

1 **Elemental concentration and migratability in bioplastics derived from organic waste**

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16

17 **Abstract**

18

19 In line with the Circular Economy approach, the production of polyhydroxyalkanoate (PHA) with organic
20 waste as the feedstock may a biotechnological application to reduce waste and recover high-value materials.

21 The potential contaminants that could transfer from bio-waste to a PHA include inorganic elements, such as
22 heavy metals. Hence, the total content and migratability of certain elements were evaluated in several PHA
23 samples produced from different origins and following different methods. The total content of certain
24 elements in PHA ranged between 0.0001 (Be) and 49500 mg kg⁻¹ (Na). The concentrations of some alkaline
25 (Na and K) and alkaline earth (Ca and Mg) metals were highest, which are of little environmental concern.

26 The feedstock type and PHA stabilisation and extraction procedures affected the element contents. Several
27 sets of experiments were conducted to evaluate the migration of elements from the PHA samples under
28 different storage times, temperatures, and pH levels. The total contents of some heavy metals (As, Cd, Fe,

29 Hg, Ni, Pb, and Zn) in PHA produced from fruit waste or crops (commercial PHA) were lower than those in
30 the PHA samples produced from the mixture of the organic fraction of municipal waste and sludge from
31 wastewater treatment. Both the PHA obtained by extraction from wet biomass (acid storage) with aqueous
32 phase extraction reagents and commercial PHA were below the migration limits stipulated by the current
33 Toy Safety Directive and by Commission Regulation (EU) 10/2011 on plastic materials and articles intended
34 to come into contact with food under frozen and refrigerated conditions.

35

36 **Keywords**

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38 Biopolymers; polyhydroxyalkanoates; bioaccessibility; toxic elements; inductively coupled plasma mass
39 spectrometry; cold vapour generation atomic fluorescence spectrometry.

40

41 **1. Introduction**

42

43 Polyhydroxyalkanoates (PHAs) can be considered to be the sustainable polymers of the future due to their
44 physicochemical properties, biodegradability, and biocompatibility (Kourmentza et al., 2017; Tsang et al.,
45 2019; Valentino et al., 2017). In line with the Circular Economy approach (European Commission, 2015),
46 the production of PHAs may be a biotechnological application to reduce waste through reuse and recovering
47 high-value substances. PHAs are naturally polymerised by bacteria using organic waste as a low-cost carbon
48 source (Khiewwijit et al., 2015; Kourmentza et al., 2017). In recent years, the organic fraction of municipal
49 solid waste has been used as a new feedstock for microorganism-based PHA production, which has become a
50 valuable strategy for reducing biopolymer production costs and increasing their sustainability (Valentino et
51 al., 2018; Valentino et al. 2019).

52 One of the main concerns relating to the use of waste as a raw material is the content of priority pollutants
53 that can migrate from the waste, through the technology chain, and into the end-products (i.e., PHA). The
54 possible chemical contaminants include inorganic elements, such as heavy metals. In recent years, ecological
55 and public health concerns associated with environmental contamination by these elements have increased
56 (Astolfi et al., 2019a; He et al., 2005). Heavy metal (As, Cd, Hg, and Pb) toxicity can damage the
57 functioning of the brain, lungs, kidney, liver, blood composition, and other important organs (Alina et al.,
58 2012; Chowdhury et al., 2000; Henson and Chedrese, 2004; Martin and Griswold, 2009; Smith et al., 2000).
59 Repeated long-term exposure to some metals and their compounds may even cause cancer (Järup, 2003).
60 Toxic elements can originate from geogenic, industrial, agricultural, pharmaceutical, domestic, and
61 atmospheric sources (Astolfi et al., 2017, 2006; Canepari et al., 2018; Manigrasso, et al., 2019; Marconi et
62 al., 2011; Vitali et al., 2019). Sources of heavy metals from household municipal solid waste include
63 household dust, batteries, disposable household materials, paints and inks, body care products and medicines,
64 and household pesticides (Bardos, 2004). Significant concentrations of heavy metals are also present in
65 industrial wastewater and municipal sludge (Altaş, 2009).

66 Under waste law, Italy has a national end-of-waste system that regulates the procedure for the authorisation
67 of certain non-hazardous waste recovery options and establishes the conditions under which biodegradable
68 waste can cease to be considered waste (Italian Regulation, 1998).

69 The European Commission has issued several directives on plastic materials, including Directive 94/62/EC
70 Packaging and Packaging waste (European Parliament, Council of the European Union, 1994), Directive
71 2009/48/EC on the safety of toys (European Parliament, Council of the European Union, 2009), and
72 Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact
73 with food (European Commission, 2011). In particular, the presence of As, Cd, Cr, Hg, and Pb in plastics is
74 regulated by Annex XVII of the EU REACH Regulation on chemicals (European Parliament, Council of the
75 European Union, 2006). Moreover, Directive 2009/48/EC on the safety of toys specifies limits for the
76 migration of 19 elements (Al, Sb, As, Ba, B, Cd, Cr(III), Cr(VI), Co, Cu, Pb, Mn, Hg, Ni, Se, Sr, Sn, organic
77 Sn, and Zn) from toy materials and parts.

78 To our knowledge, the elements of PHA biopolymers have not yet been determined. This study describes the
79 first investigation of the occurrence and migratability of heavy metals and other elements in several PHA
80 samples with different sources and produced following different process steps. The contents of 40 elements
81 (Al, As, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, K, La, Li, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Rb,
82 Sb, Se, Si, Sn, Sr, Te, Ti, Tl, U, V, W, Zn, and Zr) and Hg were quantified by inductively coupled plasma
83 mass spectroscopy (ICP-MS) and cold-vapour generation atomic fluorescence spectrometry (CV-AFS),
84 respectively. Although there is currently no specific PHA regulation, our results were compared with current
85 regulations and guidelines for similar materials and/or applications.

86

87 **2. Materials and methods**

88

89 **2.1 PHA samples**

90

91 Two types of feedstock were considered, including a) a mixture of the organic fraction of municipal solid
92 waste and biological sludge from urban wastewater treatment (collected from the Treviso pilot plant in Italy;
93 PHA TV) and b) fruit waste (collected in from Lisbon pilot plant in Portugal; PHA L). Details regarding the
94 production of PHA TV have been reported in previous studies (Valentino et al., 2019a, 2019b). The
95 production of PHA L was similar to that for PHA TV. The main difference was the type of feedstock, which
96 was organic waste originating from fruit processing.

97 The extraction step of the production technologies (especially PHA extraction from biomass) received the
98 most attention. Thus, both PHA-rich biomass before extraction and purified PHA after extraction from
99 biomass were considered. Three different types of extraction for the latter were considered.

100 After PHA accumulation, the PHA-rich biomass was stabilised for storage via overnight thermal drying
101 (biomass from Treviso only; PHA TV-d) or acidification with H₂SO₄ (biomass from both Treviso and
102 Lisbon; PHA TV-a and PHA L-a, respectively).

103 For the type-a) feedstock samples, four different PHA samples were analysed: 1) raw PHA-rich biomass
104 (dried biomass, no extraction; PHA TV-d-ne); 2) PHA after extraction from dried biomass with CHCl₃
105 (reference method; PHA TV-d-CHCl₃); 3) PHA after extraction from dried biomass with NaClO (PHA TV-
106 d-NaClO); and 4) PHA after extraction from wet biomass (acid storage) with aqueous-phase extraction (PHA
107 TV-a-ap). For each type, at least two different samples were collected from the pilot plant at different
108 operation times.

