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 Molina[1], Maria Desamparados Soriano[3],**

5 **Antonio Gallardo[4], Leonor Lapeña[4]**

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_{humus}/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_{humus}/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 hydrolisable 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 30ºC 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 $HNO_3:HClO_4$

146   (Polkowska-Motrenko et al., 2000).

147   Compost samples were extracted with 0.1 M NaP_2O_7 (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 H_2SO_4 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: H_2O (shaking during 2h at 20 ºC, three times); KNO_3 0.5

171   M (shaking during 16 h at 20 ºC); NaOH 0.5 M (shaking during 16 h at 20 ºC),

172   and EDTA 0.05 M (shaking during 16 h at 20 ºC). Finally, to collect the low-

173   bioavailable fraction LB (bound to sulphides; hardly mobilisable) the samples

174   were treated with HNO_3 4 M (shaking during 16 h at 80 ºC). 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 35ºC. 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 CO_2 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_{humus}/TOC ratio explains both Cu_{MB} and Cd_{MB} fractions. Cu_{MB}

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 Cu_{MB}

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), Cd_{MB} increases at expenses

360   of the residual form of this metal.

361   Ni_{MB} increases when C_{humus}/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 Cu>Ni>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 Cd_{MB}>Ni_{MB}=Cu_{MB}. 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 Cu>Ni>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

5. Acknowledgements

402

403

410

411

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., Sperni, 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 α = 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_{humus}/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 mg kg^{-1}	Total Ni	Total Cd
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 α = 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 x^2 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_{FA}	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_{FA}	-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_{FA}	-0.069	0.032	0.065	