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152 **Carbon based nanomaterial interactions with metals and metalloids in terrestrial**  
153 **environment: a review**

154  
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164  
165 **Abstract:** The use of carbon based nanomaterials (CNMs) is diversifying and is being  
166 considered in so many sectors that their release into the environment is inevitable. Terrestrial  
167 ecosystems and particularly agrosystems are considered a major sink for CNMs due to their  
168 accumulation in sewage sludge widely used for crop production. These ecosystems already  
169 accumulated other contaminants such as metals and metalloids which are more closely  
170 monitored to date. Although reviews about these contaminants considered individually have  
171 already been published, no review focused on their interactions and the impact of these  
172 interactions on their respective transfer and impacts on dwelling organisms (bacteria,  
173 earthworms and plants). Indeed, CNMs and metal(loid)s can interact together as metal(loid)s  
174 are able to adsorb on the surface of CNMs under the influence of diverse parameters such as  
175 pH, organic matter content or intrinsic CNM properties, among others. In ecotoxicity studies,  
176 interactive mechanisms (synergy and antagonism) were most of the time highlighted even

177 though the underlying mechanisms are not yet fully understood. This work questions the  
178 current paradigm in use for risk assessment related to the additivity of the risks in presence of  
179 a cocktail of contaminants and *in fine* on the strategies to be applied in order to guarantee  
180 food safety.

181

182 **Keywords:** adsorption, carbon nanotube, cocktail effect, organism, metal(loid), toxicity, soil

183

184 **Highlights:**

185 - Metal(loid) adsorption on CNM depends on pH, contact time, temperature, CNM properties.

186 - Most studies demonstrate a change in metal transfer in organisms due to CNM presence.

187 - Most studies showed synergic and/or antagonist effect on the toxicity of the cocktail.

188 - Additive effect model for risk assessment is not appropriate in many cases.

189 - More integrative markers should be considered to properly evaluate ecosystem health.

190

## 191 **1. Introduction**

192 Scientific and industrial interest in carbon-based nanomaterials (CNMs) continues to grow  
193 because of their remarkable properties and the applications they make possible<sup>1</sup>. A CNM is a  
194 nanomaterial essentially composed of carbon with any external dimension in the nanoscale or  
195 having internal structure or surface structure in the nanoscale (*i.e.* nanometric scale particles  
196 aggregated together)<sup>1</sup>. Among CNMs, most have a structure based on graphene, a sheet of  
197 carbon atoms joined together in a hexagonal lattice, one atom thick, sp<sup>2</sup> bonded<sup>2-4</sup>. Over the  
198 years, the number of derived materials using graphene as a precursor grew up<sup>5</sup>. Examples  
199 include multilayer graphene, functionalized graphene materials such as graphene oxide (GO),  
200 carbon nanotubes (CNTs), fullerene, and many metal-CNT composite

201 The potential applications of CNMs are such that models already predict the evolution of their  
202 concentrations in natural ecosystems after release at the end of life of commercial goods in  
203 which they are included, or expected during their life cycle. For example, Sun al.<sup>6</sup> predicted  
204 that agricultural soils treated with sewage sludge are the main sink for CNTs with an annual  
205 increase of 74 ng.kg<sup>-16</sup>. Furthermore, the use of CNMs as nanofertilizers<sup>7</sup>, nanopesticides<sup>8</sup> and  
206 as decontamination agents<sup>9</sup> is also increasingly being considered.

207 Indeed, CNMs are capable of adsorbing different compounds on their surface. Among these  
208 many compounds are metal(loid)s, which are elements present in natural conditions in biotope  
209 at concentrations below 100 mg.kg<sup>-1</sup> including copper (Cu), zinc (Zn), iron (Fe), manganese  
210 (Mn), molybdenum (Mo), boron (B), cobalt (Co), nickel (Ni), lead (Pb), cadmium (Cd),  
211 chromium (Cr), arsenic (As), and selenium (Se)<sup>10</sup>. Cu, Zn, Fe, Mn, Mo, and B are essential for  
212 plant growth, and Cu, Zn, Fe, Mn, Mo, Co and Se are essential to the growth and health of  
213 animals including human beings<sup>11</sup> while others are toxic (Ni, Pb, Cd, As). For example, it has  
214 been largely reported that CNTs can adsorbed metal(loid)s in aqueous solution<sup>12-17</sup> and this

215 ability raises questions about its influence on heavy metal transfer and subsequent impacts  
216 along food webs.

217 Agrosystems seems to be particularly suitable for the coexistence of both CNMs and  
218 metal(loid)s (among others) due to cultural practices. Indeed, the continuous spreading of  
219 organic and inorganic fertilizers and the application of sludge from wastewater treatment  
220 plants have progressively led to the accumulation of both CNMs and metal(loid)s in  
221 agrosystems<sup>18-20</sup>.

222 Toxicological risk assessment of contaminants is usually carried out using additive models  
223 that do not take into account the interactions between contaminants and their underlying  
224 toxicological effects. For several years, metal(loid)s are submitted to a close monitoring in  
225 sludge and food to ensure safety for populations, but the emerging contaminants such as  
226 CNMs are not yet regulated. Studies report that interactions (both synergy and/or antagonism)  
227 between contaminants can modify their transfer and toxicity to different organisms<sup>21-30</sup>  
228 (Figure 1).

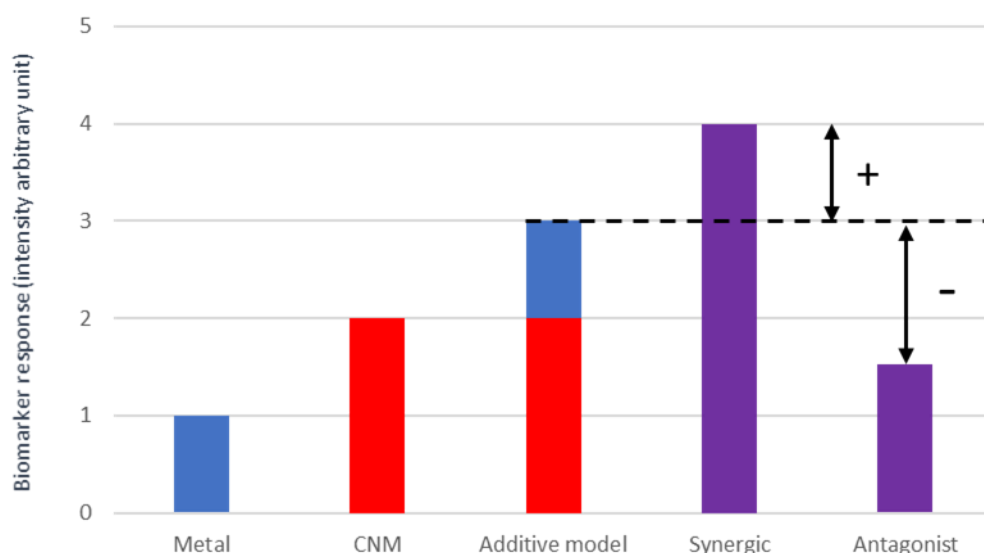


Figure 1 : Schematic differences on additive model compared to synergic and antagonist due to exposure to a cocktail of metal(loid) and CNM substances.

229

230 Tools have been developed to reveal possible interactions between stressors such as mixture  
231 of substances<sup>31-33</sup>. A meta-analysis by Vijver *et al.* aimed at predicting the response of  
232 organisms exposed to mixtures of metals (Cd, Cu and Zn) and showed that the frequency of  
233 occurrence of antagonism was 51%, and a synergistic response was detected in 28% of  
234 cases<sup>31</sup>. Thus, in nearly 80% of the cases, interactive and non-additive patterns were  
235 demonstrated. This finding may lead to a profound questioning of the current risk assessment  
236 performed by the authorities.

237 The main goal of this work was to extensively review articles related to 1) CNM-metal(loid)  
238 interactions in aqueous medium to better understand implied mechanisms and the influencing  
239 parameters and 2) ecotoxicological studies related to simultaneous CNM-metal(loid)  
240 contamination and their consequences on the transfer and impacts on agrosystem organisms to  
241 reveal the extent of the interactive phenomenon.

242

## 243 **2. Interaction CNMs - metal(loid)s**

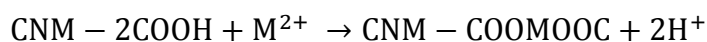
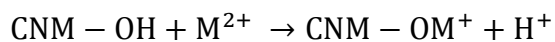
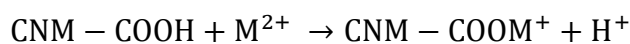
### 244 **2.1. Mechanisms of interactions between CNMs and metal(loid)s**

245

246 Several interaction mechanisms have been described between CNMs and metal(loid)s and  
247 their predominance depends on the material considered. Interactions can be related to physical  
248 adsorption, chemical interaction, electrostatic attraction and sorption-precipitation<sup>34-36</sup>.