109 For the type-b) feedstock samples, one type of PHA sample was analysed, i.e., PHA after extraction from
110 wet biomass (acid storage) with aqueous-phase extraction reagents (PHA L-a-ap).

111 Overall, 24 different PHA samples produced under six different conditions were analysed. For comparison,
112 three types of commercial PHA obtained from different producers (PHA comm) that were produced from
113 fruit waste or crops were analysed, and their results were expressed as the average.

114

115 **2.2. Materials and reagents**

116

117 Analytical reagent-grade concentrated HNO₃ (67–70%; superpure) was obtained from Carlo Erba Reagents
118 S.r.l. (Milan, Italy), CH₃COOH (100%; superpure) was purchased from Merck KGaA (Darmstadt,
119 Germany), while HCl (assay >36%; residue <3 mg L⁻¹) and H₂O₂ (assay >30%) were obtained from
120 Promochem, LGC Standards GmbH (Wesel, Germany).

121 The 5% HCl was used as a carrier, and 0.05% NaBH₄ (Sigma-Aldrich Chemie GmbH, St. Louis, USA) in
122 0.05% NaOH (assay >98%, anhydrous pellets, RPE for analysis, ACS – ISO; Carlo Erba Reagents, Milan,
123 Italy) was used as a reducing agent for CV-AFS.

124 Deionised water (resistivity $\leq 18.3 \text{ M}\Omega \text{ cm}$) was obtained using an Arioso Power I RO-UP Scholar UV
125 system from Human Corporation (Songpa-Ku, Seoul, Korea). Graduated tubes (2.5–5–10 mL in
126 polypropylene) were purchased from Artiglass S.r.l. (Due Carrare, PD, Italy). Syringe filters with cellulose
127 nitrate membranes (GVS Filter Technology, Indianapolis, USA; pore size, $0.45 \mu\text{m}$) were used to filter the
128 samples prior to analysis.

129 All plastic containers, polypropylene tubes, pipette tips, quartz digestion tubes, and reagents that came into
130 contact with the samples or standards were checked for contamination.

131

132 **2.3. Standard solutions**

133

134 An ICP-MS multi-element standard solution ($1.000 \pm 0.005 \text{ mg L}^{-1}$ As, Al, Ba, Be, Bi, Cd, Cr, Cs, Cu, Ga,
135 La, Li, Mn, Mo, Nb, Ni, Pb, Rb, Sb, Se, Sn, Te, Ti, Tl, U, V, W, and Zr; $5.00 \pm 0.03 \text{ mg L}^{-1}$ Ce and Co;
136 $10.00 \pm 0.05 \text{ mg L}^{-1}$ Fe and Zn; $50.00 \pm 0.25 \text{ mg L}^{-1}$ P and Si; $55.00 \pm 0.25 \text{ mg L}^{-1}$ B and Sr; $500.0 \pm 2.5 \text{ mg}$
137 L^{-1} K, Mg, and Na; and $1000 \pm 5 \text{ mg L}^{-1}$ Ca) was purchased from Ultra Scientific/Agilent Technologies
138 (North Kingstown, RI, USA), while a CV-AFS standard solution of Hg ($1002 \pm 7 \text{ mg L}^{-1}$) was obtained from
139 SCP Science (Baie D'Urfé, Canada). All standard solutions were diluted with the same percentage of acid
140 present in the samples [3% (v/v) HNO_3 for the digests, and 1% (v/v) HNO_3 , 3% (v/v) CH_3COOH , or 0.07 M
141 HCl for the extracts obtained by the migration tests].

142 To maintain the daily ICP-MS performance, a multi-standard tuning solution with 0.005 mg L^{-1} of Ba, Be,
143 Ce, Co, In, Pb, Mg, Tl, and Th was prepared from a multi-standard stock solution ($10.00 \pm 0.05 \text{ mg L}^{-1}$)
144 obtained from Spectro Pure/Ricca Chemical Company (Arlington, TX, USA). A standard solution of Y (at
145 0.005 mg L^{-1} from $1000 \pm 2 \text{ mg L}^{-1}$), purchased from Panreac Química (Barcelona, Spain), and a multi-
146 element standard solution of Sc, Rh, In, and Th (at 0.010 mg L^{-1} from $1000 \pm 5 \text{ mg L}^{-1}$), obtained from
147 Merck KGaA (Darmstadt, Germany), in 1% (v/v) HNO_3 were employed as the internal standards to control
148 the nebuliser efficiency, as previously reported (Astolfi et al., 2020a, 2020b).

149 A solution of trace SRM 1643e elements in water (National Institute of Standards and Technology, NIST;
150 Gaithersburg, MD, USA) was used for quality control.

151

152 **2.4. Instrumentation**

153

154 A quadrupole ICP-MS (820-MS Bruker; Bremen, Germany) equipped with a collision-reaction interface
155 (CRI) and glass nebuliser (0.4 mL min^{-1} ; MicroMistTM; Analytik Jena AG, Jena, Germany) was used to
156 analyse all elements, excluding Hg, which was analysed by CV-AFS (AFS 8220 Titan, FullTech
157 Instruments; Rome, Italy). The operating parameters for the ICP-MS and CV-AFS analyses are summarised
158 in Table S1 and are detailed in previous studies (Astolfi et al., 2020; 2019b).

159 A Gibertini Elettronica (Milan, Italy) Europe 60 analytical balance (sensitivity, 0.1 mg) was used to weigh
160 all samples.

161 A Milestone (FKV; Sorisole, Bergamo, Italy) Ethos1 Touch Control microwave system was used for acid
162 digestion, and a water bath (WB12; Argo Lab, Modena, Italy) with electronic temperature control was used
163 for the migration tests.

164

165 **2.5. Sample preparation**

166

167 **2.5.1 Total acid digestion**

168

169 Weighed amounts ($\sim 50 \text{ mg}$ or $\sim 200 \text{ mg}$ for CV-AFS or ICP-MS analysis, respectively) of the PHA samples
170 were transferred into quartz vessels; to these, 1 mL 67% HNO_3 , 0.5 mL 30% H_2O_2 , and 1.5 mL deionised
171 water were added. The resulting solutions were subsequently heated with microwave energy for 40 min. The
172 following heating program was used: first step, heating to $50 \text{ }^\circ\text{C}$ for 5 min; second step, hold temperature at
173 $50 \text{ }^\circ\text{C}$ for 5 min; third step, heating to $100 \text{ }^\circ\text{C}$ for 5 min; fourth step, hold temperature at $100 \text{ }^\circ\text{C}$ for 5 min;
174 fifth step, heating to $150 \text{ }^\circ\text{C}$ for 5 min; sixth step, hold temperature at $150 \text{ }^\circ\text{C}$ for 5 min; seventh step, heating
175 to $180 \text{ }^\circ\text{C}$ for 5 min; and eighth step, hold temperature at $180 \text{ }^\circ\text{C}$ for 15 min. Prior to use, the quartz vessels
176 were cleaned with the same reagents and digestion program used for the PHA samples, rinsed with deionised
177 water, and thoroughly dried with absorbent paper. The solutions obtained from digestion were diluted to 5
178 mL with 3% HCl or 10 mL with deionised water and filtered for CV-AFS or ICP-MS analysis, respectively.
179 Prior to ICP-MS analysis, the digests were further diluted 1:10 and 1:50 with deionised water.

180 Blanks (3% HNO₃) were periodically analysed alongside the samples to check for any losses or cross-
181 contamination.

