Metals in microplastics: determining which are additive, adsorbed, and bioavailable

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Letter: Metals in microplastics: determining which are additive, adsorbed, and bioavailable

Charlotte Catrouillet[a*], Mélanie Davranche[a], Imane Khatib[a], Corentin Fauny[a], Aurélie Wahl[a], Julien Gigault[a,b*]

[a]*Univ. Rennes, CNRS, Géosciences Rennes - UMR 6118, F-35000 Rennes, France*

[b]*TAKUVIK CNRS/ULaval, UMI3376, Université Laval, Quebec City, QC, Canada*

*Corresponding authors: julien.gigault@takuvik.ulaval.ca and charlotte.catrouillet@univ-rennes1.fr

Abstract: Microplastics from the North Atlantic Gyre deposited on Guadeloupe beaches were sampled and characterized. A new method was developed to identify which elements were present as additives in these microplastics. The method used both acidic leaching and acidic digestion. Using this original method, several elements (Al, Zn, Ba, Cu, Pb, Cd, Mn, Cr) were identified as pigments. Furthermore, some elements used as additives to plastics (especially the non-essential elements) seem to contribute to most of the acidic leaching, suggesting that these additives can leach and readsorb onto the surface of microplastics, becoming bioavailable. Based on the element content in the acidic leaching, only Cd should represent a danger for fish when ingested. However, further studies are needed to determine the potential synergetic effect on health caused by the ingestion of several elements and microplastics.

Keywords: microplastics, metals, metalloids, bioavailability, additive, adsorption, leaching

Introduction

Thousands of metric tons of plastics debris[1] have been released into the environment since the 1950s, and the public is becoming ever-more aware of the negative impacts of plastic debris on living organisms. These impacts can occur directly through the presence or accumulation of plastics, or indirectly via their associated pollutants (organic and inorganic), also known as the Trojan horse effect[2]. From the various chemicals possibly carried by plastics debris, metal and metalloid elements are most used, by relative mass, in plastics[3]. Metals and metalloids are added as plasticizers, flame retardants, antioxidants, pigments, and more.[4] (Throughout this paper, when the word "elements" is used, it is to mean metal and metalloid elements.) As other carbon-based particles are released in the environment, aging plastics debris can act as a support for biofilms or, in response to (photo)chemical oxidation of their surface functional groups, both biofilms and surface groups can adsorb metalloids [5,6].

Metals and metalloids can be highly toxic when inadvertently consumed in aging plastic debris, as these elements are released from the sorbent under acidic digestive conditions (i.e., they become bioavailable through biological digestion processes). There is now clear evidence that chemicals associated with nanoplastics impact aquatic organisms' metabolism much more than the same pristine polymer without metal additives[7]. Measuring metals' relative bioavailability remains challenging, given their surface and/or core distribution in the plastics. From the surface to the core, element exchangeability and bioavailability decrease. However, after a long residence time in the environment, plastics can undergo highly oxidative conditions, which partly alter the surface of plastics and can induce the release of additives (i.e. elements). To evaluate the potential health risks induced by the ingestion of plastics by the biota, it is thus crucial to determine the total concentrations of each element and to discriminate between which elements are labile, and which are incorporated into

plastic structures. However, the most important point is to determine which additives can be leached over time and become bioavailable for the biota.

The additive elements can be identified by measuring the depth distribution of element concentrations in the plastic by laser ablation (LA) inductively coupled plasma mass spectrometry (ICP-MS)[8]. However, only a few elements can be measured in plastic reference materials this way, and it is a time-consuming method. Another possibility is to perform a double extraction of elements: (i) from an acidic leaching to determine which elements are labile and (ii) from an acidic digestion to determine total element concentrations. Numerous studies have determined total metals concentrations in plastics by X-ray fluorescence spectrometry (XRF)[9–28], but this will be the first time for this joint approach consisting of coupling acidic leaching and digestion to ICP-MS or ICP-AES (atomic emission spectrometry) measurements. Our innovative approach aims to determine which elements are additive or adsorbed to the plastics, and their bioavailability to the biota.