249 For CNMs with surface functional groups, the chemical interactions between CNMs and  
250 metal cations is the most observed mechanism<sup>16</sup>. Metal cations are adsorbed onto the surface  
251 of CNMs via oxygenated functional groups (*e.g.*, -COOH, -OH, or C=O) and then release a  
252 H<sup>+</sup> ion from the surface of the CNMs (Figure 2, example for GO and CNT). The release of H<sup>+</sup>  
253 causes a decrease in the pH of the medium. The adsorption mechanism of divalent metal ions

254 on CNMs is illustrated according to the following equations (where CNM stands for carbon-  
 255 based nanomaterials, and M for metal(loid)):



256

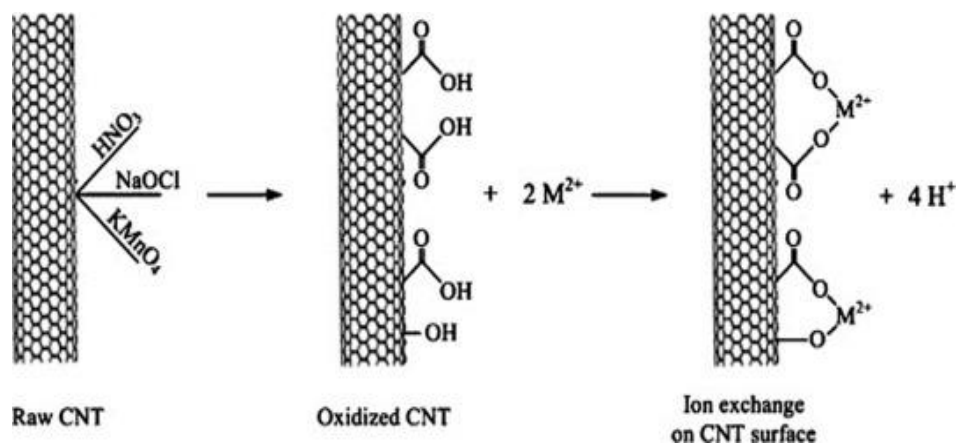


Figure 2 : Structure of carbon nanotubes (CNT) and their interactions with metal cations ( $=\text{M}^{2+}$ ) (Figure adapted from Rao et al. (2007)<sup>37</sup>)

257

258

259 Metal ion adsorption can also result from the combined effects of electrostatic attraction and  
 260 sorption-precipitation processes<sup>38,39</sup>. Liang *et al.*<sup>39</sup> observed that  $\text{Cd}^{2+}$  ions were adsorbed on  
 261 the surface of modified  $\text{Al}_2\text{O}_3$ -CNTs *via* physical interactions as electrostatic interactions and  
 262 van der Waals interactions occurred between  $\text{Cd}^{2+}$  ions and carbon atoms in the CNTs<sup>39</sup>. On  
 263 another study, following an increase in the pH value of the medium,  $\text{Zn}^{2+}$  ions were adsorbed  
 264 more on the surface of the CNTs due to the prevalence of carboxylate functions on their  
 265 surface. The authors attributed this observation to the electrostatic interactions between the  
 266 surface and divalent  $\text{Zn}^{2+}$  ions<sup>40</sup>.

267 In addition, CNMs have also been explored for the removal of anionic pollutants, such as  
 268 phosphates ( $\text{PO}_4^{3-}$ ), perchlorates ( $\text{ClO}_4^-$ ) and fluorides ( $\text{F}^-$ ), from aqueous solutions<sup>41-43</sup>. In  
 269 contrast to the immobilization of cationic metal species, the mechanism of anion adsorption

270 was previously attributed to electronic interactions with  $\pi$  electrons of the carbon  $sp^2$   
271 network<sup>43</sup>. This anion  $-\pi$  association is based on the interaction between the negatively  
272 charged anion with an electron-deficient aromatic ring on a graphene layer<sup>43</sup>.

273 Finally, new adsorbents have also been prepared by modifying CNMs with organic molecules  
274 that have a natural ability to complex metal ions. For instance, intrinsic functional groups on  
275 GO present a large variety and thus the specificity of interaction with given species of interest  
276 may be limited. To counteract this, the number of adsorption sites may be increased by  
277 grafting compounds such as ethylenediaminetetraacetic acid (EDTA)<sup>44</sup> and chitosan<sup>45</sup>.

278

## 279 **2.2. Factors influencing the CNM - metal(loid) interactions in simplified media**

### 280 **2.2.1. CNM intrinsic properties**

281 Due to the predominance of the adsorption mechanism by chemical interaction, it is obvious  
282 that the oxidation state of CNMs influences the sorption of metal(loid)s on their surface. The  
283 greater the oxidation of the CNMs, the more it provides oxygenated functional groups capable  
284 of interacting with the surrounding metal ions, regardless of CNM shape and size<sup>9,46,47</sup>.

285 Similarly, following the same idea, the degree of reduction of the CNMs acts on their  
286 adsorption capacity. The maximum adsorption capacity of rGO (12.6% of O) was much lower  
287 than that of GO (51.6% of O) due to the decrease in available oxygenated functional groups<sup>48</sup>.

288 If the oxidation state can be tuned upon synthesis, it is very unlikely that it evolves in the  
289 natural environment (with the exception of fullerenes). Their reduction may however be easier  
290 to occur in environmental conditions. There are many examples of partial reduction of GO by  
291 natural molecules extracted from plant leaves<sup>49</sup>. However, enzymatic transformations of  
292 CNMs are unfortunately often investigated using only pure enzymes and under laboratory



293 conditions and they are consequently not very representative of enzymatic transformations in  
294 the natural environment<sup>50</sup>

295 The specific surface area (SSA, m<sup>2</sup>.g<sup>-1</sup>) strongly influences the adsorption of metals. In the  
296 case of graphene, most interaction sites with pollutants are present at the top and bottom  
297 faces<sup>51,52</sup>, even if sites located at the edge may also play some role. While all the surface of  
298 monolayer graphene is available for the adsorption, multilayer graphene and CNTs have  
299 internal layers respectively little or not accessible by adsorbates<sup>51</sup>. Similarly, it has been  
300 observed that single walled CNTs are good adsorbents, which can be attributed to their high  
301 SSA. Practically, the measured SSA of graphene-based CNMs is generally very disappointing  
302 (often below a few tens of m<sup>2</sup>.g<sup>-1</sup>) when compared to the expected theoretical values (> 2600  
303 m<sup>2</sup>.g<sup>-1</sup> for graphene)<sup>53</sup> due to their significant agglomeration.

304

### 305 **2.2.2. Environmental parameters**

306 The relative concentrations of CNMs and metal ions in the medium is an important parameter  
307 controlling adsorption phenomenon. To better understand these interactions, some theoretical  
308 model isotherms were developed, the most common being the Langmuir and the Freundlich  
309 models<sup>54</sup> according to the following equations:

$$\text{Langmuir : } q_e = k_L q_m C_e / (1 + k_L C_e)$$

$$\text{Freundlich : } q_e = k_F C_e^{1/n}$$

310 where  $q_e$  and  $q_m$  (mg.g<sup>-1</sup>) represent equilibrium and maximum adsorption capacities,  
311 respectively;  $C_e$  (mg.L<sup>-1</sup>) is the equilibrium aqueous concentration;  $k_L$  (L.mg<sup>-1</sup>) the  
312 equilibrium constant and  $k_F$  and  $n$  the Freundlich constant.

313 These two models differ in particular by the fact that Langmuir model is considering the  
314 adsorption of a monolayer of atoms on a homogeneous surface and is empirical while  
315 Freundlich considers the adsorption of a multilayer of atoms on a heterogeneous surface and  
316 is a theoretical model.

317 Adsorption kinetics are generally described by diffusion models such as the pseudo-first-order  
318 and pseudo-second-order following the equations<sup>54</sup>:

$$\textit{Pseudo first order} : \log(q_e - q_t) = \log q_e - k_1 t / 2.303$$

$$\textit{Pseudo second order} : q_t = k_2 q_e^2 t / (t k_2 q_e + 1)$$

319 where  $q_t$  and  $q_e$  are the adsorption capacities ( $\text{mg}\cdot\text{g}^{-1}$ ) at time  $t$  and equilibrium,  $k_1$  ( $\text{min}^{-1}$ ) and  
320  $k_2$  ( $\text{min}^{-1}$ ) are the rate constants of the pseudo-first-order, pseudo-second-order, respectively.

321 Results extracted from these models show that the main environmental factors affecting the  
322 adsorption of metal ions on the surface of CNMs include the initial metal(loid) ion and CNM  
323 concentrations, pH, contact time, temperature, ionic strength and competition with other  
324 molecules. Table S1 summarizes the data found in the literature regarding CNM interactions  
325 with different metal(loid)s.

326 Regarding the intrinsic properties of the CNMs and the properties of the experimental  
327 medium, which both influence the adsorption of metal(loid)s, a criticism can be raised related  
328 to the lack of reported experimental details in the literature. Indeed, out of the 102 CNMs  
329 tested, the SSA was reported for only 48 of them. Furthermore, as the Freundlich and  
330 Langmuir models are thermodynamic processes, it is unfortunate that the study temperature  
331 was provided only in 56 cases out of 102. This lack of reporting makes the intercomparability  
332 of the studies impossible and thus weakens the conclusions and slows down the scientific  
333 progress, especially for the safer-by-design and nanoecotoxicity fields.

334 The average adsorption values from the Langmuir model for CNTs, oxidized CNTs and  
 335 graphene oxide have been extracted from related articles for different metal(loid)s (Cd, Cr,  
 336 Cu, Hg, Ni, Pb and Zn; Table 1).

337

338 *Table 1 : Mean of maximum adsorption ( $q_m = \text{mg.g}^{-1}$ ) of metal(loid)s (Cd, Cr, Cu, Hg, Ni, Pb and Zn) on the*  
 339 *surface of carbon nanotubes (CNT), oxidized carbon nanotubes (CNT-oxidized) and graphene oxide (GO) in*  
 340 *mono-elemental conditions extracted from the reported studies in Table S1 described with Langmuir model.*  
 341 *Data were selected on restricted pH range ( $4 < \text{pH} < 8$ ) in which adsorption is favored ( $n_{\text{tot}}=117$ ).*

	Cd (n=20)	Cr (n=13)	Cu (n=17)	Hg (n=19)	Ni (n=9)	Pb (n=20)	Zn (n=19)
$q_{m_{\text{CNT}}}$	12.4 ( $\pm 9.9$ )	3.1 (n=1)	22.9 ( $\pm 2.6$ )	24.4 ( $\pm 38.9$ )	11.1 ( $\pm 3.8$ )	30.9 ( $\pm 2.6$ )	35.6 ( $\pm 9.4$ )
$q_{m_{\text{CNT-oxidized}}}$	35.9 ( $\pm 28.2$ )	21.3 ( $\pm 11.7$ )	37.4 ( $\pm 27.3$ )	27.3 (n = 1)	49.2 (n = 1)	75.7 ( $\pm 31.3$ )	24.4 ( $\pm 28.8$ )
$q_{m_{\text{GO}}}$	306.9 ( $\pm 315.7$ )	-	183.3 ( $\pm 156.6$ )	-	84.4 ( $\pm 19.7$ )	1280.7 ( $\pm 516.5$ )	345.0 (n=1)

342

343 The adsorption of each metal(loid) appears to be always higher for GO than for CNTs and  
 344 oxidized CNTs. Similarly, the oxidation of CNTs promotes the adsorption of metal(loid)s  
 345 since the average adsorption of metal(loid)s on the surface of oxidized CNTs is higher for  
 346 each metal (except Zn, probably related to the high variability for Zn adsorption onto oxidized  
 347 CNTs) than on the surface of raw CNTs. On the basis of the data in Table 1, the sequence of  
 348 adsorption affinities in mono-elemental conditions can be drawn up:

349 - for CNTs:  $\text{Zn} \geq \text{Pb} > \text{Hg} \geq \text{Cu} > \text{Cd} \geq \text{Ni} > \text{Cr}$

350 - for CNTs oxidized:  $\text{Pb} > \text{Ni} > \text{Cu} \geq \text{Cd} > \text{Hg} \geq \text{Cr} \geq \text{Zn}$ .