182

183 **2.5.2 Migration tests**

184

185 Ten blank solutions were tested with each sample set extracted during the different migration tests to subtract
186 the background signal from the reagents and trace and control the contributions from possible sample
187 contamination. The blanks were deducted from all measurements. The procedure for each treatment is
188 detailed in the following sections. At the end of each migration test, the solution was filtered and analysed by
189 CV-AFS or ICP-MS. Prior to CV-FS analysis, the extracts were further diluted with 6% HCl in a ratio of 1:2.

190

191 **2.5.2.1 H₂O treatment experiments**

192

193 Weighed amounts (~25 mg) of the PHA samples were transferred into polypropylene tubes, to which 5 mL
194 of deionised H₂O (pH ~7) was added. The tubes were then covered with a cap and heated at 70 °C for 2 h or
195 stored in the dark at 20 °C for 10 days.

196

197 **2.5.2.2 CH₃COOH treatment experiments**

198

199 Weighed amounts (~25 mg) of the PHA samples were extracted in dilute CH₃COOH (food simulant B; pH
200 ~4.5) according to Commission Regulation (EU) No 10/2011 (European Commission, 2011) on plastic
201 materials and articles intended to come into contact with food. Five millilitres of a 3% CH₃COOH solution,
202 simulating food with a pH below 4.5, were pipetted into each tube with the PHA samples, which were then
203 capped and placed in a water bath at 70 °C for 2 h or stored in the dark at 20 °C for 10 days.

204

205 **2.5.2.3 HCl treatment experiments**

206

207 Weighed amounts (~25 mg) of the PHA samples were transferred into polypropylene tubes mixed with 5 mL
208 of 0.07-M HCl and then heated in a water bath for 2 h to 37 °C according to European standard EN 71-3
209 (British Standards Institution, 1994).

210

211 **2.6. Method performance**

212

213 Method performance parameters, such as the trueness [per cent bias (%bias) or recovery (%R)], precision
214 (intraday and interday within-laboratory precision), linearity range, and detection and quantification limits
215 (LODs and LOQs, respectively), were assessed. Fortified PHA TV-a-ap samples were used for the quality
216 control of the total digestion treatment, while a fortified reference water solution (SRM 1643e) was used for
217 the migration tests. Detailed information on the calibration graphs and quality control for elemental analysis
218 was shown in the Supplementary Material. Briefly, the instruments were calibrated externally using mixed
219 standards prepared by serial dilution in the same matrix as the final samples (3% HNO₃ for digests, and 1%
220 HNO₃, 3% CH₃COOH, and 0.07 M HCl for extracts). The linear ranges of the 41 elements examined in this
221 study are listed in Table S2.

222 Owing to the lack of certified reference materials and a blank matrix for PHA biopolymers, the analytical
223 quality control was verified by conducting recovery experiments. The obtained recoveries of the spiked PHA
224 samples ranged from 90 to 102% (Table S2), confirming that there was no significant loss during digestion
225 or contamination, or interference during analysis.

226 A reference water solution (SRM 1643) was fortified with 2 µg L⁻¹ Hg; 25 µg L⁻¹ Ce, Cs, Ga, La, Nb, Sn, Ti,
227 U, W, and Zr; and 2500 µg L⁻¹ P and Si to assess the quality of the analysis of extracts obtained by the
228 migration tests. Fortified SRM 1643e was analysed after each calibration curve and revealed element
229 concentrations that were within 10% of the certified values and ranged from 90 to 96% of spiked elements,
230 excluding Al, As, Ba, Se, and Te (within 15%; Table S2).

231 The method was determined to be within-laboratory precision, both intra-day (repeatability) and inter-day
232 (reproducibility), at the concentration used for the recovery study by calculating the per cent coefficient of
233 variation (%CV). Three spiked samples were analysed on the same day to assess the repeatability and on
234 three different days to assess the reproducibility. The results are listed in Table S2.

235 The LODs and LOQs were calculated as three and ten times the standard deviation of the blank samples,
236 respectively (Table S3). The method blanks were treated as samples to subtract the background signal from
237 the reagents. The LODs and LOQs ranged from 0.0001 (Be and Nb) to 100 mg kg⁻¹ (Ca) and 0.0004 (Be) to
238 350 mg kg⁻¹ (Ca), respectively.

239

240 **2.7. Statistical analysis**

241

242 Statistical analyses were conducted using IBM SPSS Statistics 25 software (IBM Corp., Armonk, NY, USA).
243 For each element, values below the LOD were replaced with a value equal to half the LOD. When the
244 percentage of values <LOD exceeded 20%, the element was excluded from the statistical elaboration.

245 The differences in the PHA concentration were tested by Kruskal-Wallis and pairwise post-hoc tests. The
246 results (Table 1) were considered to be statistically significant with p-values of <0.05.

247

248 **3. Results and discussion**

249

250 **3.1 Total elemental content**

251

252 Table 1 shows the results of the Kruskal Wallis test, which was conducted to verify the presence and
253 concentrations of differences elements among the six different types of PHA samples. Nb exhibited no
254 significant differences between all of the PHA samples, while the levels of Bi, Se, Te, Tl, V, W, and Zr were
255 very low or below the LODs, with an inadequate number of observations for multivariate analysis (Table 1).
256 Thus, these elements were eliminated from the multivariate analysis data set.

257 Focusing on PHA-rich biomass with no extraction (PHA content around 50% w/w, after thermal drying), the
258 concentrations of alkaline and alkaline earth metals were highest, with the levels of Na, Ca, Mg, and K
259 ranging between 0.2% and 5% on a dry-weight basis. Furthermore, P was present at a concentration of
260 approximately 0.4%, while Fe and Mn were present at concentrations of approximately 0.1% and 0.01%,
261 respectively. However, all of these elements are of no or little concern from environmental or health
262 viewpoints. Zn was the heavy metal present at the highest concentration of approximately 300 mg kg⁻¹

263 (0.03%). The concentration of Cu was approximately 70 mg/kg (0.007%), while those of other toxic metals,
264 such as Ni and Pb, were below 10 mg kg⁻¹ (0.001%). Moreover, Cd is below 0.1 mg kg⁻¹ (0.00001%). The
265 contents of metalloids, such as and Se, were below 1 mg kg⁻¹ (0.0001%).

266 PHA extraction from dried biomass using hypochlorite destroys the organic matter, causing it to concentrate
267 or dilute the metals, depending on how strongly sorbed to the remaining solid phase they are. For example,
268 the contents of Al, As, Hg, K, Na, Mo, and U were strongly reduced, while those of Cd, Fe, Mn, Pb, and Zn
269 were increased. Furthermore, chloroform dissolves the PHA, causing the metals to remain in the residual
270 solid phase, i.e., chloroform extraction also acts as a method of purifying PHA from inorganic elements (Ca,
271 Cd, Cu, Mn, Na, Rb, Sr, and Zn). The contents of all investigated elements decreased, excluding B, Cr, Nb,
272 Sb, Se, Te, Tl, V, W, and Zr, which were already at a low concentration.

273 The metal concentrations of biomass that underwent extraction after acid stabilisation and aqueous-phase
274 extraction were typically lower than those of biomass that underwent stabilisation by thermal drying. This
275 could be expected as inorganic elements were retained in the solid phase during thermal drying, while they
276 could be desorbed more easily in the liquid phase during acidic stabilisation.