Material & Methods

Geographic area and collection

Fragments and pellets of plastic were collected in November 2018 on the beaches of Sainte Marie Bay in Guadeloupe (16°23'43.6"N 61°24'21.9"W). This bay is exposed to the North Atlantic Gyre, which is known to be an area of plastic accumulation. The top 3 cm of sand composed of sargassum, sand, and plastics were manually collected. Size separation was performed using 2 sieves, with 1 cm and 2 mm grids. Microplastics and pellets from 2 mm to 1 cm were separated by visual morphologic aspects. Only microplastics were used in the present study. The collected microplastics were subdivided in 7 colours: Blue, Green, Orange, Red, Yellow, Black, Grey, and White.

Acidic leaching and acidic digestion

Microplastics were first washed with deionized (DI) water under agitation to desorb (detach) any biofilms and natural organic matter residues that often occur with microplastics in the environment[25]. Then 1 g of each coloured microplastic was mixed with 0.1 M HNO_3 (ultrapure grade) for 48 h. Microplastics were then removed from the solution by filtration through a 0.2 µm filter. The solution (<0.2 µm) was further analysed by ICP-MS. Finally, microplastic pieces were acid-digested using a multistep procedure with a microwave oven (UltraWAVE system from Millestone; 110°C for 10 min, then 180°C for 10 min, 230°C for 20 min and 230°C for 5 min). Prior to elemental analyses, samples were evaporated, dried, and then resolubilized with HNO_3 at 0.37 M.

Metal concentrations analysis

Metal concentrations were measured by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) from Agilent Technologies (7700x Model, Agilent). The ICP-MS analyses introduced He gas into a collision cell to suppress any interference from Ar. All limits of quantification are presented in Table S1. The digestion process was validated using reference materials (ERM-EC 680 and ERM-EC 681) from the Joint Research Centre of the European Commission (JRC, Ispra, Italy) (see SI Section S1 for further information).

Results and discussion

In the present work, only microplastics from 2 mm to 1 cm, a size that can be easily ingested by the marine and terrestrial biota, were studied[31,32]. Figure 1 presents the workflow and the hypotheses for the bioavailability of microplastic-associated elements. The percentage of elements released by the washing step was less than 0.1% of the total element concentrations (see Figure 1). This fraction of water-leached elements can be considered to be the most easily exchangeable fraction, and is thus negligible. The acidic leaching releases elements that can be desorbed from the microplastic surface. The pH conditions are aligned

to those encountered in the stomach digestion of animals (i.e. bioavailable fraction by ingestion route). The surface layer of environmentally-aged microplastics can be altered by wear and tear in the environment. For those altered microplastics, the acidic leached fraction represents both (i) the adsorbed environmental elements and (ii) the elements released from the microplastic alteration that were subsequently bound in the altered layer, namely a part of the additive metals. The acidic digestion is a total mineralization of the microplastic debris under extreme conditions (i.e., microwave process using concentrated acid and high temperatures). Such conditions are not environmentally relevant, but they facilitate the determination of less bioavailable metals. Microplastic debris is therefore providing a metal source rather than transfer vector. Based on this metal screening, acid digestion increases the bioavailable metals by four orders of magnitude (Figure 1), illustrating the importance of additives in the metal distribution in microplastics.

Identification of the elements nature in the different coloured microplastics

All elements can potentially be adsorbed on the microplastic surface through specific sites (i.e., carboxylic). Comparing the concentrations of elements determined after acidic leaching and total digestion can provide information on the additive or adsorbed origin of metals.

In Figure S2, the concentrations of (a) Cu (b) Cd (c) Zn (d) Pb (e) Ni (f) Ba (g) Cr (h) V and (i) As were compared for the acidic leaching and digestion, by subtracting the acid-leached concentrations from the total digestion concentrations (S =[Digestion]-[Leaching]) for each colour (Figure 2 and S1-2 for Al). High S values of this microplastics suggested that S could be used to distinguish between additive or adsorbed elements.

Due to their close behaviour, a linear relationship between Fe and Mn concentrations can be established. Only one sample is far from the linear relationship, so the relationship is good enough to determine the additive origin of some samples for Fe, Mn and Al (SI Section S2 for

125 further information). Thus, using Figure 2 and elements identified in the literature as additives
126 (Table S2 and S3), the additives elements can be identified as follows:

