

1 **INFLUENCE OF ORGANIC MATTER TRANSFORMATIONS ON THE**
2 **BIOAVAILABILITY OF HEAVY METALS IN A SLUDGE BASED COMPOST**

3

4 **Florencio Ingelmo^{1*,2}, Maria José Molina¹, Maria Desamparados Soriano³,**
5 **Antonio Gallardo⁴, Leonor Lapeña⁴**

6

7 ¹ *Centro de Investigaciones sobre Desertificación, CIDE (CSIC-UVEG-GV). C/*
8 *Camí de la Marjal s/n 46470 Albal (Valencia, Spain). Tel: 34961220540. Fax:*
9 *34961270967. E-mail: florencio.ingelmo@uv.es ; maria.j.molina@uv.es*

10 ² *Instituto Valenciano de Investigaciones Agrarias, IVIA. Apartado Oficial. 46113*
11 *Moncada (Valencia, Spain)*

12 ³ *Escuela Técnica Superior del Medio Rural y Enología. Avda. Blasco Ibáñez*
13 *21, (46010 Valencia, Spain).*

14 ⁴ *Universitat Jaume I de Castelló, Campus de Riu Sec, (12071 Castellón,*
15 *Spain).*

16

17 **Corresponding author*

18

19 **Abstract.**

20

21 The agricultural use of anaerobically digested sewage sludge (ADSS) as stable,
22 mature compost implies knowing its total content in heavy metals and their
23 bioavailability. This depends not only on the initial characteristics of the
24 composted substrates but also on the organic matter transformations during
25 composting which may influence the chemical form of the metals and their

26 bioavailability.

27 The objective of this work was to examine the relationships between the
28 changes in the organic matter content and humus fractions, and the
29 bioavailability of heavy metals.

30 A detailed sampling at 0, 14, 84, and 140 days of the composting process was
31 performed to measure C contents in humic acids (HAs), fulvic acids, (FAs) and
32 humin, the total content of Zn, Pb, Cu, Ni, and Cd, and also their distribution
33 into mobile and mobilisable (MB), and low bioavailability (LB) forms.

34 Significant changes of C contents in HA, FA, and Humin, and in the FA/HA,
35 HA/Humin and $C_{\text{humus}}/\text{TOC}$ ratios were observed during composting. The MB
36 and LB fractions of each metal also varied significantly during composting. The
37 MB fraction increased for Zn, Cu, Ni, and Cd, and the LB fraction increased for
38 Pb. Stepwise linear regressions and quadratic curve estimation conducted on
39 the MB and LB fractions of each metal as dependent on the measured organic
40 variables suggested that Zn bioavailability was mainly associated to percentage
41 of C in FAs. Bioavailability of Cu, Ni and Cd during composting was associated
42 to humin and HAs. Pb concentration increased in the LB form, and its variations
43 followed a quadratic function with the $C_{\text{humus}}/\text{TOC}$ ratio. Our results suggest that
44 the composting process renders the metals in more available forms. The main
45 forms of metal binding in the sludge and their availability in the final compost
46 may be better described when metal fractionation obtained in sequential
47 extraction and humus fractionation during composting are considered together.

48

49 **Keywords:** sewage sludge, compost, humic substances, humin, heavy metals,
50 bioavailability.

51 **Abbreviations:** C_{HA} (Carbon in humic acids), C_{FA} (Carbon in fulvic acids), C_{Humin}
52 (C in hydrolysable humin), C_{humus} (sum of C contents in FAs, HAs and
53 hydrolyzable humin), TOC (Total organic carbon), X_{MB} (mobile and mobilisable
54 metal form), X_{LB} (low bioavailability metal form).

55

56 **1. Introduction**

57

58 The agricultural use of anaerobically digested sewage sludge as an
59 organic amendment to improve soil fertility is becoming increasingly important.
60 The management of the raw sludge involves many problems such as
61 pathogens, plant seeds, odours, and a pasty structure with high water content.
62 This later feature makes it hard to store and may lead to immobilization and
63 volatilization of plant nutrients (Cambardella *et al.*, 2003). One of the
64 possibilities to convert sewage sludge into a marketable organic amendment or
65 fertilizer is to co-compost it with different bulking agents, such as wood chips,
66 thus obtaining a humus-like material that is easy to store (Gallardo *et al.*, 2007).
67 The addition of such a bulking agent for composting may optimize substrate
68 properties such as air space, moisture content, C/N ratio, particle density, pH
69 and mechanical structure, affecting positively the decomposition rate. In this
70 sense, lignocellulosic by-products such as wood chips and sawdust are
71 commonly used as bulking agents (Maboeta and van Rensburg, 2003; Pasda *et*
72 *al.*, 2005; Neves *et al.*, 2009). In the case of anaerobically digested sewage
73 sludges with high contents of nitrogen, heavy metals, and other toxic or
74 phytotoxic substances, bulking agents like sawdust are recommended because
75 of the dilution effect (Banegas *et al.*, 2006).

76 Composting of organic wastes has been defined as a well-established
77 method, which leads to a stabilized product rich in humic substances that
78 resemble native soil humic substances (De Bertoldi *et al.*, 1996). In addition, the
79 agricultural use of stable, mature sewage sludge-based compost means
80 knowing its content (CEC, 1986; Royal Decree, 1990), and the biogeochemical
81 forms of the heavy metals present (Hsu and Lo, 2001).