277 The effects of different feedstock types (municipal waste/sludge vs fruit waste) under the same acid
278 stabilisation and aqueous-phase extraction were compared. The comparison of PHA TV-a-ap and PHA L-a-
279 ap indicates that more inorganic elements are present in the PHA derived from municipal waste/sludge than
280 that derived from fruit waste (excluding As, Na, and Si, although the difference was not significant). The
281 concentration of inorganic elements in commercial PHA, which is industrially obtained from crop substrates,
282 is usually lower than that of waste-based PHA.

283 The contents of some environmentally relevant elements (Al, As, Cd, Hg, Ni, Pb, and Zn) were highest in the
284 PHA derived from municipal waste. This was not surprising, as this source also included a significant
285 fraction of sludge from wastewater treatment (approximately one-third). However, there are still
286 environmental benefits to recovering organic carbon from waste in the form of biodegradable plastics that
287 can counteract the higher level of contaminants, if the content of contaminants is sufficiently low. The
288 contents of most relevant contaminants were at the mg kg⁻¹ or µg kg⁻¹ level, which can be considered
289 acceptable. The European Commission has issued several directives on requirements for plastic materials
290 intended to be used in different ways, such as Directive 94/62/EC on packaging and packaging waste

291 (European Parliament, Council of the European Union, 1994), Directive 2009/48/EC on the safety of toys
292 (European Parliament, Council of the European Union, 2009), and Commission Regulation (EU) No 10/2011
293 on plastic materials and articles intended to come into contact with food (European Commission, 2011). In
294 particular, Annex II of Directive 94/62/EC (European Parliament, Council of the European Union, 1994) on
295 packaging and packaging waste determines the essential requirements that all packaging on the market
296 within the European Community should comply with. Article 11 of Directive 94/62/EC (European
297 Parliament, Council of the European Union, 1994) states that Member States shall ensure that the sum of the
298 concentrations of Pb, Cd, Hg, and Cr(VI) in packaging or packaging components shall not exceed 100 mg
299 kg^{-1} . The sum of these metals was well below this level in selected PHA samples.

300 According to regulation 1907/2006/EC (European Parliament, Council of the European Union, 2006), Cd
301 contamination receives particular attention, and the regulation states that mixtures and articles composed of
302 plastic materials shall not be placed on the market if the concentration of Cd is equal to or greater than 0.01%
303 (100 mg kg^{-1}) of the plastic material. The Cd contents of all waste-based PHA samples in our study are well
304 below this limit. Commission Regulation (EU) No 10/2011 establishes a set of specific requirements for
305 PHA obtained through microbial fermentation by using *Alcaligenes eutrophus*. According to this regulation,
306 PHA should not contain more than 100, 5, 2, 1, and 1 mg kg^{-1} of Zn, Cu, Pb, As, and Cr, respectively (Table
307 2). By comparing these values with Table 1, it can be seen that the contents of Cr, Cu, and Pb in PHA
308 produced from municipal waste/sludge exceeded these limits, while only the content of Cu exceeded the
309 limit in PHA from fruit waste. Additionally, the content of Cr in commercial PHA exceeded the limit. Hence,
310 in terms of heavy metals content, these samples did not comply with the regulations for plastics intended to
311 come into direct contact with food, and the extraction techniques need to be further improved.

312 Inorganic species may be present as residues from the catalysts or additives used in the production of
313 polyethylene terephthalate (PET), the most favourable packaging material for drinking water (Bach et al.,
314 2012). Sb_2O_3 is the preferred catalyst for the synthesis of PET due to its low cost and sufficient catalytic
315 activity (Keresztes et al., 2009), and PET typically contains Sb levels in the range of 170-300 mg kg^{-1}
316 (Nishioka et al., 2002; Keresztes et al., 2009; Westerhoff et al., 2008). The maximum average content of Sb
317 ($0.131 \pm 0.085 \text{ mg kg}^{-1}$; Table 1) of the studied PHA samples was approximately 1000 times lower than that
318 in PET. Other metals, such as Co, Cr, Fe, and Mn, have also been found in PET bottles, with contents of 27,

319 0.1, 1.3, and 0.3 mg kg⁻¹, respectively (Westerhoff et al., 2008). In this case, the content of Co in all the PHA
320 samples was at least 100 times lower, while that of Mn was lower than or similar to the values in literature
321 for PHA L-a-ap and PHA comm.

322

323 **3.2 Migratable concentrations**

324

325 Extraction tests were conducted on all PHA samples to assess compliance/noncompliance with the European
326 Directive migration limit (Table 2) and explore possible controls and variations in element migratability.

327 Table 3 summarises the results obtained for all analysed elements by the different migration tests in terms of
328 concentration, and detailed data are provided in the Supplementary Material (Tables S4-S8). The migratable
329 concentrations ranged from <0.2 µg kg⁻¹ for Be, Cs, La, Nb, Tl, and U to >1000 mg kg⁻¹ for Ca, K, Mg, Na,
330 and P, and was highly variable, ranging from less than 1% to approximately 100%. The results for each
331 treatment are discussed in detail in the following sections.

332

333 **3.2.1 Element migratability in water**

334

335 The elemental analysis results for the H₂O experiment are presented in Tables 3, S4, and S5. The
336 concentrations of many of the minerals commonly found in H₂O, including Ca, K, Mg, Na, and P, were
337 elevated, and the levels of many trace elements were lower. Heating can increase the migration speed and
338 concentrations of migrated elements than the storage of solutions at room temperature (20 °C). The
339 concentrations of Cu, Mn, and Sb in PHA TV-d-ne and Sb in PHA TV-a-ap after heating to 70 °C for 2 h
340 were higher than those achieved 10 days at 20 °C. Small differences were detected for the other elements in
341 all PHA samples.

342 The migration test for materials and articles intended for food contact was conducted using the time and
343 temperature specified in Commission Regulation (EU) No 10/2011 (European Commission, 2011). We
344 conducted the following overall migration tests: 1) test for 10 days at 20 °C, covering any contact with food
345 under frozen and refrigerated conditions; and 2) tests for 2 h at 70 °C, covering all other contact conditions,
346 including heating up to 70 °C for up to 2 h, or up to 100 °C for up to 15 min, which are not followed by long-

347 term storage at room or refrigerated temperatures. The contents of Ba, Co, and Cu in PHA TV-d-ne; Mn in
348 PHA TV-d-ne, PHA TV-d-CHCl₃, and PHA TV-d-NaClO; and Pb in PHA TV-d-NaClO exceeded the
349 migration limits of Commission Regulation (EU) No 10/2011 for both tests. The contents of Ba in PHA TV-
350 d-CHCl₃ and Fe in PHA TV-d-ne exceeded the limits for test 1), and those of Ba in PHA TV-d-NaClO and
351 Sb in all PHA samples, excluding PHA TV-d-CHCl₃ and PHA comm, exceeded the limits for test 2). The
352 concentrations of all the selected elements in PHA comm were below the migration limits, thus, these PHA
353 samples are food (water)-contact safe under frozen and refrigerated conditions, at 70 °C for 2 h, or at 100 °C
354 for up to 15 min.

355 The lower levels of elements observed in polymeric materials than Sb explain the lack of studies conducted
356 on elemental migration into bottled water (Bach et al., 2012). The specific migration limit for Sb is 0.04 mg
357 kg⁻¹ (European Commission, 2011). Consistent with our results, temperature significantly affects the release
358 of Sb (Bach et al., 2012; Cheng et al., 2010; Keresztes et al., 2009; Westerhoff et al., 2008). There were no
359 significant traces of inorganic elements other than Sb in PET-bottled water subjected to different conditions
360 (Cheng et al., 2010; Reimann et al., 2010), and more metals (Al, Ce, Pb, and Zr) can leach from glass into
361 water than PET bottles (Reimann et al., 2010).