127 • **Blue**: Both Cu and Ba present large S values (814 and 2360 nmol g^{-1}, respectively) in
128 blue plastics compared to other colours of microplastics. This might be explained by the
129 use of the inorganic blue pigments $BaCuSi_2O_6$ and $2CuCO_3 \cdot Cu(OH)_2$.
130 • **Red**: Ba and V have high S values (189 and 8 nmol g^{-1}, respectively) in red plastics
131 compared to other colours of microplastics. However, to our knowledge those elements
132 are not used as red pigments, but Ba is a well-known filler in plastics. Unfortunately, very
133 little information is available on V as an additive. However, trace elements were found in
134 fly ash used for building materials, concrete modification, composite and polyester
135 mortar, which might explain its presence as additive[3].
136 • **Green**: Five elements present large S values in the green microplastics: Pb (615.90
137 nmol g^{-1}), Cr (485.68 nmol g^{-1}), Cu (344.21 nmol g^{-1}), Ba (141.40 nmol g^{-1}) and Cd (60.50
138 nmol g^{-1}). The presence of Cd, Cr and Cu should be explained by the use of green
139 pigments, such as phthalocyanine green (Cu organic complex), $Cu(CH_3COO)_2 \cdot H_2O$,
140 $CuCO_3 \cdot Cu(OH)_2$, Cr_2O_3 $2H_2O$, and/or a mix of CdS and Cr_2O_3.
141 Pb and Ba are not used as green pigments, but they are used as yellow pigments as
142 $Pb_3(SbO_4)_2$, $PbCrO_4$ or Pb_2SnO_4 and $BaCrO_4$, which likely explains their presence. Since
143 Sb and Sn were not analysed in our study, it is impossible to know if $Pb_3(SbO_4)_2$ and
144 Pb_2SnO_4 were used in this sample. The presence of Cr as an additive might confirm the
145 use of $PbCrO_4$ and $BaCrO_4$.
146 The use of Pb and Ba for other properties can not also be excluded. Pb and Ba are also
147 largely used in plastic formulations as heat stabilisers, antioxidants, UV stabilisers (Pb),
148 and fillers (Ba). The presence of Cd and Pb as additives in green microplastics from this
149 sampling site was confirmed by El Hadri et al.[8] using LC-ICP-MS (Table S4).

- **Orange**: Five elements have large S values in orange plastics: Al (122.82 µmol g^{-1}), Zn (8820.68 nmol g^{-1}), Cd (426.48 nmol g^{-1}), Ba (68.32 nmol g^{-1}), and V (53.20 nmol g^{-1}). The presence of Cd might be explained by the use of the orange pigment CdS. The presence of Zn, Ba and V might be explained either by their use as yellow pigments or for other properties. Other yellow pigments include $BiVO_4$, $K_2O4ZnCrO_4(H_2O)_3$, $BaCrO_4$. However, Ba and Zn are also well-known fillers and Zn is also a flame retardant. To our knowledge, no common orange, red or yellow pigments contain Al. Thus, Al's presence in this sample as an additive is likely due to its use as flame retardant. Note that El Hadri et al.[8] showed that Cd was present as an additive in orange microplastics by LC-ICP-MS analysis (Table S4).
- **Grey and Black**: Five elements have large S values in grey and black plastics: Cu (402.12 nmol g^{-1}), Pb (180.13 nmol g^{-1}), Cr (174.77 nmol g^{-1}), Mn (75.72 nmol g^{-1}) and Cd (49.77 nmol g^{-1}). Large Mn and Cd concentrations could be explained by the use of Cd-Mn pigments. The presence of Cu, Pb and Cr as additives are either explained by their use as a mix of different pigments to obtain the black colour, or their use for properties other than pigments in this sample. Note that the presence of Pb as an additive was also highlighted by El Hadri et al. [8] in grey microplastics from the same field site analysed by LC-ICP-MS (Table S4).
- **Yellow and White**: No element has a large S in yellow or white microplastics. Therefore, no additive elements are highlighted in these microplastics.

Release of additives

The S calculation helped to identify which elements are additives in the microplastics we collected from the Guadeloupe beaches. However, this method does not give any information about the source of the acid-leached elements: are they coming from the environment or are they coming from the alteration of microplastics? Looking at the $[metal]/[metal]_{acidic\ leaching}$ and $[metal]/[metal]_{acidic\ digestion}$ ratio for metals identified as additives in

176 the orange and grey and black samples (see Section 3.1), two different behaviours are
177 exhibited (Figure 3). Cu, Pb, Cr and Cd exhibit similar [metal]/[metal]$_{\text{acidic leaching}}$ and
178 [metal/[metal]$_{\text{acidic digestion}}$ values. In these microplastics, the acidic leached concentrations,
179 namely adsorbed Cu, Pb, Cr and Cd, may therefore originate from the alteration of plastics
180 and the subsequent adsorption of released additives.