351 It is also important to note that these adsorption experiments were carried out in mono-  
 352 elemental conditions (one adsorbent + one ionic species) thus not considering potential ionic  
 353 repulsions possibly induced if several ionic species would be present in the solution.

354

### 355 2.2.2.1. pH

356 The pH is a parameter that greatly influences the interactions between CNMs and metal  
357 cations because it determines not only metal speciation but also surface charge of CNMs and  
358 complexation behavior of functional groups<sup>55,56</sup>. Most of the studies in Table S1 evidenced  
359 that the adsorption capacity of CNMs was affected by variations in the pH of the  
360 solution<sup>34,37,57,58</sup>. Adsorption of the metal(loid) is partly related to the point of zero charge  
361 ( $\text{pH}_{\text{PZC}}$ ) of a given CNM; when  $\text{pH} > \text{pH}_{\text{PZC}}$ , cation adsorption is higher due to the  
362 electrostatic interactions between the cations and the negative surface charge of CNM. On the  
363 other hand, the adsorption of cations is lower when  $\text{pH} \leq \text{pH}_{\text{PZC}}$  due to the neutralization of  
364 the surface charge of CNM, or even the presence of repulsive positive charges. A change in  
365 pH also affects competing complexing reactions and metal ion species<sup>36</sup>. Generally, the state  
366 of the divalent metal ions in solution depends on the pH. The most common species would be  
367 present in the form of  $\text{M}^{2+}$ ,  $\text{M}(\text{OH})^+$ ,  $\text{M}(\text{OH})_2$  et  $\text{M}(\text{OH})_3^-$  depending on the pH of the  
368 medium due to hydrolysis<sup>16,34,36</sup>. It is important to note that precipitation of metal cations  
369 may occur when the pH increases, which also affects the removal of metal ions<sup>59</sup>.

370

### 371 2.2.2.2. CNM concentration

372 Increasing the concentration of CNM up to a certain point, increases the overall surface  
373 available for reactions and thus increases the sorption of metal(loid)s. For instance, adsorption  
374 of  $\text{Ni}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  increased with increasing CNT concentration in different  
375 experiments<sup>35,37,38,60</sup>. Similar results were also observed for  $\text{GO}^{61}$ . However, beyond a  
376 threshold mass (specific to the CNM studied), the adsorption capacity reaches saturation and  
377 then decreases<sup>15,61,62</sup>. For a exposition of  $50 \text{ mg.L}^{-1}$  of oxidized SWCNT, Moradi et al.<sup>15</sup>  
378 observed a maximum adsorption capacity up to 55, 68 and  $90 \text{ mg.g}^{-1}$  for Cd, Cu and Pb

379 respectively, while this adsorption decreased at 45, 58 and 82 mg.g<sup>-1</sup> with a exposition to 100  
380 mg.L<sup>-1</sup>. Indeed, beyond the threshold, an increase in the concentration of adsorbent could  
381 reduce the available sites because the effective surface area is likely to decrease due to homo-  
382 agglomeration, entanglement and/or folding of the CNMs (depending on its morphology)<sup>15,63</sup>.  
383 In addition, metal ions can induce CNM agglomeration due to their interactions with  
384 carboxylic groups<sup>64</sup>.

385

### 386 **2.2.2.3. Ionic strength**

387 Increasing the ionic strength of the solution can be achieved by increasing the concentration  
388 of an ion or in multi-elemental conditions by having several types of metal ions in the  
389 medium. However, this last experimental set-up even though more relevant in environmental  
390 context has been much less studied. The adsorption capacities of ions by CNMs decreased  
391 with increasing ionic strength for two reasons : (i) the formation of double-layer electrical  
392 complexes between cations and CNMs and (ii) the influence of the ionic strength on  
393 the activity coefficients of ions, which limit their transfer to the CNM surfaces<sup>38</sup>. In multi-  
394 elemental conditions, the increase in number of ions present in the media decreases the part of  
395 metal ions adsorbed onto CNMs. Affinity sequences of metal ions for adsorption sites could  
396 be deduced from these multi-elemental adsorption experiments (Table 2). For instance, Salam  
397 *et al.*<sup>65</sup> showed that ion binding affinity followed the order of Cu(II) > Zn(II) > Pb(II) > Cd(II)  
398 for MWCNTs (initial metal concentrations = 1 mg.L<sup>-1</sup>, pH = 7, contact time = 2h). For GO,  
399 Sitko *et al.*<sup>66</sup> observed another ranking of affinity: Pb(II) > Cu(II) > Cd(II) > Zn(II) (initial  
400 concentration = 1 mg.L<sup>-1</sup>, GO = 0.1 g.L<sup>-1</sup>, pH = 5, contact time = 2h). Metal precipitation  
401 related to the pH of the solution could explain differences observed on affinity sequences<sup>67</sup>.  
402 Furthermore, the addition of metals in the medium could increase the co-precipitation<sup>68</sup> and

403 could modified the fitting of experimental adsorption measured with Langmuir and/or  
404 Freundlich modelling. To date, very little studies dealing with competitive adsorptions are  
405 available and more work needs to be carried out to understand the underlying mechanisms  
406 responsible for the establishment of these adsorption affinity sequences.

Table 2: Maximum adsorption capacity of different CNMs established by adsorption models for various metal(loid)s in multi-elemental conditions extracted from the cited articles (SSA = specific area ( $m^2.g^{-1}$ ),  $q_m$  = maximum modelled adsorption ( $mg.g^{-1}$ ),  $R^2$  = correlation coefficient, C = metal concentration range ( $mg.L^{-1}$ ), A = Adsorbent dosage ( $mg.L^{-1}$ ), T = temperature ( $^{\circ}C$ ), Ct = Contact time (hours), RPM = agitation (Rotation Per Minutes))

M <sup>n+</sup>	Adsorbent	SSA	Isotherm model		C	A	Seq affinity	Parameters				References
			Langmuir					T	Ct	pH	RPM	
			qm	R2								
Cd(II), Cu(II), Pb(II), Zn(II)	MWCNT	61.5			1	2500 to 15 000	Cu>Zn>Pb>Cd		2	7	400	Salam <i>et al.</i> <sup>65</sup>
Cu(II), Pb(II), phenol	N <sub>2</sub> H <sub>4</sub> -SH-Fe <sub>3</sub> O <sub>4</sub> /o- MWCNT		Pb: 40, Zn: 21, Phenol: 56	Pb: 0.91, Zn: 0.96, Phenol: 0.9	20 to 60	200	Pb>Cu	25	12	6		Jiang <i>et al.</i> <sup>69</sup>
Pb(II), Cu(II), Ni(II)	carbonaceous nanofibers	150.3	Pb: 470.3, Cu: 98.5, Ni: 64.6		0.1 to 0.6 mmol.L <sup>-1</sup> (Pb: 20.7 to 124.3 ; Cu: 6.5 to 39.3 ; Ni: 5.9 to 35 mg.L <sup>-1</sup> )	200	Pb>Cu>Ni	25		5.5		Ding <i>et al.</i> <sup>70</sup>
Cd(II), Hg(II), As(II)	GO		Cd: 66.8, Hg: 55.4, As: 23.7			333.3	Cd>Hg>As					Alimohammady <i>et al.</i> <sup>71</sup>
Cd(II), Hg(II), As(II)	GO-3- aminopyrazole		Cd: 85.3, Hg: 74.6, As: 38.3		20	333.3	Cd>Hg>As		2	8	250	
Cd(II), Cu(II), Pb(II), Zn(II)	GO				5 $\mu$ mol.g <sup>-1</sup> of GO	100	Pb>Cu>Cd>Zn					Sitko <i>et al.</i> <sup>66</sup>
Cd(II), Cu(II), Ni(II)	GO membrane				0.1 to 1 mmol.L <sup>-1</sup> (Cd: 11 to 112 ; Cu: 6 to 64 ; Ni: 6 to 59 mg.L <sup>-1</sup> )	200	Cu>Cd>Ni	30	12	5.5	150	Tan <i>et al.</i> <sup>72</sup>

Cd(II), Cu(II), Pb(II)	O-MWCNT		Cd: 3.3, Cu: 17.0, Pb: 34.0	Cd: 0.21, Cu: 0.97, Pb: 0.97	5 to 30	1000	Pb>Cu>Cd			5		Li <i>et al.</i> <sup>38</sup>
Ni(II), Zn(II)	SWCNT	700	Ni: 28.4, Zn: 29.5	Ni: 0.98, Zn : 0.98	10 to 80	500	Ni=Zn		12		180	Lu <i>et al.</i> <sup>73</sup>
Ni(II) and Zn(II)	MWCNT	459	Ni: 18.6, Zn: 19.4	Ni: 0.98, Zn: 0.98	10 to 80	500	Ni=Zn		12		180	



#### 210 **2.2.2.4. Contact time**

211 As the contact time increases, the percentage of metal removal increases until an equilibrium  
212 is established<sup>16,36</sup>. The equilibrium depends on all the experimental parameters of the medium,  
213 including the initial concentration of metal ions. However, the two most important parameters  
214 on the adsorption kinetics of metals is the number of adsorption sites available for a given  
215 metal concentration and contact time<sup>16,36</sup>.