362

363 **3.2.2 Element migratability in CH₃COOH**

364

365 Tables 3, S6, and S7 show the results of testing PHA samples with CH₃COOH, i.e., food simulant B listed in
366 Commission Regulation (EU) No. 10/2011 (European Commission, 2011). Food simulants must be used to
367 test migration from materials and articles not yet in contact with food. Food simulant B is used for foods
368 with hydrophilic characteristics, can extract hydrophilic substances, and have a pH below 4.5 (beverages,
369 such as clear fruit or vegetable juices of normal strength or concentrated, fruit nectars, lemonades; and milk
370 products, such as fermented milk including yoghurt, buttermilk and similar products, and processed and
371 preserved cheese) The data show that the concentrations of elements such as Ba, Co, Cu, Fe, Mn, and Zn
372 increased under acidic conditions (Tables 3, S6, and S7). The maximum levels of the analysed elements in
373 CH₃COOH increased by more than double the concentration obtained in H₂O (Table 3). The levels of
374 elements released from PHA L-a-ap and PHA comm were below the migration limits; thus, these materials

375 are safe for contact with food under frozen and refrigerated conditions, while PHA comm is safe for contact
376 with food at 70 °C for 2 h, or at 100 °C for up to 15 min.

377 In contrast with our results, for all analysed elements (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Se,
378 Tl, and V), excluding Sb, in all PET bottle samples, Cheng et al. (2010) detected no significant leaching due
379 to low pH, and the levels of metals were well below the specific migration limit listed in Commission
380 Regulation (EU) No 10/2011 (European Commission, 2011). Keresztes et al. (2009) also demonstrated that
381 the Sb dissolution rate into water is higher into sparkling water than that into still water due to the lower pH
382 of carbonated water.

383

384 **3.2.3 Element migratability in HCl**

385

386 The original European Council Directive 88/378/EEC (Council of the European Union, 1988) on toy safety
387 stipulated migratable limits for some hazardous elements based on the ingestion of a small amount of
388 material and defined by 2 h of extraction under simulated gastric conditions (dilute HCl at 37 °C) according
389 to European standard EN 71-3, as listed in Table 2 (British Standards Institution, 1994; Turner, 2018). An
390 amended directive (European Parliament, Council of the European Union, 2009) provided revised limits on
391 migration in dilute HCl that depended on the matrix being tested (dry, brittle, powder-like, or pliable toy
392 materials; liquid or sticky toy materials; and scraped-off toy materials), as well as limits for additional
393 elements and different oxidation states of Cr (Table 2). Furthermore, there is currently a proposal to reduce
394 the concentration limits of Pb (Council of the European Union, 2017). The results of this study (Tables 3 and
395 S8) reveal low concentrations of the elements listed by the original Toy Safety Directive (European Council
396 Directive 88/378/EEC). Instead, the extracted concentrations of Cd in PHA TV-d-NaClO and Pb in all PHA
397 samples excluding PHA L-a-ap and PHA comm exceeded the current or proposed European migratable
398 limits.

399 The contents of Cd and Pb in second-hand plastic toys also exceeded their migration limits stipulated by the
400 current EU Toy Safety Directive, with the release of Cd from yellow and red Lego bricks exceeding the limit
401 by one order of magnitude (Turner et al., 2018). Cd and Pb were used as stabilisers in polyvinyl chloride
402 (PVC) or, in association with other elements (Cr, Mo, and S) as coloured pigments (Turner et al., 2018).

403

404 **3.2.4 Bioaccessibilities of elements**

405 Exposure to elemental concentrations and their health hazards are related to their migratable levels rather
406 than their total contents. The percentage migration relative to the total elemental content can be a measure of
407 the bioaccessibility. The results shown in Figures 1 and S1-S4 indicate wide variations in the bioaccessibility
408 of the toxic elements listed in Table 2. Sb and Se were not considered because all data were <LOQs. The
409 standard deviation of the mean bioaccessibility percentage values in each PHA was very high as the
410 elemental composition of each PHA sample collected at different operation times from the pilot plant (batch)
411 was highly variable. Therefore, we reported all the results for each batch of the different types of PHA.
412 Generally, the migration of elements is higher in acidic solutions and with heating (Figures 1 and S1-S4). For
413 all considered conditions, the bioaccessibility percentages of Al, Cr, and Fe were lower than 60% for all
414 types of PHA, and that of Sn was below 40% (Figure S1). As, Cd, and Hg appeared to be more bioaccessible
415 in PHA TV-d-ne; PHA TV-d-ne and PHA TV-NaClO; and PHA TV-NaClO and PHA TV-a-ap (Figure 1),
416 respectively.

417

418 **4. Conclusions**

419

420 In the context of the European Circular Economy strategy, an analytical method for determining the presence
421 and migration of 41 elements, including alkaline metals, alkaline earth metals, heavy metals and metalloids,
422 in PHA biopolymers was optimised. The obtained LODs were suitable for the identifying element
423 concentrations well below the limits set for plastic materials by European regulations.

424 PHA samples produced from different origins and with different process steps were analysed. The elemental
425 content of PHA was generally low, ranging from ppb to a few ppm, except for alkaline and alkaline earth
426 metals (which are of little environmental concern). The feedstock type affected the contaminant levels, with
427 the PHA from fruit waste having a lower element content than PHA obtained from the mixture of the organic
428 fraction of municipal waste and sludge from wastewater treatment. The crop-derived commercial PHA was
429 found to have lower concentrations of heavy metals than the waste-based PHA samples. The PHA
430 stabilisation and extraction process also affected the contents of contaminants, where acid stabilisation and

431 extraction with aqueous extractants resulted in lower heavy metal contents than thermal stabilisation and
432 extraction with either hypochlorite or chloroform.

433 Although there is currently no specific regulation, the PHA samples were compared to the regulations and
434 guidelines for similar materials and/or applications. The total content of the analysed elements for all tested
435 PHA types complied with the current regulations and guidelines, such as the limits for Cd in plastic materials
436 based on REACH regulation, including toys. According to Commission Regulation (EU) No 10/2011, PHA
437 TV-a-ap and PHA L-a-ap are food (water)-contact safe under frozen and refrigerated conditions and PHA
438 comm in all tested conditions; PHA L-a-ap and PHA are safe for contact with food under frozen and
439 refrigerated conditions, and PHA comm is safe for contact with food at 70 °C for 2 h, or at 100 °C for up to
440 15 min. The levels of elements released from PHA L-a-ap and PHA comm were below the migration limits
441 as stipulated by the current and proposed EU Toy Safety Directive.

442

443 **Declaration of competing interest**

444

445 The authors declare that they have no known competing financial interests or personal relationships that
446 could have appeared to influence the work reported in this paper.

447

448 **Acknowledgments**

449

450 This work was supported by the “REsources fromURban Bio-waSte” - RES URBIS (GA 7303499) project in
451 the European Horizon 2020 (Call CIRC-05-2016) program.

452

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621 **Table 1**622 Number of valid data (N), descriptive statistics (mg kg⁻¹) and Kruskal-Wallis test for each analysed element in the different PHA samples.