181 In contrast, Mn, Al and Zn present larger [metal]/[metal]$_{\text{acidic digestion}}$ values than
182 [metal]/[metal]$_{\text{acidic leaching}}$ values. These additives are therefore either not leached in significant
183 amounts, or they are progressively released from the plastic surface to the external
184 environment. All these elements are known as oligo-elements. Manganese is usually used as
185 Mn(IV) oxide for grey pigment in plastic formulations. Microorganisms are able to reduce
186 Mn(IV) to Mn(II), releasing the Mn from the plastic to the solution. Aluminium and Zn as
187 additives are also solubilized and potentially consumed by the biota as oligo-elements,
188 disappearing from the system as a consequence. By contrast Pb, Cr and Cd are non-
189 essential elements for living organisms and should therefore not be preferentially consumed.

190 **Bioavailability of adsorbed and additive elements**

191 Numerous studies have shown that plastics are ingested accidentally by animals. Plastics
192 can injure animals through both the plastic itself, and through pollutants adsorbed on the
193 plastic surface that can be released by digestion processes (conditions similar to acidic
194 leaching). If the largest concentrations measured in the plastics correspond to additive
195 elements, additive elements are less (or not) released by acid than adsorbed elements.
196 Ecotoxic parameters only discuss acidic leached concentrations of elements that may be
197 dangerous to animals due to their release in acidic stomach conditions.

198 The ECOTOX (https://cfpub.epa.gov/ecotox/search.cfm) database contains the Lowest
199 Observed Effect Concentrations (LOEC [mg (kg-food)$^{-1}$]) that have been determined for Fe,
200 Cu, Zn, As, Cd and Pb for several fish (Table S5). Note that LOEC were determined for

201 acute tests by ingesting one dose of each element. Concentrations measured after acidic
202 leaching for Fe, Cu, Zn, As and Pb are lower than the LOEC (minimal and maximal values)
203 for all microplastics samples (Table S5). Cadmium concentrations in the blue and grey and
204 black microplastics were below the LOEC minimal value but higher for the green, orange,
205 red, yellow and white microplastics. However, fish do not only eat plastics (100% food ≠
206 100% plastic); they tend to ingest the plastic along with real food. From Cd concentrations
207 measured in the acidic leaching and for an LOEC of 0.07 mg (kg food)$^{-1}$, it is possible to
208 calculate the maximal percentage of plastic in food for which the LOEC is attained. The
209 percentage of microplastic in the fish's food has be higher than 72% (Green), 71% (White),
210 24% (Yellow), 21% (Orange) and 16% (Red) to observe effects due to Cd from plastics.
211 These percentages show that it is highly improbable that fish will eat sufficient plastics by
212 accident to present a danger for them.

213 Our results suggest that our microplastic samples are not very dangerous for fish, when
214 ingested in one dose. However, here, we only studied the possible danger posed by (i) the
215 ingestion of a single dose of metal adsorbed by plastics, whereas chronic ingestion could
216 also be a potential danger for biota. We did not consider the potential synergetic health
217 effects of the elements being in mixtures in plastics. As well, plastics themselves can hurt
218 ecosystems. It would thus be interesting to study the effects of chronic ingestion of metals
219 adsorbed onto plastics and verify their toxicity or lack of it.

220 ## Conclusion

221 It is crucial to determine the origin of elements contained in plastic debris, since the response
222 of additives and sorbed metalloids to physico-chemical conditions can vary. In our study, we
223 provided a new and simple method to determine the additive *versus* sorbed elements in
224 microplastics. This method consists of measuring and comparing the concentrations of
225 elements released from two processes: acidic leaching and total digestion. Using this

method, a large number of element concentrations can be determined simultaneously. Our method also determines the bioavailable elements for living organisms.

The colour of the microplastic debris of the North Atlantic Gyre that is deposited on Guadeloupe beaches depends on the heterogeneous elements it contains. Several elements were probably used as pigments (Cd, Cu, Cr, and possibly Pb, Ba, and V). When plastics are altered by the addition of these elements, the additives seem to be mobile. Only Cd seems to be a danger when ingested by fish. Considering the percentage of plastic potentially ingested by fish, Cd levels do not seem to reach the LOEC. However, further research is needed to determine if microplastics and associated elements affect the health of the biota, and what effects the chronic consumption of plastics with additive elements might have.