82 Most of the studies on the speciation of heavy metals have been carried out in
83 raw or composted sludges-amended soils (Petruzzelli *et al.*, 1994; Kunito *et al.*,
84 2001; Maboeta and van Rensburg, 2003; Hanc *et al.*, 2009). Only a few were
85 dedicated specifically to study the bioavailability of the heavy metals during
86 composting of sewage sludges (Amir *et al.*, 2005; Liu *et al.*, 2007; Tandy *et al.*,
87 2009). Studies relating changes in bioavailability of heavy metals with changes
88 in humus fractions during composting are scarce (Amir *et al.*, 2005; Liu *et al.*,
89 2007).

90 The speciation of each metal in the sewage sludge-based compost may
91 depend not only on its initial chemical state in the sewage sludge which also
92 depends on their nature and processing (Fuentes *et al.*, 2004; Walter *et al.*,
93 2006), but also on the organic matter transformations during composting. These
94 could influence the metal distribution through metal interaction with the newly
95 formed humic substances (Petruzzelli *et al.*, 1994; Amir *et al.*, 2005; Liu *et al.*,
96 2007).

97 The objective of this work was to examine the relationships between the
98 changes in the organic matter content and humus fractions, and the
99 bioavailability of heavy metals. This was tested in a 70:30 (on wet basis)
100 mixture of ADSS and wood chips with an initial C/N ratio of 30.4, during its

101 aerobic batch composting at 30°C of external temperature in an open type lab-
102 scale reactor without lixiviation.

103

104

105 **2. Materials and methods**

106

107 2.1. Composting

108

109 The raw material used in the composting process was a mixture of
110 sewage sludge and wood chips as bulking agent, in the ratio 70:30 (on a wet
111 basis). Sewage sludge was an anaerobically digested dewatered cake of
112 sludge (FACSA Sewage Treatment Plant in Castellón, Spain). The
113 characteristics of the raw sludge were 94.3 % moisture content, pH 8.51, EC
114 1.51 dS m⁻¹; 42.2 % TOC; 6.37 % total N; C/N 6.62; and total Zn, Pb, Cu, Ni,
115 and Cd contents of 1660, 310, 256, 16.0, and 1.95 mg kg⁻¹, respectively (all
116 results expressed in dry basis). The C/N ratio of the wood chips was 64.5, its
117 moisture content was 8.5 %, and its total N content was 0.83 % (Gallardo *et al.*,
118 2007). Normally, bulking agents have high C/N ratios, which can compensate
119 for the low values of the sewage sludge because of the dilution effect (Banegas
120 *et al.*, 2006; Neves *et al.*, 2009).

121 The pilot-scale composting experiments were carried out in five 65 L capacity
122 open type lab-scale reactor without drainage of lixiviates. Aeration was
123 controlled daily, moisture every five days, and mixture turned every 15 days.

124 Composting was monitored for 140 days, when oxygen consumption finished
125 (García *et al.*, 1992). According to temperature measurements (Gallardo *et al.*,

126 2007) composting developed in a first very active phase with high oxygen
127 consumption until day 20; a second phase in which the activity dropped to a
128 medium level until day 90; and a third phase with low activity, which lasted until
129 day 140.

130

131 2.2. Physico-chemical analysis

132

133 To obtain representative samples for the physico-chemical analysis of
134 the sludge based compost during the time of composting, good homogenization
135 was ensured, and five aliquots of about 80 g (on dry basis) were taken and
136 mixed at every sampling date. Three replicates of each composite sample were
137 analysed at 0, 14, 84, and 140 days of composting. The time intervals were
138 determined according to the changes of composting temperature and oxygen
139 consumption (Gallardo *et al.* 2007). To determine their main physico-chemical
140 properties we followed standard methods (MAFF, 1986): organic carbon by
141 partial oxidation with potassium dichromate, total nitrogen by the Kjeldahl
142 method, and pH and electrical conductivity (EC), respectively, in a 1/2.5 and a
143 1/ 5 sample/water ratios. The total concentrations of metals were determined
144 through inductively coupled plasma-ICP (EPA, 1990) using a Perkin Elmer ICP/-
145 5 500 after the microwave digestion of the samples with HNO₃:HClO₄
146 (Polkowska-Motrenko *et al.*, 2000).

147 Compost samples were extracted with 0.1 M NaP₂O₇ (pH 9.8) at room
148 temperature using a sample/extractant ratio of 1/10. Each extraction was
149 repeated 3 times. For each extraction step, the mixture was shaken for 3 h,
150 centrifuged at 15 000 g for 15 minutes and the supernatant was filtered through

151 a Whatman 31 filter paper. The combined alkaline extracts (soluble humic
152 substances) were then acidified with concentrated H₂SO₄ to pH 1, left standing
153 for 24 h in a refrigerator to allow the complete precipitation of HAs, and then
154 centrifuged at 15 000 g for 30 min to separate the supernatant FAs fraction.
155 Since the alkali-insoluble humin fraction may contain humic-like substances (i.e.
156 proteinaceous compounds linked to decomposed ligno-cellulosic materials), we
157 determined the hydrolyzed humin (Zaccheo *et al.*, 2002). The hydrolyzed humin
158 was obtained after acidification of the sample retained in the filter paper with
159 concentrated 6 N HCl for 10 h, filtration and washing with deionized water. This
160 fraction was considered as forming part of the humus in the compost. The total
161 alkali extractable (soluble humic substances), the FAs, and the hydrolyzed
162 humin were analyzed for C. The C in HAs was obtained by the difference
163 between C in the total alkali extractable and C in FAs (MAFF, 1986). C_{humus} was
164 obtained as the sum of C contents in FAs, HAs and hydrolysable humin.