623

Types of feedstock		Municipal waste/sludge									Fruit waste			Crops					
Element	N	PHA TV-d-ne ^a			PHA TV-d-CHCl ₃ ^b			PHA TV-d-NaClO ^c			PHA TV-a-ap ^d			PHA L-a-ap ^e			PHA comm ^f		
		Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD
Al	23	548 ^{f,c}	1090	1500	52	49	37	9.6 ^a	9.0	5.9	51 ^f	52	33	6.11	6.11	0.92	2.76 ^{a,d}	2.76	0.62
As	20	0.79 ^{d,e,f}	0.73	0.13	0.145	0.152	0.096	0.206 ^d	0.213	0.091	0.029 ^{a,c}	0.035	0.018	0.072 ^a	0.072	0.070	0.028 ^a	0.036	0.019
B	19	4.2 ^f	4.5	1.1	5.0 ^f	10	11	4.1 ^f	5.5	2.6	2.1	3.2	3.0	1.93	1.93	0.19	<1 ^{a,b,c}	<1	-
Ba	21	28 ^{e,f}	23	15	2.2	2.6	1.3	28 ^{d,e,f}	38	28	1.92 ^c	1.89	0.59	1.31 ^{a,c}	1.31	0.30	<1 ^{a,c}	<1	-
Be	18	0.031 ^{b,f}	0.027	0.018	<0.0001 ^{a,c}	<0.0001	-	0.053 ^{b,c,f}	0.056	0.025	0.0032	0.0043	0.0032	0.000657 ^c	0.000657	0.000071	<0.0001 ^{a,c}	<0.0001	-
Bi	12	0.16	<0.02	-	<0.02	<0.02	-	0.52	0.41	0.27	<0.02	<0.02	-	<0.02	<0.02	-	<0.02	<0.02	-
Ca	24	20400 ^{d,f}	19200	7600	827 ^c	848	220	28900 ^{c,d,e,f}	28000	2500	393 ^{a,c}	719	850	467 ^c	467	320	422 ^c	420	8.7
Cd	17	0.101 ^{d,e,f}	0.096	0.070	0.015 ^c	0.019	0.015	0.36 ^{b,d,e,f}	0.37	0.22	<0.005 ^{a,c}	0.0088	0.0085	<0.005 ^{a,c}	<0.005	-	<0.005 ^{a,c}	0.006	0.003
Ce	21	0.66 ^f	0.57	0.42	0.062 ^c	0.073	0.042	1.29 ^{b,d,e,f}	1.10	0.80	0.052 ^c	0.067	0.041	0.046 ^c	0.046	0.032	<0.006 ^{a,c}	<0.006	-
Co	21	0.46 ^{b,d,f}	0.46	0.13	0.03 ^{a,c}	<0.01	-	0.36 ^{b,d,f}	0.53	0.35	0.051 ^{a,c}	0.062	0.046	0.052	0.052	0.020	<0.01 ^{a,c}	<0.01	-
Cr	24	3.2 ^b	2.8	1.1	0.53 ^{a,c}	0.55	0.16	2.64 ^b	2.66	0.70	2.6	4.2	4.2	0.95	0.95	0.49	1.3	1.2	1.0
Cs	22	0.081 ^{b,f}	0.071	0.051	0.0013 ^{a,c}	0.0023	0.0021	0.076 ^{b,f}	0.085	0.064	0.0059	0.0065	0.0042	0.0036	0.0036	0.0016	<0.0004 ^{a,c}	<0.0004	-
Cu	24	50 ^{b,f}	74	43	3.3 ^{a,c}	3.8	1.5	77 ^{b,f}	88	23	18	22	18	14	14	13	0.211 ^{a,c}	0.203	0.057
Fe	24	779 ^{e,f}	805	590	43.5 ^c	45.0	5.1	1430 ^{b,d,e,f}	1660	1200	82 ^c	77	35	19.4 ^a	19.4	4.3	22 ^{a,c}	19	17
Ga	21	0.20 ^{b,f}	0.29	0.28	0.011 ^{a,c}	0.014	0.012	0.35 ^{b,f}	0.38	0.21	0.087	0.086	0.031	0.0490	0.0490	0.0092	<0.001 ^{a,c}	<0.001	-
Hg	24	0.22 ^{d,e,f}	0.18	0.12	0.023	0.025	0.017	0.066 ^{e,f}	0.071	0.020	0.012 ^a	0.023	0.027	0.0062 ^{a,c}	0.0062	0.0053	0.0034 ^{a,c}	0.0033	0.0020
K	23	1810 ^{b,c,d,e,f}	2280	1700	59 ^a	63	22	150 ^a	129	56	41 ^a	42	24	29.3 ^a	29.3	9.6	18.7 ^a	21.0	5.2
La	21	0.39 ^f	0.34	0.24	0.034 ^c	0.041	0.025	0.80 ^{b,d,e,f}	0.66	0.47	0.027 ^c	0.032	0.017	0.017 ^c	0.017	0.015	<0.004 ^{a,c}	<0.004	-
Li	24	0.59 ^{b,f}	0.57	0.32	0.016 ^{a,c}	0.026	0.026	0.75 ^{b,f}	0.78	0.56	0.061	0.063	0.045	0.0230	0.0230	0.0045	0.0042 ^{a,c}	0.0078	0.0069
Mg	24	3490 ^{d,e,f}	4360	3300	514	548	350	3570 ^{d,e,f}	6470	5600	169 ^{a,c}	220	160	141 ^{a,c}	141	23	19 ^{a,c}	24	10
Mn	22	83 ^{e,f}	75	13	5.8 ^c	5.3	3.1	166 ^{b,d,e,f}	171	14	1.26 ^c	1.38	0.42	0.18 ^{a,c}	0.18	0.15	0.24 ^{a,c}	0.32	0.30
Mo	24	0.56 ^{b,f}	0.60	0.19	0.0173 ^{a,c,d}	0.0196	0.0058	0.058 ^b	0.062	0.013	0.074 ^b	0.104	0.080	0.044	0.044	0.016	0.0262 ^a	0.0247	0.0068
Na	24	49500 ^{b,d,f}	51100	36000	205 ^a	202	80	753	1140	870	299 ^a	878	1200	1290	1290	190	65 ^a	231	290
Nb	16	0.056	0.052	0.038	0.00512	0.00538	0.00065	0.058	0.069	0.033	0.0105	0.0103	0.0014	0.00530	0.00530	0.00001	<0.005	<0.005	-