216

#### 217 **2.2.2.5. Temperature**

218 The most common observation is that sorption capacity increases with rising temperature<sup>16,36</sup>.  
219 For example, the sorption of  $\text{Pb}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  on CNTs is an endothermic process and  
220 increases with increasing temperature<sup>14,74</sup>. Similar results were observed for the same metals  
221 for GO<sup>66</sup>. Authors explained that the increase in temperature could lead to an increase in the  
222 rate of diffusion of ions through the outer boundary layer and into the pores of CNMs due to a  
223 temperature-induced decrease in the viscosity of the solution<sup>36,66</sup>.

224

### 225 **2.3. Metal(loid) adsorption by CNMs in complex media**

226 Due to the increasing use of CNMs, their potential in the agricultural and environmental fields  
227 and their interactions with metal(loid)s in simplified environments (aqueous solution), it is  
228 important to investigate how interactions investigated in simplified medium can be transposed  
229 in more complex environments. Various studies have thus recently been carried out (i) to  
230 characterize interactions of CNMs with compounds that are widely found in ecosystems, such  
231 as organic matter (OM) and sedimentary matrices and (ii) to investigate the role of some key  
232 parameters.

233

### 234 **2.3.1. Organic Matter**

235 Humic substances generally represent a predominant part of the natural OM distributed in  
236 soils, sediments and water<sup>75</sup>. It has been reported that 3 to 28% of the OM dissolved in  
237 wastewater treatment plant effluents are humic substances<sup>76</sup>. They are hydrophobic organic  
238 acids mainly derived from soil humus and plants. These humic substances are often  
239 encountered in agrosystems especially due to the use of wastewater treatment plant effluents  
240 for fertilizing agricultural soils. Humic acid (HA) and fulvic acid (FA) are the two most  
241 important components of humic substances. It is expected that HA/FA will have a significant  
242 influence on the sorption of metal ions by CNMs because the hydrophobic parts as well as the  
243 aromatic monomers of HA/FA have a strong affinity for CNMs via hydrophobic and  $\pi - \pi$   
244 interactions. At the same time, the hydrophilic fractions of HA/FA contain various functional  
245 groups (carboxyl, phenol, hydroxyl, amine and quinine) which can bind to metal(loid)s<sup>77</sup>. For  
246 instance, Lin *et al.*<sup>77</sup> observed that HA bound to the surface of MWCNTs significantly  
247 favoured Pb(II) sorption (pH = 5.0 ) (Figure 3A). Similar results were also observed for the  
248 sorption of Cd(II) (pH = 6.0) on HA-coated MWCNTs<sup>78</sup>. Additionally, OM can also increase  
249 the stability of CNMs in suspension as the OM polymeric chains adsorbed to their surface  
250 tend to avoid each other in solution so as not to constrain the conformational space available  
251 to each of them inducing repulsive forces and thus decreasing the chances of homo-  
252 agglomeration<sup>79,80</sup> (and in turn increasing the potential available sites for metal(loid)  
253 adsorption) (Figure 3B and 3C). These interactions also depend on both pH and OM  
254 composition. Indeed, Sheng *et al.* reported an increase in the adsorption of Cu(II) on  
255 MWCNTs due to dissolved HA/FA at pH < 7.5 (up to 90%), while a decrease was found at  
256 pH > 7.5 (to 80%)<sup>81</sup>. In parallel, Sun *et al.*<sup>82</sup> found that there was a higher binding affinity  
257 between Cu(II) and FA compared to HA, due to more oxygenated functional groups present

258 in FA that could also complex with Cu(II) (pH = 6). Thus, it seems that there are different  
259 OM-metal(loid)- CNM interaction processes.

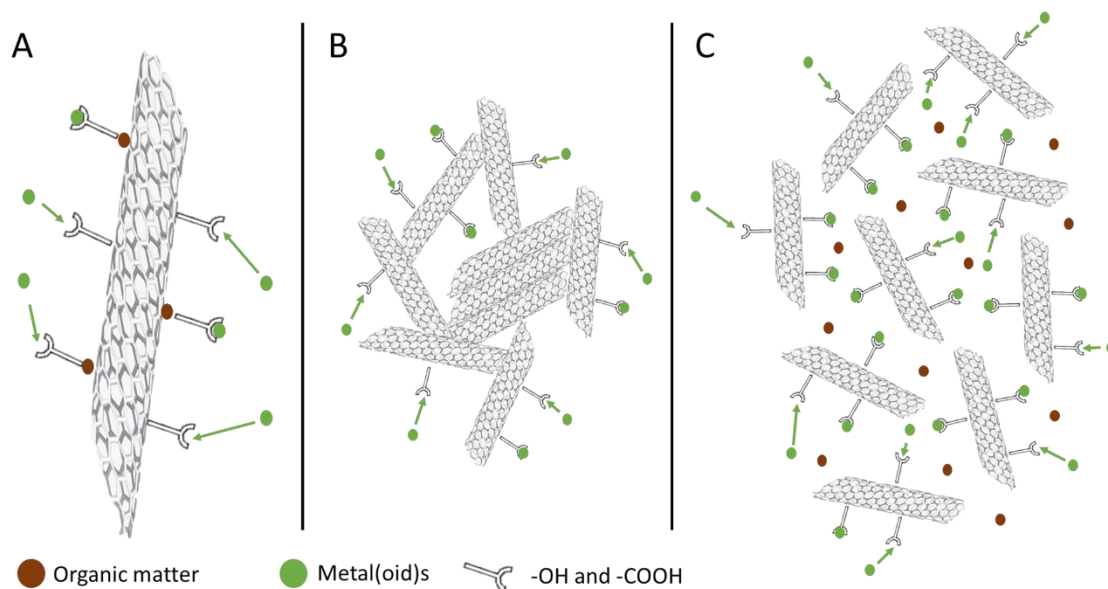


Figure 3 : Effects of organic matter (A) bound on CNTs which increase metal(loid) adsorption onto CNTs and (B-C) on the dispersion of CNTs in media (B : without OM, C : with OM)

260

### 261 2.3.2. Soil

262 First of all, it should be reminded that due to the complexity of both soil and sediment, very  
263 few studies have been conducted to understand (i) what are the properties of these matrices  
264 that influence the mobility and/or agglomeration of CNMs and (ii) the related mechanisms.

265 However, as in simplified conditions, CNMs influence the fate of metal(loid)s in soil. For  
266 instance, a study revealed that Pb and Cu immobilization in soil increased with increasing  
267 MWCNT concentration correlated with the increased adsorption sites on the surface of CNTs  
268 available for Pb and Cu ion adsorption<sup>83</sup>.

269 These interactions between CNMs and metal(loid)s in soil vary according to CNM intrinsic  
270 properties such as their morphology (diameter, length) and their surface chemistry (surface

271 charge and functionalization)<sup>84-86</sup>. While it can be expected that the smaller the CNM, the  
272 higher their mobility in soils, it remains difficult to support this assumption from available  
273 data in the literature.

274 In addition, properties such as soil texture, structure, porosity, permeability, or type of clay  
275 contribute to the actual flow of CNMs through the soil<sup>84</sup>. It appears that MWCNTs<sup>87,88</sup> and  
276 GO<sup>89</sup> generally tend to have higher retention (low transport) in clay than in sandy soils. For  
277 example, the retention profile indicated that retention was higher in soil with smaller size sand  
278 particles (240  $\mu\text{m}$ ) than soil with higher size sand particles (607  $\mu\text{m}$ )<sup>90</sup>. Lu *et al.* showed that  
279 the type of clay influenced CNM transport because the addition of 5% of kaolinite inhibited  
280 more graphene transport in soil column than montmorillonite and illite which presented the  
281 same particle size (Kaolinite: 28.0  $\mu\text{m}$ , montmorillonite: 20.0  $\mu\text{m}$  and illite: 23.9  $\mu\text{m}$ ) due to  
282 greater amounts of positively charged edge sites. Furthermore, the ionic strength of the soil  
283 solution is also a key parameter to consider. Indeed, Qi *et al.* demonstrated that the addition of  
284 0.5 mM of  $\text{Ca}^{2+}$  significantly inhibited GO transport in clay soil in contrast to sandy soil by  
285 complexing with the surface oxygen of GO and soil components leading to enhance GO  
286 agglomeration and link GO with soil grains<sup>91</sup>.

287 Several studies using Toxicity Characteristic Leaching Procedures (TCLP) determined the  
288 leachability of metal(loid)s in CNT-amended soils. For instance, Yang *et al.*<sup>92</sup> observed that  
289 the bioaccessible Sb content in a loamy soil was significantly reduced (20.7%) with the  
290 addition of 0.9 wt.% MWCNTs compared to control (without CNT addition). In sandy soil,  
291 Vithanage *et al.*<sup>93</sup> did not observe any significant difference in extractable Sb at the tested  
292 MWCNT concentrations (0.5, 1 and 2.5 wt.%). The immobilisation and, consequently, the  
293 bioavailability of Sb differed between the two studies, which could be related to different  
294 texture and other related parameters (cation exchange capacity, structure, water  
295 holding capacity).

296 As in simplified medium, the interaction of CNMs and metal(loid)s is metal dependent.  
297 Indeed, a study using 0.2, 1 and 5 wt.% GO in a historically polluted soil showed that the  
298 availability of Pb and Cu followed a dose-dependent relationship so that at the highest  
299 concentration of GO, their availabilities relative to the controls were found to be reduced by  
300 2.5% and 5.6% respectively. This decrease was much smaller for Zn and Cd (*ca.* 1%) and As  
301 availability was increased by 191%<sup>94</sup>. Regarding the kinetics, it seems that reaching the  
302 equilibrium takes much longer in more complex environments than in simplified medium. For  
303 example, Song *et al.*<sup>95</sup> observed that in soil solution the adsorption of Cd<sup>2+</sup> on MWCNTs  
304 reached 65% after 7 days and up to 87% and 88% after 30 and 60 days, respectively. To  
305 compare with aqueous media, the time to reach equilibrium does not exceed 12 hours (Table  
306 S1).