165 Heavy metal fractionation for Zn, Cu, Pb, Ni, and Cd was determined
166 according to Sposito's procedure (Amir *et al.*, 2005). In each of the three
167 replicates taken from the composite samples, a series of reagents were
168 sequentially applied with a compost/extractant ratio of about 1/4. The sequence
169 of reagents application to collect the medium -bioavailable fraction MB (mobile
170 and mobilisable) was: H₂O (shaking during 2h at 20 °C, three times); KNO₃ 0.5
171 M (shaking during 16 h at 20 °C); NaOH 0.5 M (shaking during 16 h at 20 °C),
172 and EDTA 0.05 M (shaking during 16 h at 20 °C). Finally, to collect the low-
173 bioavailable fraction LB (bound to sulphides; hardly mobilisable) the samples
174 were treated with HNO₃ 4 M (shaking during 16 h at 80 °C). Metal concentration
175 was measured after each step treatment, and referred to dry weight. All filtered

176 supernatants were analyzed by ICP (EPA, 1990).
177 The levels of bioavailability considered in this work are:

178 1) Medium, MB (mobile and mobilisable fractions):

179 $MB = X-H_2O + X-KNO_3 + X-NaOH + X-EDTA$

180 2) Low, LB (Sulphides. Hardly mobilisable fraction):

181 $LB = (X-HNO_3)$

182

183 2.3. Statistical analysis

184 Statistical analyses were performed with the SPSS v.17.0 statistical
185 software. A one-way ANOVA was used to detect the significant effect of time of
186 composting on different compost parameters. The Tukey's t- test was used for
187 mean comparison and significant differences at 95% level on data obtained at
188 the different composting times. To describe more clearly the metal and humus
189 fraction variations through time, also linear and curvilinear adjustments were
190 performed. In order to ascertain the best-fit model between variations in the
191 metal fractions during composting and changes in the organic fractions,
192 stepwise linear regressions and quadratic curve estimations were performed on
193 the MB and LB fractions of each metal as dependent variables. The
194 independent organic variables were C_{FA} , C_{HA} , C_{Humin} , and C_{FA}/C_{HA} , C_{HA}/C_{Humin} ,
195 and C_{humus}/TOC ratios.

196

197

198 **3. Results and discussion**

199

200 3.1. Compost properties

201

202 The main physico-chemical properties of the composted sludge at
203 different times of the process, the ANOVA and the Tukey's t-test results are
204 presented in Table 1.

205 (Table 1)

206 Because of the high moisture content of the raw sludge, the ratio of
207 sewage sludge and wood chips on a dry weight basis was 15:85. As shown by
208 Pasda et al. (2005) this product is not easy to decompose because its high
209 lignin / tannins ratio. This fact likely provoked that temperature in the reactors
210 during the composting process was always below 35°C. No significant changes
211 were detected for pH, EC and total N. The high value of pH in the raw sewage
212 sludge may compensate for the decrease of this parameter during composting
213 (Amir *et al.*, 2005; Liu *et al.*, 2007). The pH during composting was in the
214 optimal range for the development of bacteria and fungi (Zorpas *et al.*, 2003).

215 Total organic C content (TOC) decreased significantly during composting
216 (Table 1), which is consistent with the decomposition of the organic matter
217 through microbe respiration in the form of CO₂ and even through mineralization.
218 The overall decomposition observed in this work (37%) contrasts with the 60%
219 observed by Jouraiphy *et al.* (2005) during 135 days of composting of a mixture
220 of sewage sludge and green plant waste, and the 19.6 % of Amir *et al.* (2005)
221 during 180 days with straw as bulking agent. At difference with other authors
222 (Soumaré *et al.*, 2002), the organic matter decomposition during composting did
223 not cause an increase in total N during the process. Although N variations were
224 not significant, the trend was to decrease. In agreement with the results by
225 García *et al.* (1995), it is interpreted because heavy metal concentration of the

226 sewage sludge may have affected to certain extent the mineralization rate of N
227 in our sewage sludge based compost.

228 The C/N ratio significantly decreased from 30.4 in the initial mixture to
229 21.6 at 140 days. This relatively high C/N ratio at the end of composting
230 indicates that organic matter has not achieved an adequate stabilization (De
231 Bertoldi *et al.*, 1996), likely due to the quality of the bulking agent (Pasda *et al.*,
232 2005).