Ni	23	9.0^{e,f}	8.7	1.3	2.23^c	2.24	0.62	11.3^{b,d,e,f}	13.5	5.9	1.7^c	2.3	1.8	0.55^{a,c}	0.55	0.22	0.093^{a,c}	0.072	0.043
P	24	4057^{b,d,e,f}	4300	790	341^a	330	160	3190^{d,f}	3020	1200	64^{a,c}	128	160	108^a	108	13	24^{a,c}	29	15
Pb	21	5.9^f	7.4	4.2	0.73^c	0.87	0.58	26^{b,e,f}	59	89	2.4	3.8	4.1	0.260^c	0.260	0.084	<0.1^{a,c}	<0.1	-
Rb	24	1.3^{b,f}	1.8	1.1	0.032^{a,c}	0.035	0.010	0.81^{b,f}	0.91	0.65	0.107	0.107	0.067	0.043	0.043	0.017	0.0112^{a,c}	0.0116	0.0041
Sb	22	0.088^{b,f}	0.106	0.058	0.019^{a,d}	0.021	0.013	0.049^f	0.066	0.033	0.133^{b,f}	0.131	0.085	0.034	0.034	0.021	<0.002^{a,c,d}	<0.002	-
Se	1	<0.2	<0.2	-	<0.2	<0.2	-	<0.2	<0.2	-	<0.2	<0.2	-	<0.2	<0.2	-	<0.2	<0.2	-
Si	24	855^{b,f}	790	320	181^{a,c}	188	63	686^{b,f}	620	220	329	332	52	564^f	564	170	125^{a,c,e}	113	23
Sn	24	1.06^f	1.01	0.79	0.101^c	0.104	0.026	1.8^{b,e,f}	2.0	1.4	0.14	0.50	0.79	0.070^c	0.070	0.037	0.011^{a,c}	0.036	0.044
Sr	24	110^{d,e,f}	114	42	4.25	4.31	0.73	112^{d,e,f}	112	16	3.5^{a,c}	6.5	6.6	2.5^{a,c}	2.5	1.1	0.485^{a,c}	0.475	0.023
Te	2	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-	<0.005	<0.005	-
Ti	24	10.3^{b,e,f}	11.5	6.8	0.88^{a,c}	0.83	0.36	17^{b,e,f}	21	13	2.18	2.17	0.61	0.48^{a,c}	0.48	0.27	0.078^{a,c}	0.098	0.043
Tl	15	0.012	0.015	0.010	0.00165	0.00164	0.00085	0.023	0.029	0.019	<0.0003	<0.0003	-	<0.0003	<0.0003	-	<0.0003	<0.0003	-
U	19	0.130^{b,d,e,f}	0.131	0.077	0.0106^a	0.0110	0.0030	0.058^{e,f}	0.069	0.035	0.0047^a	0.0086	0.0092	<0.001^{a,c}	<0.001	-	<0.001^{a,c}	<0.001	-
V	14	1.00	0.86	0.35	<0.03	<0.03	-	0.83	0.75	0.45	0.062	0.072	0.051	<0.03	<0.03	-	<0.03	<0.03	-
W	11	0.078	0.066	0.021	<0.02	<0.02	-	<0.02	<0.02	-	0.09	0.11	0.10	0.0251	0.0251	0.0088	<0.02	<0.02	-
Zn	24	178^{e,f}	330	270	26^c	46	47	766^{b,d,e,f}	1070	870	39^c	48	41	12.0^{a,c}	12.0	2.9	2.3^{a,c}	3.6	2.4
Zr	14	0.76	0.61	0.44	0.108	0.123	0.048	1.14	1.36	0.68	0.18	0.20	0.16	0.24	0.24	0.25	<0.1	<0.1	-

624

625 a,b,c,d,e,f Different superscript letters within rows are significantly different (p <0.05). a = PHA TV-d-ne; b = PHA TV-d-CHCl₃; c = PHA TV-d-NaClO; d = PHA TV-a-ap; e = PHA L-

626 a-ap; f = PHA comm.

627 **Table 2**628 Migratable limits (mg kg⁻¹) of elements in food and toys and as defined by the original and amended EC toy
629 safety directives.

630

Element	CR (EU) No 10/2011 ^a	88/378/EEC ^b	2009/48/EC ^c		
			in dry, brittle, powder-like or pliable toy material	in liquid or sticky toy material	in scraped-off toy material
Al	-	-	5625	1406	70000
As	1 ^e	25	3.8	0.9	47
B	-	-	1200	300	15000
Ba	1	1000	4500 (1500)	1125 (375)	56000 (18750)
Be	-	-	-	-	-
Bi	-	-	-	-	-
Ca	-	-	-	-	-
Cd	-	75	1.9 (1.3)	0.5 (0.3)	23 (17)
Ce	-	-	-	-	-
Co	0.05	-	10.5	2.6	130
Cr	1 ^e	60	Cr(III) 37.5; Cr(VI) 0.02	Cr(III) 9.4; Cr(VI) 0.005	Cr(III) 460; Cr(VI) 0.2
Cs	-	-	-	-	-
Cu	5 ^e	-	622.5	156	7700
Fe	48	-	-	-	-
Ga	-	-	-	-	-
Hg	-	-	7.5	1.9	94
K	-	-	-	-	-
La	-	-	-	-	-
Li	0.6	-	-	-	-
Mg	-	-	-	-	-
Mn	0.6	-	1200	300	15000
Mo	-	-	-	-	-
Na	-	-	-	-	-
Nb	-	-	-	-	-
Ni	-	-	75	18.8	930
P	-	-	-	-	-
Pb	2 ^e	-	13.5 (2.0)	3.4 (0.5)	160 (23)
Rb	-	-	-	-	-
Sb	0.04	60	45	11.3	560
Se	-	-	37.5	9.4	460
Si	-	-	-	-	-
Sn	-	-	15000	3750	180000
Sr	-	-	4500	1125	56000
Te	-	-	-	-	-
Ti	-	-	-	-	-
Tl	-	-	-	-	-
U	-	-	-	-	-
V	-	-	-	-	-
W	-	-	-	-	-
Zn	25 (100 ^e)	-	3750	938	46000
Zr	-	-	-	-	-

631 ^a Commission Regulation (CR) (EU) No 10/2011 (European Commission, 2011) on plastic materials and articles to come into
632 contact with food establishes a set of specific requirements for PHA (which has been obtained through microbial fermentation by
633 using *Alcaligenes Eutrophus*.634 ^b The original European Council Directive 88/378/EEC (Council of the European Union, 1988) on toy safety stipulated migratable
635 limits for eight hazardous elements.636 ^c The amended Directive on toy safety (European Parliament, Council of the European Union, 2009) with revised values shown in
637 parentheses. There is currently a proposal to reduce the concentration limits of Pb (Council of the European Union, 2017).

638 **Table 3**639 Summary of the migratable concentrations^a ranges [minimum and maximum mean; mg kg⁻¹] of each
640 element detected in the extracts of different PHA samples.

