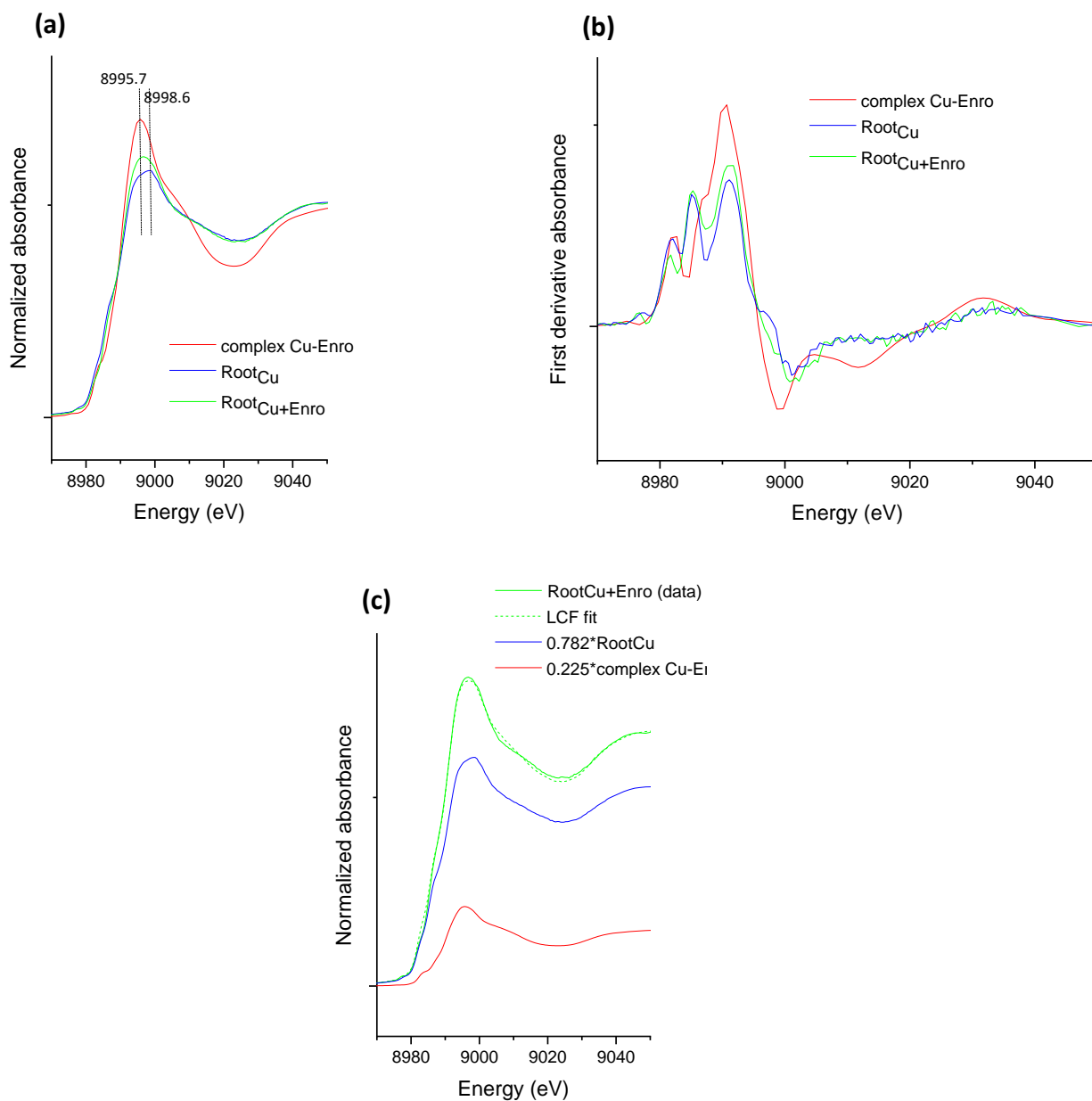
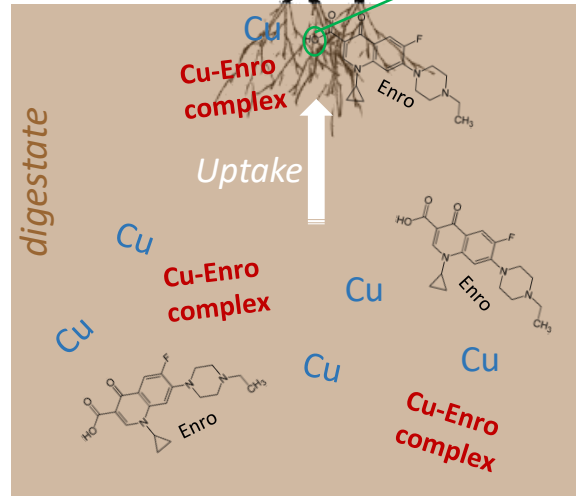
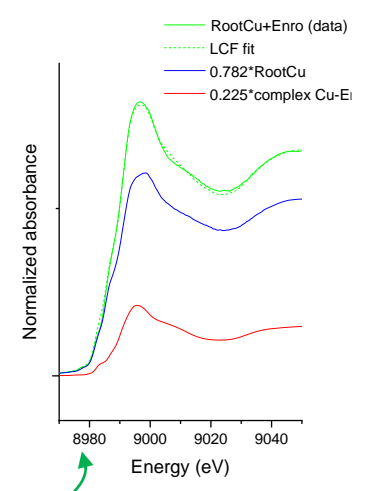


Figure 15: Normalized Cu K-edge XANES spectra of Cu taken up by *Phragmites australis* roots when singly added (Root_{Cu}) and simultaneously added with Enro ($\text{Root}_{\text{Cu+Enro}}$), and of the reference solid complex $\text{Cu}(\text{Enro})_2(\text{H}_2\text{O})_2$ (a), and their corresponding first derivative (b); Linear Combination Fit (LCF) of the $\text{Root}_{\text{Cu+Enr}}$ spectrum using the spectra of Root_{Cu} and of the complex Cu-Enr as components (c).



Simultaneous presence of Cu and Enro:

Cu uptake ↘ Enro uptake ↗

Cu-Enro complexation