233 Since metal loss by leaching did not occur in our experiment, we
234 observed a continuous increase of total heavy metal concentration in the
235 compost due to the weight loss during composting, the release of carbon
236 dioxide and water, and the mineralization process as shown by Lazzari *et al.*
237 (2000). Although the trend was to increase, no significant differences with time
238 were detected for total Zn. The increase of the total metal concentration during
239 composting was significant for other metals which had high concentration in the
240 sludge (Pb and Cu), but also for metals with low concentrations (Ni, Cd). The
241 total heavy metal concentrations in the obtained compost were below the
242 maximum permitted in Spain for application of sewage sludge in soils (Royal
243 Decree, 1990).

244

245 3.2. Heavy metals bioavailability

246

247 The sum of the amounts extracted by sequential extraction (MB and LB
248 fractions, Table 2) for the most abundant metals (Zn, Pb, Cu, Ni), and also for
249 Cd, was, respectively, almost four or two times lower than the total amount of
250 metal. This result indicates that most metals are mainly bound to residual forms.

251 Our results agree with those by Amir *et al.* (2005), who found recoveries of 20-
252 30% using this sequential extraction procedure. The fact that the residual
253 fraction is so abundant indicates that an important proportion of metal is
254 probably occluded in minerals present in the sludge as has been referred by
255 Wong *et al.* (2001) for some metals such as Pb.

256 For all metals except Pb, the concentration of the MB forms is higher
257 than that in the LB forms (Table 2). This suggests that composting enhances
258 the availability of most of metals. The MB and LB fractions of Zn vary in a
259 quadratic function with time. The amount of Zn_{MB} increases to a maximum at
260 day 84 and decreases thereafter to concentrations that are similar to those at
261 day 14. Zn_{LB} follows the reverse trend (Table 2). It decreases to a minimum at
262 day 84, and increases at day 140 to concentrations that are similar to those at
263 the beginning of composting. This result suggests that important changes in the
264 Zn speciation occur in the final period.

265 For Cu, Ni and Cd, the concentrations of the MB fraction follow a linear
266 significant increase with time of composting. Cu_{LB} also linearly decreases with
267 time although with the b parameter (absolute value) lower than the
268 corresponding parameter of the Cu_{MB} model. This result indicates that the
269 increase in the MB fraction of Cu occurs at expenses of both the LB fraction
270 and the residual fraction. The changes in the LB fractions of Ni and Cd during
271 composting follow a curvilinear trend, reaching a maximum at day 84 and
272 decreasing thereafter, especially Ni. The decrease of the Ni_{LB} in the last period
273 of composting suggests that some moieties of the Ni_{LB} become more available
274 and increase the Ni_{MB} fraction, whereas some other could join the residual
275 fraction. For Cd, the decrease in the LB fraction is lower than the corresponding

276 increase of the MB fraction, and suggests that some Cd_{MB} forms also at
277 expenses of residual Cd. The MB fraction of Pb remains constant during
278 composting, but the curvilinear trend of increase observed for Pb_{LB} indicates
279 that this form likely increases at expenses of the residual forms during
280 composting and reaches a maximum at the end of the process. Although the
281 quadratic fit may indicate a further decrease, this cannot be tested because of
282 the lack of data beyond 140 days. Overall, the results for Pb indicate that this
283 metal accumulates in the very stable organic fractions and unavailable mineral
284 forms.

285 Increased availability for Zn and Cu through composting is in agreement
286 with results obtained by several authors (Wong et al., 2001, Amir *et al.* 2005).
287 The observed increase of Cd availability is in agreement with results by
288 McGrath and Cegarra (1992), who found high extractable Cd levels in sludge-
289 amended soils. For composted sludges Walter *et al.* (2006) found increased
290 mobility for Zn, Cu and Cd during composting. Richards *et al.* (1997) found
291 reductions in Pb mobility and an increase in Cd and Cu mobility because of the
292 composting process.

293

294 3.3. Humic substances

295

296 The changes in C in humic (FAs, HAs) and humic-like substances
297 (hydrolyzed humin) and their ratios during composting are shown at the bottom
298 of Table 2. All of them changed significantly with time of composting indicating
299 that transformations of the organic matter and humification have occurred. C in
300 FAs increased mainly during the initial phases of composting. The best model

301 describing the FAs changes is quadratic and reflects that stop increase beyond
302 day 84 of composting. C in FAs was higher than that in HAs thus indicating that
303 among the soluble humic substances the most abundant are those of low
304 molecular weight. Some of them may have polymerized in the last phases of
305 composting likely forming more condensed structures such as HAs thus
306 explaining some of the increase of C in HAs and also the linear decrease of the
307 C_{FA}/C_{HA} ratio (Table 2).

308 C in HAs increased linearly during composting. Its rate of increase was
309 similar or even higher than that of C in FAs, as deduced from the b parameters
310 of models. The linear rate of increase observed for C in hydrolyzed humin is
311 higher than that of C in HAs as deduced by comparison of the corresponding b
312 parameters. This suggests that both FAs and HAs of the sludge may
313 polymerize in the form of humin. The abundance of aliphatic compounds in
314 sewage sludges may have a negative effect on the formation of the condensed
315 structures typical of the true HAs (García *et al.*, 1989). Likely, the dilution of the
316 sludge with the bulking agent in our compost has lowered this negative effect
317 and even facilitated HAs and likely humin formation through polymerization of
318 FAs with some ligno-cellulosic derivates coming from the partial degradation of
319 the wood chips. The slope of the linear model describing the changes of the
320 HA/Humin ratio (Table 2) is an order magnitude lower (absolute value) than that
321 of FA/HA ratio. This result may indicate that the transformation of FAs into HAs
322 is higher than that of HAs into humin, but also that some compounds in humin
323 may transform into HAs.