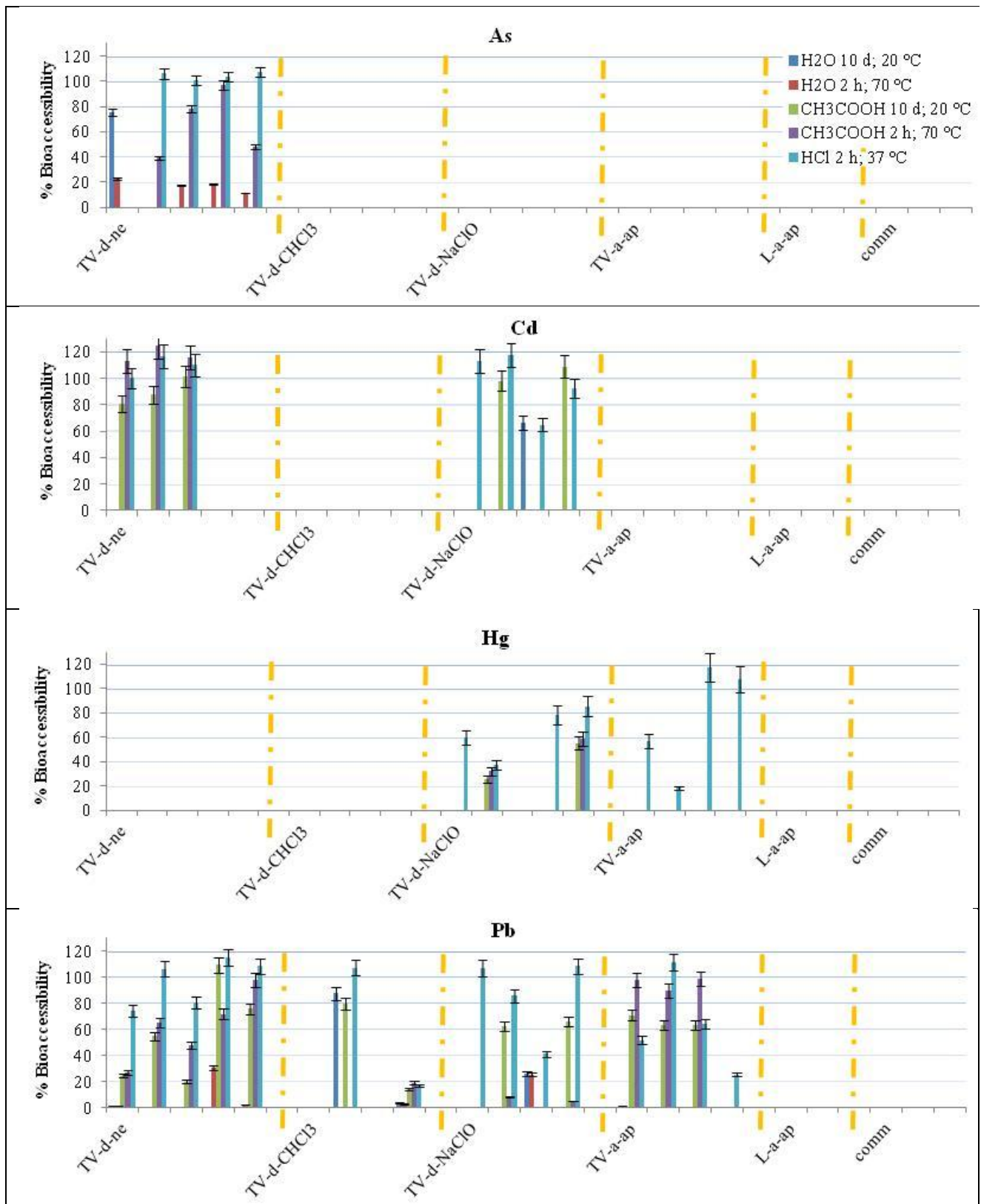
641

Element	H ₂ O (10 d; 20 °C)		H ₂ O (2 h; 70 °C)		CH ₃ COOH (10 d; 20 °C)		CH ₃ COOH (2 h; 70 °C)		HCl (2 h; 37 °C)	
	min	max	min	max	min	max	min	max	min	max
Al	<0.2	3.8	0.321	3.4	<0.04	7.4	0.88	9	<0.1	9
As	<0.1	0.16	<0.02	0.107	<0.05	0.210	<0.06	0.46	<0.2	0.87
B	<0.2	3.9	0.56	3.5	0.211	3.2	0.160	3.8	0.168	3.7
Ba	0.39	1.01	0.401	1.4	<0.1	18.8	0.364	18.7	0.285	25.6
Be	<0.0003	0.00044	<0.0002	0.0024	<0.0002	0.021	<0.0002	0.0217	<0.0003	0.037
Bi	<0.002	0.0058	<0.001	0.00733	<0.0007	0.17	0.00074	0.064	<0.002	0.19
Ca	28	6190	100	7510	69	15200	65	15400	244	21300
Cd	<0.01	0.08	<0.003	0.09	<0.01	0.27	0.00228	0.28	<0.005	0.31
Ce	<0.001	0.0024	<0.001	0.012	0.00061	0.22	<0.001	0.2180	0.0014	0.42
Co	0.0020	0.201	0.00236	0.244	0.0033	0.468	0.0058	0.48	0.0055	0.43
Cr	<0.02	0.15	0.0369	0.60	<0.02	0.56	0.058	1.09	<0.3	4.0
Cs	<0.001	0.0126	<0.0002	0.0153	<0.0006	0.0107	<0.001	0.0230	<0.0002	0.017
Cu	0.26	21	0.28	35	0.26	56	0.22	65	0.24	67
Fe	0.21	54	0.25	3.9	<0.3	156	1.29	171	0.7	520
Ga	0.0081	0.027	0.00983	0.08	0.0107	0.47	<0.01	0.34	<0.01	0.43
Hg	<0.001	0.0028	0.0042	0.0118	0.00149	0.0239	0.0041	0.0272	0.0042	0.0396
K	5.5	909	4.0	1245	7	896	12.4	1650	11.5	1400
La	<0.0002	0.0027	<0.001	0.006	0.00077	0.17	0.0014	0.14	0.0012	0.35
Li	<0.006	0.104	0.0069	0.149	0.0034	0.118	0.00184	0.166	<0.01	0.115
Mg	14	2210	23	2860	21	4040	18	4110	26	6300
Mn	<0.1	21.7	<0.1	17	<0.1	102	0.16	103	0.219	168
Mo	<0.01	0.146	<0.02	0.32	<0.003	0.027	0.0058	0.076	0.0095	0.0581
Na	100	22700	195	37800	93	26800	156	35000	105	22800
Nb	<0.0002	0.00113	<0.0003	0.00059	0.000145	0.0064	<0.0004	0.00606	<0.0003	0.0074
Ni	0.10	3.2	0.11	3.8	<0.1	11.5	<0.1	11.3	<0.1	12.5
P	<2	1280	6.0	778	10.9	3020	8.7	1700	5.0	1840
Pb	0.0180	19	<0.01	18	0.036	12.5	0.064	11.4	0.040	35
Rb	0.0053	0.62	0.0020	0.83	0.0073	0.59	0.012	1.06	0.0071	0.87
Sb	<0.04	<0.04	<0.04	0.177	<0.04	<0.04	<0.04	0.062	<0.04	0.083
Se	<0.2	0.308	<0.1	0.35	<0.04	0.127	<0.08	0.18	<0.4	0.55
Si	5.0	48	<6	97	<30	102	26.7	112	<6	440
Sn	0.0012	0.012	0.004	0.016	0.00105	0.12	0.00346	0.110	<0.001	0.26
Sr	0.17	31	0.33	25	0.19	62	0.17	62	0.30	72.0
Te	<0.01	<0.01	<0.003	0.0086	<0.01	0.0158	<0.01	<0.01	<0.03	0.038
Ti	<0.1	13	0.0517	13	<0.03	8.3	0.0353	13.6	<1	28
Tl	<0.0003	0.0032	<0.0002	0.0080	<0.0003	0.0156	<0.0006	0.0135	<0.004	0.010
U	<0.0002	0.0011	<0.0003	0.0045	<0.0003	0.019	<0.0003	0.039	<0.0003	0.038
V	<0.03	0.138	<0.04	0.55	<0.02	0.25	<0.04	0.62	<1	<1
W	<0.001	0.012	0.0056	0.08	<0.001	0.011	<0.002	0.017	<0.001	0.0103
Zn	0.43	19.5	0.471	11.6	1.2	607	1.01	620	1.4	715
Zr	0.00040	0.018	<0.001	0.019	0.00119	0.09	0.00040	0.0901	0.0014	0.13

642 ^a Note that numbers in bold denote non-compliance according to the Commission Regulation (EU) No 10/2011 (European
643 Commission, 2011) for migration test with H₂O and CH₃COOH; and the European Union Toy Safety Directive (Council of the
644 European Union, 1988; European Parliament, Council of the European Union, 2009; Council of the European Union, 2017) for
645 migration test with HCl. Contact time in days (d) or hours (h) at contact temperature in °C.

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649 **Figure 1** Percentage of bioaccessibility of As, Cd, Hg and Pb in the extracts of different PHA samples taken
 650 at different operation times of the pilot plant (batch). The data not shown are lower than the quantification
 651 limits.