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Supporting information:

Metals in microplastics: determining which are additive, adsorbed, and bioavailable

Charlotte Catrouillet[a*], Mélanie Davranche[a], Imane Khatib[a], Corentin Fauny[a], Aurélie Wahl[a], Julien Gigault[a,b*].

[a]*Univ. Rennes, CNRS, Géosciences Rennes - UMR 6118, F-35000 Rennes, France*

[b]*TAKUVIK CNRS/ULaval, UMI3376, Université Laval, Quebec City, QC, Canada*

*Corresponding authors: julien.gigault@takuvik.ulaval.ca and charlotte.catrouillet@univ-rennes1.fr

Section S1: Materials and methods

Quantitative analyses were performed using a conventional external calibration procedure (7 external standard multi-element solutions were purchased from Inorganic Venture, USA). A 300 ppb mixed solution of rhodium and rhenium was injected with the sample in-line in the nebulizer. This solution was used as an internal standard for all measured samples, to correct any instrumental drift and matrix effects. Calibration curves were calculated based on the intensity ratios between the internal standard and the analysed elements. An SLRS-6 water standard was used to check the accuracy of the measurement procedure.

The matrix of reference materials (ERM-EC 680 and ERM-EC 681) is composed of polyethylene supplemented with various concentrations of inorganic additives including As, Cd, Cr, Pb and Zn. Concentrations were validated for As (-0.56 and 9.26% of error), Cd (-1.04 and 0.82%), Cr (-0.8 and 3.16% of error), Pb (10.19 and 3.54% of error) and Zn (-0.68 and -7.75% of error).

Table 1: Quantification limit of the ICP-MS.

Element	*Isotope*	Mode	<1000 ppb	< 100 ppb	< 10 ppb	<1 ppb	<0,5ppb	< 0,1 ppb
Al	*27*	*No Gas*	3%	5%	5%	5%	5%	10%
V	*51*	*He*	3%	5%	5%	5%	5%	10%
Cr	*52*	*He*	3%	5%	5%	5%	5%	10%
Cr	*53*	*He*	3%	5%	5%	5%	5%	10%
Mn	*55*	*He*		3%	3%	5%	5%	10%
Fe	*56*	*He*	3%	5%	5%	10%		
Fe	*57*	*He*	3%	5%	5%	10%		
Co	*59*	*He*		3%	3%	5%	5%	10%
Ni	*60*	*He*		3%	3%	5%	5%	10%
Ni	*62*	*He*		3%	3%	5%	5%	10%
Cu	*65*	*He*	3%	3%	3%	5%	5%	10%