324 Finally, the progressive increase of the C_{humus}/TOC ratio indicates that
325 the proportion of humified organic matter (sum of FAs, HAs, and hydrolyzed

326 humin) increases linearly through composting.

327

328 3.4. Metal fraction-humic substances relationships

329

330 Table 3 summarizes the best-fit models (highest R^2) containing the
331 humus fractions in the compost that are most related to the changes in the main
332 metal forms during composting. FAs and humin explained the Zn_{MB} fraction.
333 The stepwise regression procedure selected C_{humus}/TOC ratio as the
334 independent variable predicting metal fractions of the rest of metals. The
335 C_{humus}/TOC ratio was also dependent on the three humus fractions considered
336 in this work. The best model explaining the variations of the C_{humus}/TOC is
337 shown at the bottom of the table. C_{humus}/TOC ratio increases when C in
338 hydrolyzed humin and HAs increase, and when C in FAs decreases.

339 Overall, results in Table 3 suggest that the bioavailability of metals
340 clearly depend on the organic compounds present and formed during
341 composting, which may increase or restrict it.

342

343 (Table 3)

344

345 Regressions indicate that Zn availability is positively associated with the
346 FA fraction and negatively with humin. This result agrees with those by Moreno
347 *et al.* (1996) who questioned the capability of Zn to form complexes with organic
348 compounds. Alloway and Jackson (1991) found Zn associated to organic matter
349 of low molecular weight. The negative dependence with humin may be
350 indicating a decrease in Zn bioavailability at the end of the composting process

351 due to a relative decrease of FAs amount in much more stable forms such as
352 humin.

353 The $C_{\text{humus}}/\text{TOC}$ ratio explains both C_{uMB} and C_{dMB} fractions. C_{uMB}
354 increases at expenses of the LB fraction (Table 2), and this increase is
355 explained by the increase of C in humin and HAs at expenses of
356 transformations of the sulphide forms. It follows that more than a half of C_{uMB}
357 must be attached to alkali insoluble EDTA extractable organic forms such as
358 humin, and the rest bound to alkali soluble HAs. This explanation also follows
359 for Cd, although, as deduced from data (Table 2), C_{dMB} increases at expenses
360 of the residual form of this metal.

361 Ni_{MB} increases when $C_{\text{humus}}/\text{TOC}$ ratio increases and C in FAs decreases
362 because some FAs polymerize into HAs and humin. These results suggest that
363 Ni_{MB} follows the same trend as Cu and Cd, and the reverse trend as Zn_{MB} .

364 As indicated in Table 1, the order of abundance of Cu, Ni, and Cd in the
365 sludge is $\text{Cu} > \text{Ni} > \text{Cd}$. However, comparing the amount of metal in the MB
366 fraction at the beginning and at the end of composting, their relative availability
367 increase in the order $C_{\text{dMB}} > Ni_{\text{MB}} = C_{\text{uMB}}$. This suggests that the main factor
368 explaining their bioavailability during composting was not the initial metal
369 concentration but the stability of complexes with humic-like substances and
370 HAs, which likely increase in the order $\text{Cu} > \text{Ni} > \text{Cd}$. Other authors (Canet et al.,
371 1997) have also attested to the high stability of Cu-organic matter complexes.
372 Soler Rovira *et al.* (2010) found that the complexing capacity of Cu (II)
373 increased as the humification degree increased. Our results suggest that it may
374 occur also for Ni and Cd.

375 Finally, the model for Pb differs from the rest of metals. Since the MB

376 fraction did not change through composting (Table 2), the increase of the Pb_{LB}
377 fraction in a quadratic model with the C_{humus}/TOC ratio may indicate the amount
378 of residual Pb changing to LB forms. The quadratic fit would open the question
379 to a further decrease of Pb_{LB} forms (decreasing branch of the curve) depending
380 on the compost maturation.

381

382

383 **4. Conclusions**

384

385 The 140 days composting process of a mixture of sewage sludge and
386 wood chips (C/N ratio of 30.4), resulted in a product with a relatively high C/N
387 ratio of 21.6, a relatively low stabilization of the organic matter if considered the
388 dominance of FAs over HAs, and total heavy metal concentrations below the
389 maximum permitted for land application. With exception of Pb, the relative
390 bioavailability of metals increased with composting. Zn bioavailability was
391 mainly associated to percentage C in FAs. Bioavailability of Cu, Ni and Cd
392 during composting was associated to percentage C in humin and HAs. Pb
393 concentration increased in unavailable forms, and followed a quadratic function
394 of the C_{humus}/TOC ratio.

395 Our results suggest that the composting process renders the metal in more
396 available forms. The main forms of metal binding in the sludge and their
397 availability in the final compost may be better described when metal
398 fractionation obtained in sequential extraction and humus fractionation during
399 composting are considered together.