307 Overall, more studies involving solid matrices under environmentally relevant conditions  
308 need to be carried out as the risk of CNM release into the environment is increasing. It is  
309 important to understand all the physico-chemical characteristics of soils affecting the  
310 interactions among contaminants in order to better predict their transfer and toxicity in the  
311 environment and along trophic chains.

312

### 313 **3. Metal(loid)s, CNM and CNM-metal(loid) complex uptake and impacts on agrosystem** 314 **organisms**

#### 315 **3.1. Uptake and impacts of metal(loid)s and CNMs as single contaminant**

316 The toxicological impacts of metal(loid)s and CNMs and their potential consequences on  
317 agrosystems have been widely studied. Thus, the scientific corpus available is abundant and  
318 compiled in several scientific articles for microorganisms<sup>96-101</sup>, invertebrates<sup>96,102-106</sup> and crop  
319 plants<sup>19,96,101,102,107-110</sup> which are the major organisms of agrosystems due to their functions

320 and interactions. This corpus is regularly updated according to the technical advances,  
321 offering a step further on the scientific knowledge in these fields of research especially for  
322 metal(loid)s due to older knowledge.

323 Microorganisms are essential for many agrosystem services like mineralization, C, N and P  
324 biogeochemical cycles and because of their interactions with plants like mycorrhizal  
325 association<sup>111</sup>. Microorganisms are capable of accumulating metal(loid)s, this accumulation is  
326 generally governed by the sorptivity of the cell envelope and the ability to absorb metal(loid)s  
327 into the cytosol<sup>112</sup>. The metal membrane transporters are not highly selective and thus  
328 metal(loid)s can be taken up through this pathway<sup>112,113</sup> leading to alterations in the  
329 microorganisms at different scales. Metal(loid) toxicity could occur through displacement of  
330 essential metals from their original binding sites or through ligand interactions and  
331 conformational structure alterations with oxidative phosphorylation and osmotic balance<sup>114</sup>.  
332 Damage to the DNA structure, the cell membrane and the activity of some enzymes was also  
333 reported<sup>97,98,115-117</sup>. Thus, metal(loid)s can affect the microbial population by decreasing their  
334 growth/proliferation, and altering their morphology and biochemical activities<sup>100,118</sup>. Due to  
335 these alterations, the functions performed by microorganisms such as the decomposition of  
336 organic matter<sup>97,98</sup>, C sequestration or N fixation could be negatively impacted<sup>98,99,119</sup>.  
337 According to a study that compiled the lowest metal(loid) amount added that resulted in a  
338 decrease in the rate of basal respiration (LOAED), the mean LOAED for Hg, C, Cu, Pb, Ni,  
339 Cd, Zn was 257, 411, 500, 506, 576, 594 and 697 mg.kg<sup>-1</sup>, respectively (Table S2)<sup>98</sup>.

340 In parallel, for CNMs, transmission electron microscopy observations have evidenced that  
341 they may interact strongly with bacterial cell membranes. These interactions may disrupt the  
342 integrity of the membrane structure by oxidative stress and/or physical damage, allowing  
343 CNMs to be internalized in bacteria<sup>120-122</sup>. In addition, a majority of studies has observed a  
344 decrease in soil microbial activity following exposure to CNMs<sup>122-127</sup>. CNMs would present

345 an antimicrobial potential since a whole set of studies reported a loss of viability following  
346 exposure<sup>123,124,128–130</sup>. Changes in microbial community structure have also been  
347 reported<sup>123,126,131,132</sup>. Finally, microbial functions such as mineralization, N and CO<sub>2</sub> fixation  
348 could be decreased<sup>133</sup>. The effect of CNMs on microbial activity<sup>122</sup> and biomass appeared at  
349 85 mg.L<sup>-1</sup> and 1-5 mg.g<sup>-1</sup>, respectively<sup>124,126,127</sup>.

350 Oligochaetes are considered as soil engineers because of their involvement in physical and  
351 chemical processes such as litter mixing, soil porosity, active soil water transport, OM  
352 mineralization and nutrient mobilization and cycles<sup>134–136</sup>. These organisms present two  
353 pathways of contamination: the oral pathway by ingestion of soil and the tegumentary  
354 pathway by contact with soil. After absorption, metal(loid)s can accumulate in the posterior  
355 alimentary canal<sup>137</sup>. Metal(loid)s may decrease the growth of earthworms through delayed  
356 biomass gain<sup>138–141</sup> and act on energy reserves by decreasing the production of lipid  
357 reserves<sup>142,143</sup> and modifying the gut microbiota<sup>144</sup>. In addition, morphological and  
358 behavioural changes have been demonstrated following exposure to metal(loid)s<sup>145</sup>. It was  
359 observed that metal(loid)s can interfere with earthworm reproduction by reducing the number  
360 of juveniles produced<sup>138,139,146</sup> induced by a reduction of number of sperm produced<sup>147</sup>, the  
361 production of cocoons<sup>138,141,146,148–150</sup> and the success of cocoon hatching<sup>139,151,152</sup>. Median  
362 lethal concentrations (LC<sub>50</sub>) of metal(loid) after a 17 day exposure were on average 97, 663,  
363 756, 791, 836, 1415, 7542 mg.kg<sup>-1</sup> for As, Cu, Ni, Cd, Zn, Cr(III) and Pb, respectively<sup>103</sup>.

364 For CNM exposure, it has been demonstrated that earthworms are able to eliminate orally  
365 ingested CNMs by depuration thus limiting the toxicity of these contaminants<sup>153–155</sup>. As a  
366 result, many studies have not observed any apparent effects on earthworms following CNM  
367 exposure<sup>154,156,157</sup>. However, few works reported DNA damages<sup>158,159</sup> and a decreased cocoon  
368 production<sup>160</sup> (up to -60% and -78% at 495 and 1000 mg DWCNT.kg<sup>-1</sup> food). According to  
369 Scott-Fordsmand *et al.*<sup>160</sup> and Hu *et al.*<sup>158</sup>, the median effective concentration (EC<sub>50</sub>) for

370 earthworms exposed to DWCNT and MWCNT would be  $>500$  and  $1480.3 \text{ mg.kg}^{-1}$  for  
371 growth and mortality, respectively.

372 Plants can take up metal(loid)s from the soil through their roots, transport them through the  
373 xylem and accumulate them in the different tissues of all organs (roots, stem, leaves, grains  
374 and fruits) by symplastic and apoplastic pathways<sup>161</sup>. Generally, the roots tend to accumulate  
375 metal(loid)s such as Al, Cd, Cr, Cu, Hg, Ni, P and Zn, vegetative parts have a lower  
376 accumulation level and the reproductive parts tend to exclude them<sup>162,161</sup>. During their  
377 transport in the plant, metal(loid)s are largely bound to the cell walls, which explains why  
378 most of the metal(loid)s are bound in the roots (about 75-90%) and a smaller fraction in the  
379 shoots<sup>161</sup>. However, some particularities exist and accumulation is a function of the stage of  
380 development of the plant and the metal(loid) considered<sup>161</sup>. The stress imposed by meta(loid)l  
381 accumulation can induce reactive oxygen species (ROS), promote DNA damage, impair  
382 functional membrane integrity, nutrient homeostasis and disrupt protein and enzyme function  
383 and activity<sup>163-165</sup>. These toxic effects lead to a slowing down of cellular parameters, *i.e.* a  
384 reduction of photosynthetic activity, lipid production<sup>107,166</sup>, plant growth parameters, and  
385 consequently of the yield<sup>107,166-172</sup>. The phytotoxicity of the metal(loid)s under their ionic  
386 form follows the trend (from most to least toxic):  $\text{Pb} \approx \text{Hg} > \text{Cu} > \text{Cd} \approx \text{As} > \text{Co} \approx \text{Ni} > \text{Zn} > \text{Mn}$ ,  
387 with median toxic concentrations of : 0.06, 0.09, 0.13, 0.56, 0.67, 1.00, 1.12, 1.63 and 2.53  
388  $\text{mg.L}^{-1}$  (hydroponic exposure)<sup>173</sup>.

389 Although the entry mechanisms of CNMs are not well known, it is established that they can  
390 interact with plants at the root system level. Once absorbed, they can share the vascular  
391 system with water and nutrients and can be transported by transpiration in the upper part of  
392 the plants<sup>96</sup>. Several studies have observed CNM accumulations in all parts of the  
393 plant<sup>110,174,175</sup>. CNMs can act on plants starting from the seed germination process with a trend  
394 to activate and increase the seed germination rate<sup>176-179</sup>. The scientific corpus of research on



395 the effects of CNMs reports contrasting effects on plant growth and physiological status. It  
396 reports an increase, a decrease or even a neutral effect on growth parameters (root system,  
397 biomass production, flowers and fruits)<sup>110,174</sup>. In case of toxicity, it was associated with  
398 generation of ROS, antioxidant activity, necrotic lesions of cells and leaf tissues as well as  
399 root and leaf morphological changes, decreased chlorophyll, and programmed cell death<sup>180–</sup>  
400 <sup>183</sup>. The inherent properties of CNMs (functionalization, length, diameter) and experimental  
401 designs (exposure medium, duration, plant species) could be a reason for the observed  
402 variability in CNM toxicity. The mean “lowest observed effect level” (LOEC) for plants  
403 exposed to CNTs was 389 mg.L<sup>-1</sup> according to Jackson *et al.*<sup>120</sup>.

404

405 Regardless to LOAED, NOEC, LOEC and EC<sub>50</sub>, it seems that metal(loid) concentrations  
406 needed to induce toxicity were much lower than those required for CNMs.

407 Table 3: Effects of simultaneous CNM-metal(loid) exposure on transfer and associated toxicity for three representative agrosystem organisms (bacteria, earthworms and  
 408 plants). The light blue lines represent studies with hydroponic exposure and the dark blue lines represent studies with soil exposure (SOD = superoxide dismutase, POD =  
 409 peroxidase, MDA = malondialdehyde, CAT = catalase, GR = glutathione reductase, GP = glutathione peroxidase, Chlo = chlorophyll, n.p = data not provided).