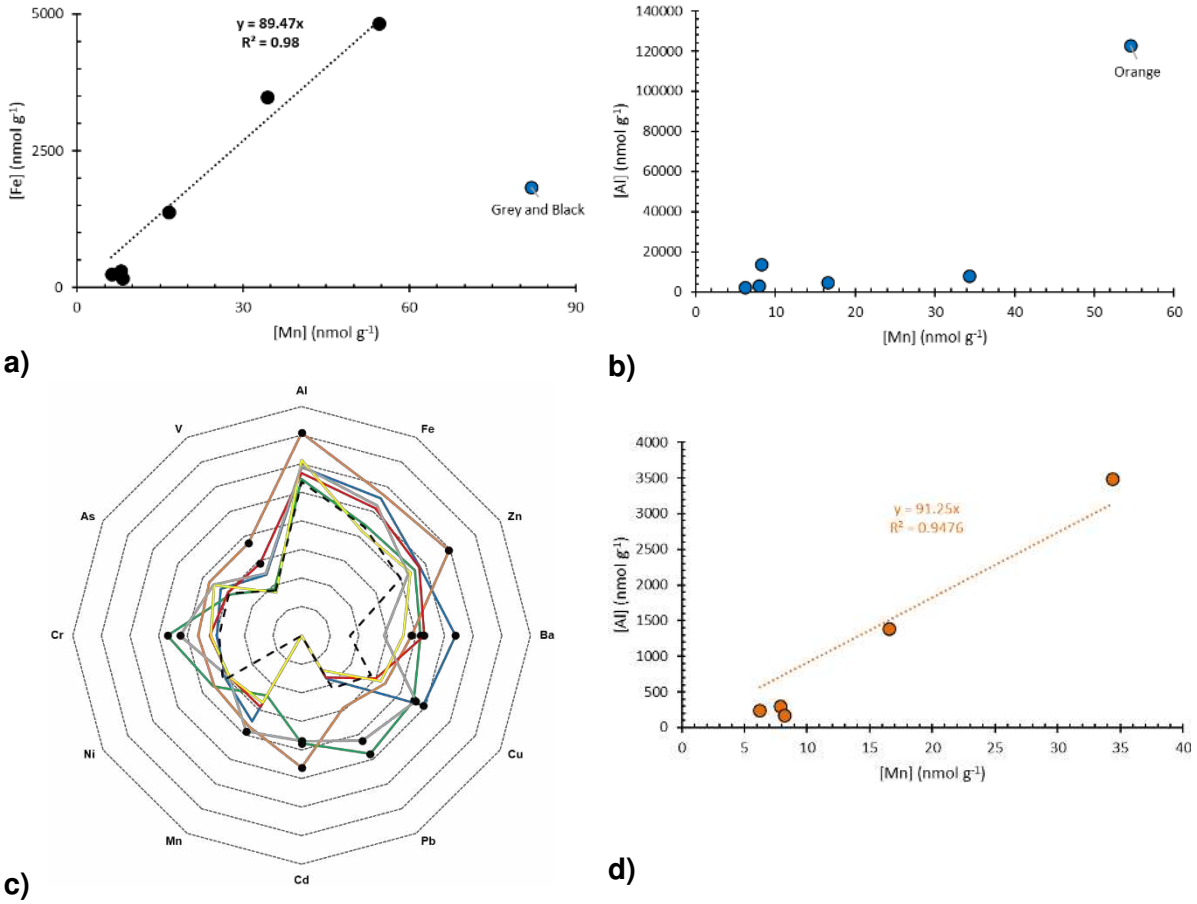
Zn	*66*	*No Gas*		3%	3%	5%	5%	10%
Zn	*66*	*He*	3%	3%	3%	5%	5%	10%
As	*75*	*He*		3%	3%	5%	5%	10%
Cd	*111*	*No Gas*		3%	3%	5%	5%	10%
Ba	*138*	*No Gas*	3%	3%	3%	3%	5%	10%
Pb	*208*	*No Gas*			3%	3%	5%	10%

330

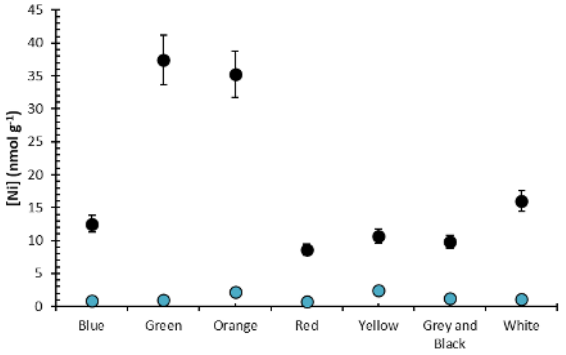
Section S2: Identification of the elements nature

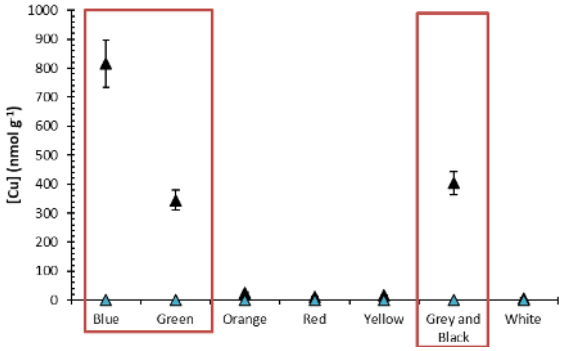
Due to their similar chemical behaviour, Fe and Mn concentrations in water are usually related by a linear relationship. Interestingly, Fe and Mn also show this linear relationship in all the coloured microplastics, except for the grey and black microplastics (Figure S1a). There are three factors that lead us to state that Fe and Mn are present as sorbent elements in all samples, except for the grey and black sample: (i) to our knowledge, Fe is used as an additive only as an inorganic pigment (Table S1 and 2), (ii) Mn is used only as an inorganic pigment for the grey colour (Table S1 and 2), and (iii) Fe and Mn concentrations are linearly linked. Similarly, a linear relationship is highlighted between Al and Mn concentrations, except for the orange microplastics (Figure S1b and c). Both microplastics samples (grey and black for Mn and orange for Al) could, therefore, be additives. Comparing the S values of Al, Mn and Fe (Figure 2a)), those two samples (grey and black for Mn and orange for Al) present high S values. We can thus identify elements as additives based on their S values.