400

401

402 **5. Acknowledgements**

403

404 This work was part of the Generalitat Valenciana projects IIARCO2004-A-196,
405 IIARCO2004-A-212, and IIARCO/2004/213, and the national project 135/2004/3
406 (Ministerio de Medio Ambiente). We thank FACSA Sewage Treatment Plant
407 (Castellón, Spain), and University Jaume I of Castellón (Spain) for providing
408 materials and technical assistance. We thank the two anonymous reviewers for
409 their useful comments on the manuscript.

410

411

412 **6. References**

413 Alloway, B.J., Jackson, A.P., 1991. The behaviour of heavy metals in sewage
414 sludge-amended soils. *Sci. Total Environ.* 100, 151-16.

415 Amir S., Hafidi M., Merlina G., Revel J.C., 2005. Sequential extraction of heavy
416 metals during composting of sewage sludge. *Chemosphere* 59, 801–810.

417 Banegas, V., Moreno, J. L., Moreno, J. L., García, C., León, G., Hernández, T.
418 2007. Composting anaerobic and aerobic sewage sludges using two
419 proportions of sawdust. *Waste Manage.* 27, 1317-1327.

420 Cambardella CA, Richard TL, Russell A., 2003. Compost mineralisation in soil
421 as a function of composting process conditions. *Eur. J. Soil Biol.* 39, 117-127.

422 Canet, R., Pomares, F., Tarazona, F., 1997. Chemical extractability and
423 availability of heavy metals after seven year application of organic waste to a
424 citrus soil. *Soil Use & Manage.* 13, 117-121.

425 CEC, Council of the European Communities, 1986. Directive on the protection

426 of the environment, and in particular of the soil, when sewage sludge is used in
427 agriculture (86/278/CEE). Official Journal of the European Communities L181,
428 6-12.

429 De Bertoldi, M., Sequi, P., Lemmes, B., Papi, T., 1996. The Science of
430 Composting. Chapman & Hall. London.

431 EPA, 1990. Standard operating procedure for the analysis of metals in soils,
432 sediments and solids by ICP. ICSY1 Dec, 3/1990. Version 11.

433 Fuentes, A., Llorens, M., Sáez, J., Soler, A., Aguilar, M. I., Ortuño, J. F.,
434 Meseguer, V. F., 2004. Simple and sequential extraction of heavy metals from
435 different sewage sludges. Chemosphere 54(8), 1039-1047.

436 Gallardo A., Bovea, M.D., Contreras, R., Lapeña, L., Ingelmo, F., Molina, M.J.,
437 2007. Production of compost with woodchips and stabilized sludge from urban
438 wastewater treatment plants. Influence of temperature on the composting
439 process. 11th International Congress of Projects Engineering. Agroforestry
440 Engineering Department. Lugo (Spain), 26-28 September. I.S.B.N. 978-84-690-
441 8134-1. (In Spanish).

442 García, C., Hernández, T., Costa, F., 1989. Study of the lipidic and humic
443 fractions from organic wastes before and after the composting process. Sci.
444 Total Environ. 81, 551-560.

445 García, C., Hernández, T., Costa F., Ayuso, M., 1992. Evaluation of the
446 maturity of municipal waste compost using simple chemical parameters.
447 Commun. Soil Sci. Plant Anal. 23, 1501-1512.

448 García, C., Moreno, J.L., Hernández, T., Costa, F., 1995. Effects of composting
449 on sewage sludges contaminated with heavy metals. Bioresource Technol. 53,
450 13-19.

451 Hanc, A., Tlustos, P., Szakova, J., Habart, J., 2009. Changes in cadmium
452 mobility during composting and after soil application. *Waste Manage.* 29 (8),
453 2282-2288.

454 Hsu, J.H., Lo, S.L., 2001. Effect of composting on characterization and leaching
455 of copper, manganese, and zinc from swine manure. *Environ. Pollut.* 114, 119-
456 127.

457 Jouraiphy, A., Amir, S., El Gharous, M., Revel, J. C., Hafidi, M., 2005. Chemical
458 and spectroscopic analysis of organic matter transformation during composting
459 of sewage sludge and green plant waste. *Int. Biodeter. Biodegr.*, 56 (2), 101-
460 108.

461 Kunito, T., Saeki, K., Goto, S., Hayashi, H., Oyaizu, H., Matsumoto, S., 2001.
462 Copper and zinc fractions affecting microorganisms in long-term sludge-
463 amended soils. *Bioresource Technol.* 79 (2), 135-146.

464 Lazzari, L., Sperti, L., Bertin, P., Pavoni, B., 2000. Correlation between
465 inorganic (heavy metals) and organic (PCBs and PAHs) micropollutant
466 concentrations during sewage sludge composting process. *Chemosphere* 41,
467 427-435.

468 Liu, Y., Ma, L., Li, Y., Zheng, L. 2007. Evolution of heavy metal speciation
469 during the aerobic composting of sewage sludge. *Chemosphere* 67 (5), 1025-
470 1032.

471 Maboeta, M. S., van Rensburg, L., 2003. Vermicomposting of industrially
472 produced woodchips and sewage sludge utilizing *Eisenia fetida*. *Ecotoxicol.*
473 *Environ. Saf.* 56 (2), 265 – 270.