Type and CNM concentration	CNM size	Type and metal(loid) concentration	Organisms	Exposure medium	Exposure duration	Transfer effects	Interactive toxicological effects	References
GO (0, 1 and 10 mg.kg <sup>-1</sup> )	n.p	Cd (0 and 1 mg.L <sup>-1</sup> )	Plant: <i>Oryza sativa</i>	Culture solution	30 days	Increase of Cd transfer: +15% and +32% of Cd in roots and shoots with addition of 10 mg.L <sup>-1</sup> GO compared to control (without GO with Cd)	No interactive effects on germination and dry biomass of roots and shoots	Li <i>et al.</i> <sup>23</sup>
GO (0.1, 1, 10 mg.L <sup>-1</sup> )	n.p	As (0 and 10 mg.L <sup>-1</sup> )	Plant: <i>Triticum aestivum</i>	Water	n.p	Compared with the 19.9% rate of As uptake recorded without GO exposure, the uptake rate of As increased to 24.6% and 32.1%, then decreased to 16.1% in the presence of GO concentrations of 0.1, 1 and 10 mg.L <sup>-1</sup> , respectively	Synergic effects in the presence of both contaminants: reduction of root number and fresh biomass, reduction of root length, increase of antioxidant enzymes activity (SOD and POD), decrease of photosynthetic pigments (chlo a/b)	Hu <i>et al.</i> <sup>24</sup>
GO (0, 1, 2.5, 5, 10, 20, and 40 mg L <sup>-1</sup> )	n.p	Cd (0, 1, and 5 mg.L <sup>-1</sup> )	Plant: <i>Triticum aestivum</i>	Water	7 days	Increase of Cd transfer in roots and leaves at every GO and Cd concentrations tested.	Synergic effects: decrease in dry mass of roots and shoots, decrease in chlorophyll concentration and rubisco, increase in H <sub>2</sub> O <sub>2</sub> and O <sub>2</sub> concentration in roots and leaves, increase in antioxidant enzymes activity (MDA, POD and SOD)	Gao <i>et al.</i> <sup>25</sup>

GO (0, 30, 60 mg.L <sup>-1</sup> )	n.p	Cd (0 and 2 mg.L <sup>-1</sup> )	Plant: <i>Lactuca sativa</i>	Culture solution. Addition of GO on foliar way.	28 days	n.p	Antagnist effects: increase in dry mass, photosynthetic parameters (quantum yield, chlorophyll a and total chlorophyll content and electron transfer rate), decrease in O <sub>2</sub> concentration in leaves and roots, activity of antioxidant enzymes (SOD, CAT, GR, GP) and glutathione concentration	Gao et al. <sup>184</sup>
GO (0, 30, 60 mg.L <sup>-1</sup> )	n.p	Cd (0 and 2 mg.L <sup>-1</sup> )	Plant: <i>Lactuca sativa</i>	Culture solution. Addition of GO on foliar way.	28 days	Decrease of Cd transfer: - 19 and -9% of Cd in roots with the addition of 30 and 60 mg.L <sup>-1</sup> of GO respectively, - 33% of Cd in leaves with 30 mg.L <sup>-1</sup> of GO	Antagnist effect on the number and diameter of roots and the concentration of vitamin C	Gao et al. <sup>26</sup>
GO (1.0, 2.5, 5.0, 10, 20, and 40 mg.L <sup>-1</sup> )	n.p	Cd solution (1.0 and 5.0 mg.L <sup>-1</sup> )	Plant : wheat seedlings	Culture solution	n.p	n.p	Decrease in roots growth and microtubule protein. Increase on cytochrome P450	Gao et al. <sup>185</sup>
MWCNT (0, 100, 500, 1000 mg.kg <sup>-1</sup> )	Length: 3–12 μm, diameter: 12–15 nm, SSA : 245,28 m <sup>2</sup> .g <sup>-1</sup>	Cd and As (Cd: 1.41 mg kg <sup>-1</sup> , As: 54.80 mg.kg <sup>-1</sup> )	Plant : <i>Solanum nigrum</i> L.	Natural soil	60 days	No effect on Cd transfer with the addition of MWCNT. Decrease on As in stem (-25%) at 100 mg.kg-1, in leaf (-31%) at 1000 mg.kg-1	Increase in antioxidant enzymes activities (MDA, PRO and APX)	Chen et al. <sup>186</sup>

FNCB (10 mg.L <sup>-1</sup> )	Diameter : 47.35 ± 1.67 nm, SSA: 635.96 m <sup>2</sup> .g <sup>-1</sup>	Cd (20 and 60 mg.L <sup>-1</sup> )	Worms ( <i>Eisenia fetida</i> ) cells (coelomocytes)	Culture plate	12H	Decrease on Cd transfer (-31%) in supernatants at 60 mg.L <sup>-1</sup> of FNCB. Increase on Cd transfer (+37%) in free Cd content inside cells	No effect on viability of coelomocytes	Xu et al. <sup>187</sup>
GO (1000 and 2000 mg.kg <sup>-1</sup> )	n.p	Cd (0.1 mg.kg <sup>-1</sup> )	Bacteria	Soil	3-15-60 days	n.p	No Interactive effect but the addition of GO changes in the composition and diversity of the bacterial community	Xiong et al. <sup>188</sup>
MWCNT (0, 10, 50, 100 mg.kg <sup>-1</sup> )	Length = 5–30 μm, diameter < 8 nm	Cd (0, 2.0 and 10 mg.kg <sup>-1</sup> )	Earthworms: <i>Eisenia fetida</i>	Soil	14 days	Decrease in Cd concentration: at 2 mg.L <sup>-1</sup> of Cd -24%, -18%, and -12% with addition of 10, 50 and 100 mg.kg <sup>-1</sup> of MWCNT. Decrease more pronounced at 10 mg.L <sup>-1</sup> of Cd (-42%, -34%, -26% of Cd with addition of 10, 50 and 100 mg.kg <sup>-1</sup> of MWCNT).	There appear to be an interaction: decreased percentage of inhibition of worm growth, detoxifying enzymes, and metabolites for the lowest the MWCNT concentration.	Yang et al. <sup>32</sup>
GO spheres (5 mg.L <sup>-1</sup> )	n.p	Cu (0 and 240 mg.L <sup>-1</sup> )	Plant: <i>Triticum aestivum</i>	Soil	14 days	Reduction of transfer: decrease of Cu concentration in roots and stems by 60% and 67% and it was similar in leaves (increase only in 2%) with addition of GO compared to the control (without GO with Cu)	No interactive effects growth parameters (biomass, root, stem and leaf lengths). cellular: photosynthesis, transpiration and stomatal conductance	Zhao et al. <sup>21</sup>
GO (1000 and 3000 mg.kg <sup>-1</sup> )	n.p	Cd (0 and 10 mg.kg <sup>-1</sup> )	Plant: <i>Oryza sativa</i>	Soil	40 days	Increase in Cd with 3000 mg.kg <sup>-1</sup> addition of 12.5% in total rice biomass compared to control (no GO with Cd)	No interactive effects on the growth parameters studied (fresh biomass and size)	He et al. <sup>22</sup>
MWCNT (0, 100, 500, 1000 and 5000 mg.kg <sup>-1</sup> )	n.p	Cd (22.4 mg.kg <sup>-1</sup> )	Plant: <i>Boehmeria nivea</i>	Natural soil	60 days	Increase in Cd concentration: in leaves by +15%, +12% and +9% at 100, 500 and 1000 mg.kg <sup>-1</sup> , in stems by +33% and +28% at 100 and 500 mg.kg <sup>-1</sup> . Decreased Cd transfer: in stems by -10% at 5000 mg.kg <sup>-1</sup> , in roots by -22%, -21%, -29% and -30% at 100, 500, 1000, 5000 mg.kg <sup>-1</sup>	Interactive effects: increase in leaf dry mass with the addition of 1000 mg.kg <sup>-1</sup> of MWCNT, stem dry mass at 500 mg.kg <sup>-1</sup> of MWCNT and root dry mass at 500 and 1000 mg.kg <sup>-1</sup> of MWCNT, increase in H <sub>2</sub> O <sub>2</sub> , MDA concentration and SOD activity from the addition of 100 mg.kg <sup>-1</sup> of MWCNT and decrease of POD activity from 100 mg.kg <sup>-1</sup> of MWCNT	Gong et al. <sup>27</sup>

MWCNT (0, 100, 500, 1000 mg.kg <sup>-1</sup> )	Length: 3–12 µm, diameter: 12–15 nm, SSA : 245,28 m <sup>2</sup> .g <sup>-1</sup>	Cd and As (Cd: 1.41 mg kg <sup>-1</sup> , As: 54.80 mg.kg <sup>-1</sup> )	Plant : Solanum nigrum L.	Natural soil	60 days	No effect on Cd transfer with the addition of MWCNT. Decrease on As in stem (-25%) at 100 mg.kg <sup>-1</sup> , in leaf (-31%) at 1000 mg.kg <sup>-1</sup>	Increase in antioxidant enzymes activities (MDA, PRO and APX)	Chen et al. <sup>186</sup>
MWCNT (0, 0.1 and 10 mg.kg <sup>-1</sup> )	Length = 1 to 20 µm, average diameter of 16.8 ± 6.7 nm	Two types of soil with two contamination level on 3 metals: 1 = Cd : 13, Pb : 524 and Zn : 689 mg.kg <sup>-1</sup> and 2 = Cd : 28, Pb : 1471 and Zn 1825 mg.kg <sup>-1</sup> )	Plant: <i>Lactuca sativa</i> , earthworms: <i>Eisenia fetida</i> and native soil bacteria	Natural soil	42 days	In lower contaminated soil: no effects on metals transfer for earthworms. Increase in Cd (+37%) and Zn (+21%) transfer with 10 mg.kg <sup>-1</sup> of MWCNT for lettuces. In higher contaminated soil: increase in Pb (+34%) and Zn (+25%) transfer with 10 mg.kg <sup>-1</sup> of MWCNT for earthworms. Decrease in Cd (-29%) and Zn (-30%) transfer with 0.1 mg.kg <sup>-1</sup> of MWCNT for lettuces.	Interactive effects on higher contaminated soil: biomass reduction for lettuces exposed to 0.1 mg.kg <sup>-1</sup> of MWCNT. Increase in flavonoid concentration with 10 mg.kg <sup>-1</sup> of MWCNT.	Leroy et al. <sup>30</sup>

410

### 411 **3.2. Impacts of the interaction between metal(loid)s and CNMs**

412 After an extensive literature survey on the topic, only articles focusing on CNTs, graphene  
413 and GO together with a proper statistical analysis were kept for this review. Considering  
414 studies evaluating both the transfer and the toxicity of metal(loid)s and CNMs (Table 3) (11  
415 studies), 18% focused on bacteria, 18% on earthworms and 82% on plants. Among these  
416 studies, about 64% reported interactive toxicological effects due to the simultaneous presence  
417 of CNMs and metal(loid)s. Within these interactive effects, when all control conditions were  
418 set-up (without metal(loid) and without CNM contamination, metal(loid) alone, CNM alone),  
419 we could distinguish antagonist (18%) and synergic (18%) effects (Figure 1).