474 MAFF (Ministry of Agriculture, Fisheries and Food), 1996. Official methods of
475 analyses. Madrid, Spain. 532 pp. (In Spanish).

476 McGrath, S.P., Cegarra, J., 1992. Chemical extractability of heavy metals
477 during and after long-term application of sewage sludge to soil. *J. Soil Sci.* 43,
478 313-321.

479 Moreno, J.L., García, C., Hernández, T., Pascual, J.A., 1996. Transference of
480 heavy metals from a calcareous soil amended with sewage-sludge compost to
481 barley plants. *Bioresource Technol.* 55, 221-258.

482 Neves, L., Ferreira, V., Oliveira, R., 2009. Co-composting cow manure with food
483 waste: The influence of lipids content. *World Acad. Sci., Eng. & Technol.* 58,
484 986-991.

485 Pasda, N., Limtong, P., Oliver, R., Montange, D., Panichsakpatana, S., 2005.
486 Influence of bulking agents and microbial activator on thermophilic aerobic
487 transformation of sewage sludge. *Environ. Technol.* 26 (10), 1127-1136.

488 Petruzzelli, G., Ottaviani-Lubrano, L., Veschetti, E., 1994. Characterization of
489 heavy metal mobile species in sewage sludge for agricultural utilisation.
490 *Agrochimica* 38, 277-284.

491 Polkowska-Motrenko, H., Danko, B., Dybczynski, R., Koster-Ammerlaan, A.,
492 Bode, P., 2000. Effect of acid digestion method on cobalt determination in plant
493 materials. *Anal. Chim. Acta* 408, 89-95.

494 Richards, B.K., Peverly, J.H., Steenhuis, T.S., Liebowitz, N., 1997. Effect of
495 processing mode on trace elements in dewatered sludge products. *J. Environ.*
496 *Qual.* 26, 782-788.

497 Royal Decree, 1990. RD 1310/1990. October, 20. BOE 0-11-1990. (In Spanish).

498 Soumaré, M., Demeyer, A., Tack, F.M.G., Verloo, M.G., 2002. Chemical
499 characteristics of Malian and Belgian solid waste composts. *Bioresource*
500 *Technol.* 81, 97-101.

501 Tandy, S., Healy, J. R., Nason, M. A., Willianson, J. C., Jones, D. L., 2009.
502 Heavy metal fractionation during the co-composting of biosolids, deinking paper
503 fibre and green waste. *Bioresource Technol.* 100 (18), 4220 – 4227.
504 Walter, I., Martínez, F., Cala, V., 2006. Heavy metal speciation and phytotoxic
505 effects of three representative sewage sludges for agricultural uses. *Environ.*
506 *Pollut.* 139, 507-514.
507 Wong, J.W.C., Li, K., Fang, M., Su, D.C., 2001. Toxicity evaluation of sewage
508 sludges in Hong Kong. *Environ. Int.* 27, 373-380.
509 Zaccheo, P., Ricca, G., Crippa, L., 2002. Organic matter characterization of
510 compost from different feedstocks. *Compost Sci. & Utilizat.* 10, 29-38.
511 Zorpas, A., Arapoglou, D., Panagiotis, K., 2003. Waste paper and clinoptilolite
512 as a bulking material with dewatered anaerobically stabilized primary sewage
513 sludge (DASPSS) for compost production. *Waste Manage.* 23, 27-35.

514

515

Captions

516

517 Table 1. Physico-chemical properties (n=3) of the sludge based compost at
518 different days of composting. All results expressed in dry basis.

519

520 Table 2. Evolution of heavy metal fractions, humic substances, and their ratios
521 during composting. Metal fractions (mg kg^{-1} dry basis): MB=Mobile+Mobilisable
522 (Bioavailable), LB=low bioavailability. FA=% C Fulvic acids; HA=% C Humic
523 acids; TOC=% Total organic carbon. Means in a row followed by the same letter
524 are not significantly different at $\alpha = 0.05$ according to the Tukey's t-test. P=P
525 values of the F test in ANOVA curvefit for linear and quadratic models. Model

526 parameters: a =constant; b= coefficient of x in linear and quadratic models;
527 c=coefficient of x^2 in the quadratic model. Independent variable =Time of
528 composting (days).

529

530 Table 3. Best-fit models for major metal fractions variations as a function of C in
531 different humus fractions during composting, and linear combination of humic
532 substances explaining the variations in the $C_{\text{humus}}/\text{TOC}$ ratio.

Table 1. Physico-chemical properties (n= 3) of the sludge based compost at different days of composting. All results expressed in dry basis.

Time*	Moisture (%)	pH	EC (dSm-1)	TOC (%)	Total N (%)	C/N	Total Zn	Total Pb	Total Cu	Total Ni	Total Cd
										mg kg ⁻¹	
0	71.8 a	7.07 a	1.06 a	50.0 a	1.64 a	30.4 a	259.8 a	45.3 a	37.7 a	2.24 a	0.29 a
14	71.0 a	7.03 a	1.14 a	45.2 b	1.54 a	29.3 ab	262.1 a	49.5 ab	41.3 a	2.38 a	0.33 ab
84	69.0 b	7.01 a	1.16 a	37.4 c	1.51 a	24.8 ab	267.1 a	53.7 ab	43.1 ab	2.69 b	0.40 ab
140	68.8 b	7.01 a	1.12 a	31.3 d	1.45 a	21.6 b	278.2 a	57.4 b	49.5 b	2.76 b	0.45 b
ANOVA											
F	15.942	1.848	0.243	1960.23	1.896	14.340	2.924	14.141	12.446	19.310	12.365
p	0.001	0.217	0.864	0.000	0.209	0.001	0.100	0.001	0.002	0.001	0.002
* Days of composting. Mean value followed by different letters is statistically different (Tukey's t-test, p < 0.05).											