420

#### 421 **3.2.1. Bacteria**

422 For bacteria, very few studies have been conducted. Furthermore, in the available articles the  
423 experimental design did not permit to highlight the existence of interactive effects since the  
424 impact of CNM alone or metal(loid)s alone were not tested (not the purpose of the study).  
425 However, a study using soil contaminated with Cd ( $0.1 \text{ mg.kg}^{-1}$ ) and GO (1000 and 2000  
426  $\text{mg.kg}^{-1}$ ) evidenced a decrease in soil microbial diversity after addition of GO, indicating that  
427 the microbial communities in the Cd-contaminated group without GO were more diverse than  
428 those in the treated groups (Cd x GO)<sup>188</sup>. In addition, authors observed some changes in  
429 bacterial communities after addition of GO with an increase in several bacterial abundances  
430 (*Acidobacteria* and *Actinobacteria*) compared to single Cd contamination and a decrease in  
431 phyla such as *WD272* and *TM6* revealing phylum-specific sensitivities<sup>188</sup>. Interestingly, the  
432 abundance of *Rhodospirillales* and *Nitrospirae* which are symbiotic N<sub>2</sub>-fixing bacteria was

433 reduced at 3 days and then restored at 15 and 60 days evoking some resilience in the  
434 ecosystem.

435 These few studies alone do not allow generalization to be made and highlight the need to  
436 initiate more studies on microorganisms with simultaneous CNM and metal(loid) exposure.  
437 Indeed, due to adsorption of metal(loid)s on CNM and to CNM strong interactions with  
438 bacterial cell membranes, CNMs could be a vector to increase metal(loid) transfer to  
439 microorganisms and associated toxicity or depending on their interactions with soil particle  
440 they could also mitigate the metal(loid) toxicity (*i.e.* the complex CNM-metal(loid) could  
441 adsorb to soil particles making metal less bioavailable to bacteria cells and thus less toxic).

442

### 443 **3.2.2. Earthworms**

444 Studies focused on earthworms report contrasting results of the addition of CNMs on  
445 metal(loid) transfer and toxicity. Yang *et al.* observed a decrease of Cd transfer (antagonist  
446 effect) ranging from -43% to -31% with the addition of 10 mg.kg<sup>-1</sup> and 100 mg.kg<sup>-1</sup> of  
447 MWCNTs, respectively after 14 days of exposure in the model *Eisenia fetida* (Cd: 10mg.kg<sup>-1</sup>  
448 )<sup>32</sup>. The decrease in Cd transfer was thus inversely proportional to the dose of MWCNTs.  
449 Additionally, the change in transfer was also metal dose-dependent: at 10 mg.kg<sup>-1</sup> MWCNTs,  
450 a -43% decrease was noticed at 10 mg.kg<sup>-1</sup> of Cd but decreased to -28% at 2 mg.kg<sup>-1</sup> of Cd. In  
451 agreement with transfer results, the addition of MWCNTs decreased earthworm growth  
452 inhibition caused by Cd, detoxifying enzymes and metabolites<sup>32</sup>. At the highest Cd  
453 concentration (10 mg.kg<sup>-1</sup>), a growth inhibition rate of 27% was registered in comparison with  
454 earthworms exposed to control conditions (no Cd, no MWCNTs), this rate was decreased to  
455 8% and 22% at 10 and 100 mg.kg<sup>-1</sup> of MWCNTs, respectively.

456 In contrast, Leroy *et al.* rather observed synergic effects of metals and CNTs when  
457 earthworms were exposed to two contaminated soils (Zn, Pb and Cd) and demonstrated either  
458 no impact of the addition of MWCNTs on metal uptake in one soil or an increase up to +34%  
459 and +25% of Pb and Zn in the second soil (with no impact on Cd transfer) after 6 weeks<sup>30</sup>.  
460 Furthermore, when an increased uptake was detected it was CNT dose dependent. Even  
461 though earthworms are able to eliminate orally ingested CNMs by depuration, it appears that CNM  
462 ingestion could lead to metal release in earthworm tissues, maybe due to changing physico-chemical  
463 conditions in the gut (especially pH) leading to desorption. Despite transfer increase, no  
464 toxicological effects were found.

465 The difference between these articles could partly be explained by different soil texture.  
466 Indeed, the first one which induced Pb and Zn transfer increase upon CNT addition had a  
467 lower OM content than the other soil. Authors reminded that a low OM is less favorable for  
468 earthworm feeding and burrowing activities<sup>189</sup> and thus metal transfer<sup>30,190</sup>.

469

### 470 **3.2.3. Plants**

471 Among studies on the transfer and toxicological impacts of simultaneous contamination with  
472 CNMs and metal(loid)s on plants (9 studies reviewed), 89% present results on the transfer of  
473 metal(loid)s from the exposure medium to the plants and 100% on toxicity. In the transfer  
474 studies, 91% of the studies showed CNM - metal(loid) interactions with either an  
475 increase<sup>24,23,25,21,22,27,30,191,192</sup> and/or a decrease<sup>21,24,26,27,29,30</sup> of metal(loid) transfer differing  
476 from the additive risk model.

477 Furthermore in the studies evidencing a synergic effect with an increased uptake of  
478 metal(loid)s, some authors demonstrated a dose dependent effect of the addition of CNMs on  
479 the transfer of metal(loid)s both in hydroponics and in soil<sup>21-29</sup>. For example, Gao *et al.* found



480 that the addition of GO to a Cd contaminated medium ( $1 \text{ mg.L}^{-1}$  Cd) led to a dose-dependent  
481 increase in Cd concentration in both roots and leaves: the addition of  $5 \text{ mg.L}^{-1}$  of GO  
482 increased by 12% the Cd concentration in leaves while an addition of  $40 \text{ mg.L}^{-1}$  led to a 50%  
483 increase<sup>25</sup>. Hypotheses such as facilitating metal entry into root cells due to interactions  
484 between CNMs and root cells and/or uptake of CNMs with surface adsorbed metals may  
485 explain the increased transfer observed in these examples. Additionally, it seems that at high  
486 concentrations, CNMs may have a different impact than at lower concentrations in the  
487 medium<sup>24,30</sup>. Indeed, wheat plants exposed to As(III) took up 20% of this As while the uptake  
488 rate of As increased to 25% and 32% in presence of GO concentrations of 0.1 and  $1 \text{ mg.L}^{-1}$ ,  
489 then decreased to 16% at  $10 \text{ mg.L}^{-1}$ <sup>24</sup>. Likewise, Leroy *et al.* also observed contrasted results  
490 depending on CNM concentrations and explained these results by CNM dispersion in the  
491 medium (soil): at low concentrations ( $0.1 \text{ mg.kg}^{-1}$ ), CNTs may be better dispersed in the soil  
492 than at higher concentration ( $10 \text{ mg.kg}^{-1}$ )<sup>193,194</sup> offering thus, a higher exchange surface and  
493 higher adsorption. This hypothesis is supported also by the study of Zhang *et al.* in which the  
494 authors observed that the agglomeration of CNTs decreased the adsorption of some organic  
495 compounds<sup>193</sup>. Several hypotheses can be suggested for this increased uptake: CNMs with  
496 adsorbed metal(loid)s can act as vectors by damaging root cell walls and facilitate  
497 metal(loid)s entry in crops tissues or this complex might accumulate in the rhizosphere and  
498 lead to an enhanced metal release with the pH modification at the vicinity of the root surface.

499

500 When an increased transfer of metals due to the presence of CNMs in the medium was  
501 detected, it was mostly accompanied by an increased toxicity. Indeed, among the studies that  
502 investigated the toxicological impacts of simultaneous contamination, 75% of them found  
503 interactive effects highlighting an increase in oxidative stress<sup>24,25,27-29,184</sup>, decrease in different  
504 plant components<sup>24-26,29,184,195</sup> (chlorophyll, protein, sugar) and in plant biomass<sup>24-29,184,195,196</sup>

505 (Table 3). For instance, synergistic effects were evidenced on rubisco concentration : indeed a  
506 decrease by 53% was detected in the presence of both Cd (5 mg.L<sup>-1</sup>) and GO (40 mg.L<sup>-1</sup>)  
507 while Cd alone reduced it by 14.5% and GO alone by 18.5%; additive model would have  
508 suggested a decrease by 33% ((-14.5%) + (-18.5%)) = -33%) much lower than the 53%  
509 observed here<sup>25</sup>. This observation was similar for other parameters (root and shoot biomass,  
510 chlorophyll, O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> concentrations) and more pronounced with higher Cd and GO  
511 contamination. Similarly, Hu *et al.* demonstrated that the addition of GO (10 mg.L<sup>-1</sup>)  
512 decreased plant development and photosynthetic pigments concentrations together with an  
513 increase in antioxidant enzymes activities (SOD and POD) in *Triticum aestivum L.* exposed to  
514 As solution (10 mg.L<sup>-1</sup>)<sup>24</sup>; for instance after exposure to the co-contamination SOD increased  
515 by 426%, while it increased by 56% after exposure to As alone and decreased by 4% after  
516 exposure to GO alone (additive model would suggest a 52% increase).

517 However, some studies also highlighted antagonist interactions between metal(loid)s and  
518 CNMs with a decrease in the transfer of metal(loid)s from soil to plants and finally lower or  
519 no toxicity symptoms<sup>26-29,184</sup>. These effects can also be organ dependent. Indeed, Zhao *et al.*  
520 showed that GO-chitosan spheres significantly decreased Cu bioaccumulation in roots (-60%)  
521 and stems (-67%) of wheat compare to single metal contamination, but not in leaves (almost  
522 similar with an increase of Cu bioaccumulation around +2%)<sup>21</sup>. Despite changes in Cu  
523 transfer with the addition of GO, the co-exposure did not impact growth parameters studied  
524 (root, stem and leaf length) but decreased the photosynthetic rate and stomatal conductance up  
525 to -58% and -34%, respectively. Likewise, Gong *et al.* found similar pattern with a decrease  
526 in Cd bioaccumulation in roots up to -22% upon MWCNT addition (100 mg.kg<sup>-1</sup>) and an  
527 increase in Cd accumulation in stem and leaves up to +15% and +33%, respectively<sup>27</sup>. These  
528 differences on metal bioaccumulation according to plant organ could reflect a plant defence  
529 strategy. It is known that plants have defence mechanisms against metal(loid) exposure like

530 accumulating metal(loid)s in specific compartments of the plant such as cell walls and leaves  
531 to reduce their toxicity<sup>197</sup>.

532 There are some disparities on transfer and toxicity of CNM-metal(loid) simultaneous  
533 exposure. The adsorption mechanism alone does not seem to explain such disparities and the  
534 concentration in CNMs, which directly affects their dispersion and aggregation in the  
535 medium, could be a possible explanation.

536 Although the studies carried out in liquid media (water, culture solution, soil solution, etc.)  
537 are a first approach privileged at the beginning of new researches on new substances, they do  
538 not allow to apprehend the complex relations between solid matrix and contaminants since  
539 some studies show their importance to determine the fate of contaminants in organisms<sup>30</sup>.  
540 Indeed, the type of soil could explain differences observed on interactive effects on transfer  
541 and toxicity due to the strong influence of soil physico-chemical parameters (clay, OM, pH  
542 *etc.*). Whatever the types of interactive effects, these largely dominate in the studies compared  
543 to additive effects used in risk assessment.

544

#### 545 **4. Conclusion – Knowledge gaps and Perspectives**

546 In 2012, the European Commission stated that they are “committed to ensuring that the risks  
547 associated with chemical mixtures are properly considered and assessed”. In parallel, in 2019,  
548 the European Environmental Agency warned again about the "cocktail effect" of chemical  
549 pollutants, especially those found in European waters, whose cocktail effects are assumed or  
550 proven<sup>198</sup>. However up to date lots of progress remain to be done on that matter.

551 Indeed, after reviewing extensively the literature, we can conclude that strong interactions  
552 exist between CNMs and metal(loid)s which have been demonstrated in simplified medium  
553 (solution) and whose impacts are visible in the environment by modifying the behaviour of

554 contaminants in cocktail and their impact on organisms. These interactions include physical  
555 adsorption, chemical interaction, electrostatic attraction and sorption-precipitation. In the  
556 environment, the vast majority of studies concluded that these interactions modify the fate  
557 (uptake and toxicity) of these contaminants in cocktail in comparison with an additive model.  
558 The nature of the impact (synergic or antagonist) is influenced by many parameters : 1) CNM  
559 nature which influences metal(loid) adsorption capabilities, 2) CNM concentration which  
560 influences their behaviour, in particular their agglomeration, 3) CNM morphology and SSA as  
561 well as adsorption abilities and CNM transfer through biological barriers, 4) metal(loid)  
562 nature and number of different metallic species because of their specific affinity and potential  
563 competition and repulsion for adsorption sites onto CNMs, 5) exposure medium which  
564 influences CNM and metal(loid) behaviour, 6) soil characteristics (clay content, OM and pH)  
565 which influence metal(loid) adsorption onto CNMs as well as organism behaviour and 7) type  
566 of organisms (crops, earthworms or bacteria) and environmental relevance of exposure  
567 conditions (exposure pathways, organism lifestyle, organism interactions, ...). So, in  
568 conclusion, the additive model used so far for risk assessment of chemical substances in  
569 mixture is not relevant; instead a multiplicative model which integrates synergy and  
570 antagonism would seem more suitable with correction factors for the different influencing  
571 parameters highlighted in this review to encompass processes which remain unclear so far and  
572 leading to seemingly contradictory results in the literature.

573

#### 574 **Knowledge gaps**

575 The knowledge gaps we identified through this literature survey are listed below:

576 (1) More information on CNM characteristics are needed in the articles to further understand  
577 contrasted results observed on transfer and toxicity.

578 (2) The use of element speciation models (such as Minteq or ChemEQL among others) in  
579 solution and their comparison with experimental data and their fitting with Langmuir or  
580 Freundlich equations would be interesting to identify if these models could give relevant  
581 predictions, especially in multi-metallic conditions.

582 (3) More studies need to be conducted on agrosystem organisms to have more information on  
583 the toxicological effects resulting from exposure to a mixture of contaminants. For instance,  
584 only 3 metal(loid)s (Cu = 1, As = 1 and Cd = 8, studies and one study with Cd, Pb and Zn  
585 simultaneously) were tested in interactions with CNMs, which represents a small proportion of  
586 the existing metals.

587 (4) Another important point is the difficulty to properly analyse the results in many studies  
588 due to incomplete experimental designs that do not permit to highlight possible interactions.  
589 On several occasions, the studies did not investigate the effects of CNMs alone or  
590 metal(loid)s alone (missing control conditions). Thus, recurrent increases and decreases of the  
591 studied biomarkers of toxicity were attributed to the addition of CNMs in the presence of  
592 metal(loid)s, but these interpretations are only partial in the absence of tests with single  
593 contaminant.

594 (5) The statistics are sometimes performed only by modality of metal(loid) contamination, not  
595 allowing to establish possible interactions between CNMs and metal(loid)s (ANOVA 1-way  
596 *vs* ANOVA 2-ways).

597 (6) More studies on ecosystem functions (mineralization and recycling) and biogeochemical  
598 cycles (C, N and P) are needed to have a more integrative view of these contaminant impacts  
599 on agrosystems. So far, these markers have only been studied after exposure to a single  
600 contaminant even though they are very good indicators of an ecosystem health.

601 (7) Studies are also needed to identify a potential bioaccumulation along food webs. Studies  
602 in micro-mesocosms with trophic chains seem to be a relevant experimental set-up to answer  
603 this question<sup>199</sup>. Due to CNM-metal(loid) interactions, the exposure duration in micro-  
604 mesocosms needs to be adapted to organism cycles, especially to know if CNM-metal(loid)  
605 exposure is putting at risk crop productions and human health.

606 (8) Technical development is also mandatory as the mechanisms explaining transfer and  
607 toxicity results are still not well understood, in particular because data about CNMs actual  
608 concentrations in the organisms are rarely available.

609

## 610 **Perspectives**

611 Using combined toxicity interaction indices could be a practice to generalize to gain a deeper  
612 understanding of these interactions among contaminants. For example, Yang *et al.*<sup>32</sup>  
613 demonstrated synergic and antagonist effects using indices such as the Biomarker Response  
614 Index (BRI) coupled with the Concentration Addition Index (CAI) and the Effect Addition  
615 Index (EAI), which together quantify the joint effects of mixtures for a range of effect levels.  
616 BRI is an index based on the level of alteration of the response of the biomarkers tested in the  
617 treatments with contamination(s) compared to the control (without contamination). The level  
618 of biomarker impairment is classified into four distinct levels, each corresponding to a score:  
619 mild = 4, moderate = 3, major = 2 and severe = 1<sup>200</sup>. The score for each biomarker is  
620 multiplied by the corresponding weighting to form an integrated index that is used to assess  
621 the overall effect of contaminants in a system submitted to multiple stress<sup>201</sup>. This allows the  
622 integration of complex biomarker responses into a single predefined class. It is thus possible  
623 to establish a dose-response relationship between contamination levels and biomarker  
624 responses<sup>202</sup>. This index has been used in the risk assessment of chemicals under the Water

625 Framework Directive in Wales<sup>200</sup>. The BRI coupled with the CAI and EAI allow to determine  
626 respectively additive ( $=0$ ), synergic ( $>0$ ) or antagonist ( $<0$ ) effects<sup>203</sup>. The CAI and EAI are  
627 derived from the commonly used concentration addition (CA) and independent action (IA)  
628 models that assess the toxicity of substances in non-interactive mixtures.

629 Another factor which is usually not considered is contamination aging. Indeed, the impact of  
630 metal(loid)s is influenced by their origin and aging<sup>204–207</sup>. The toxicological effects of  
631 metal(loid)s spiked in soil in laboratory experiments (in salt form) differ with soils with an  
632 aged contamination under the influence of natural processes<sup>204–207</sup>. For example, oxidation  
633 and precipitation of some metal(loid)s can affect their bioavailability and consequently their  
634 toxicity<sup>208</sup>. This parameter should be more considered in future experiments.

635 Although micro and nanoplastics have different properties than CNMs, they are principally made of  
636 carbon and also display adsorption capacities leading to similar effects on transfer and toxicity (Table  
637 S3). Plastic concentration in agricultural soils could be much higher than the predicted CNM  
638 concentrations related to sludge application or mulching practices in particular. Special attention  
639 should thus be paid to the potential transfer and toxicological impacts of these micro and nanoplastics  
640 in co-exposure situations with other contaminants such as metal(loid)s.

641 Finally, agrosystems interact closely with other organisms such as insects, small mammals  
642 and birds on which they depend for part or all of their life cycles. Considering how these  
643 organisms rely on agrosystems, they may be an entry point for the contamination to spread up  
644 to other ecosystems.

645

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