Table 2. Evolution of heavy metals in medium bioavailable forms (MB) and in low bioavailable forms (LB), and evolution of humic (FA, HA), humic-like substances (hydrolysable humin), and their ratios during composting. All metal concentrations are expressed in mg kg⁻¹ dry matter (n=3).

Dependent Variable	Means and Tukey`s t-test				ANOVA curvefit		Best-fit Model parameters			
	Time of composting (days)				F	P	a	b	c	R ²
	0	14	84	140						
Zn _{MB}	39.4 a	46.2 b	53.6 c	46.1 b	80.533	<0.001	40.41	0.347	-0.002	0.947
Zn _{LB}	25.6 a	19.4 b	13.2 c	23.4 a	94.132	<0.001	24.77	-0.343	0.002	0.954
Cu _{MB}	5.09 a	6.15 b	7.00 c	8.92 d	153.23	<0.001	5.349	0.024		0.939
Cu _{LB}	4.34 a	4.18 a	3.78 b	3.45 c	160.85	<0.001	4.300	-0.006		0.941
Pb _{MB}	5.27 a	5.42 a	5.43 a	5.25 a		0.7540				
Pb _{LB}	6.06 a	6.96 b	7.99 c	8.49 d	87.080	<0.001	6.263	0.031	-0.0001	0.951
Ni _{MB}	0.27 a	0.24 ab	0.29 ab	0.46 b	34.852	<0.001	0.234	0.001		0.777
Ni _{LB}	0.31 a	0.36 ab	0.38 ab	0.14 b	16.597	0.0010	0.309	0.001	-0.00004	0.787
Cd _{MB}	0.06 a	0.07 b	0.09 c	0.13 d	222.03	<0.001	0.065	0.0004		0.957
Cd _{LB}	0.08 a	0.09 b	0.11 c	0.10 d	882.99	<0.001	0.081	0.0001	-0.000004	0.995
C _{FA}	5.80 a	6.72 b	8.80 c	9.07 c	64.860	<0.001	6.217	0.023	-0.0002	0.866
C _{HA}	2.11 a	2.24 a	4.82 b	5.87 c	354.86	<0.001	2.057	0.029		0.973
C _{Humin}	7.30 a	8.34 a	11.4 b	13.0 c	100.51	<0.001	7.616	0.040		0.941
C _{FA} /C _{HA}	2.75 a	3.02 a	1.82 b	1.54 c	84.349	<0.001	2.904	-0.010		0.894
C _{HA} /C _{Humin}	0.29 a	0.27 a	0.42 b	0.45 c	104.43	<0.001	0.277	0.001		0.913
C _{humus} /TOC	0.30 a	0.38 b	0.67 c	0.89 d	1383.5	<0.001	0.315	0.004		0.993

Metal fraction (mg kg⁻¹ dry basis): MB=Mobile+Mobilisable (Bioavailable), LB=low bioavailability. FA=% C Fulvic acids; HA=% C Humic acids; TOC=% Total organic carbon. Means in a row followed by the same letter are not significantly different at $\alpha = 0.05$ according to the Tukey`s t-test. P= P values of the F test in ANOVA curvefit for linear and quadratic models. Model parameters: a =constant; b= coefficient of x in linear and quadratic models; c=coefficient of x² in the quadratic model. Independent variable =Time of composting (days).

Table 3. Best-fit models for major metal fractions varying in the sewage sludge compost and C in humus fractions during composting.

Dependent variable	Model	Best-fit Model parameters			
		Coefficient	SE	p-value	R ²
Zn _{MB}	Constant	8.271	2.897	0.019	0.975
	C _{FAs}	14.919	1.412	<0.001	
	C _{Humin}	-7.515	0.848	<0.001	
Cu _{MB}	Constant	3.538	0.304	<0.001	0.965
	C _{humus} /TOC	5.785	0.499	<0.001	
Pb _{LB}	Constant	3.578	0.533	<0.001	0.982
	C _{humus} /TOC	10.330	2.051	0.011	
	(C _{humus} /TOC) ²	-5.418	1.710	<0.001	
Ni _{MB}	Constant	0.588	0.031	<0.001	0.990
	C _{humus} /TOC	0.908	0.041	<0.001	
	C _{FAs}	-1.030	0.007	<0.001	
Cd _{MB}	Constant	0.033	0.004	<0.001	0.951
	C _{humus} /TOC	0.101	0.007	<0.001	
C _{humus} /TOC	Constant	-0.084	0.104	0.440	0.990
	C _{Humin}	0.090	0.030	0.018	
	C _{HA}	0.073	0.029	0.036	
	C _{FAs}	-0.069	0.032	0.065	