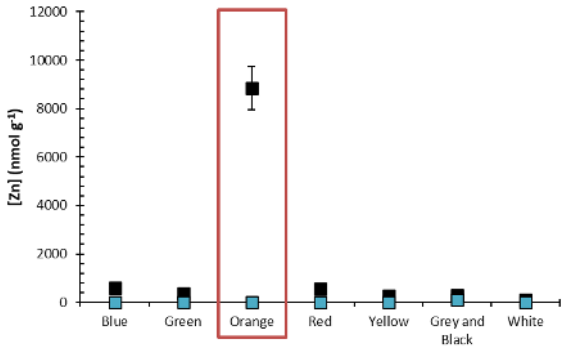
345

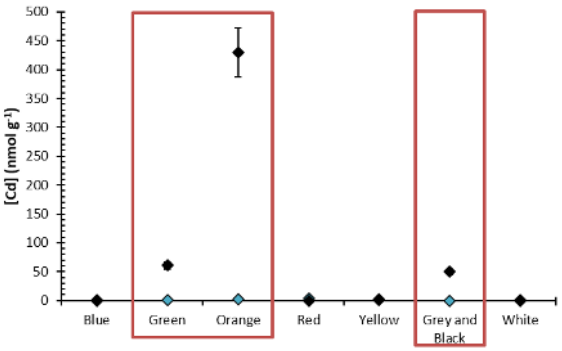


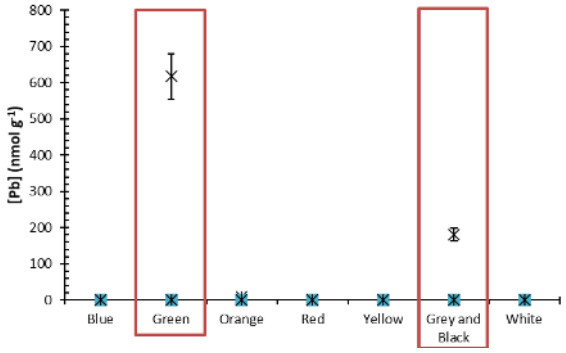
346 *Figure 1 : Linear relationship between (a) Fe and Mn and (b) Al and Mn concentrations with the orange*
347 *microplastics and (c) without the orange microplastics. d) S values obtained from acidic leaching and digestion for*
348 *Al, Fe, Zn, Ba, Cu, Pb, Cd, Mn, Ni, Cr, As and V elements; high S values indicate these are additives in*
349 *microplastics.*

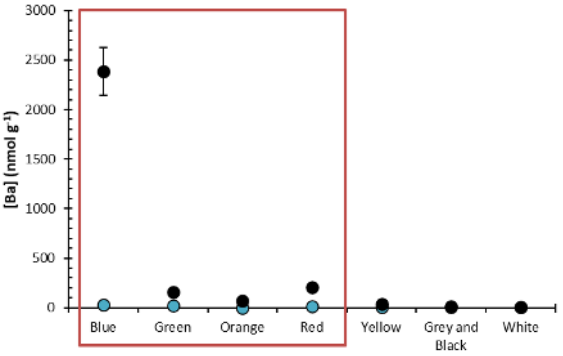

[Ni] (nmol g-1)
0
5
10
15
20
25
30
35
40
45
Blue
Green
Orange
Red
Yellow
Grey and Black
White

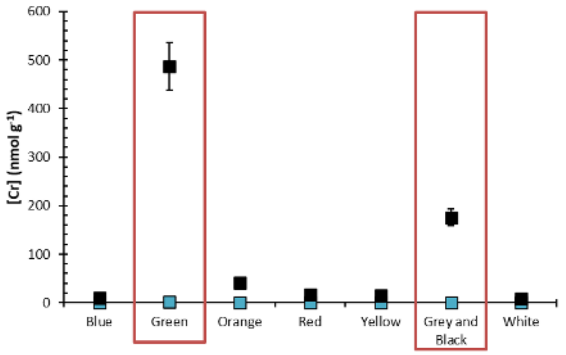

[Cu] (nmol g-1)
0
100
200
300
400
500
600
700
800
900
1000
Blue
Green
Orange
Red
Yellow
Grey and Black
White

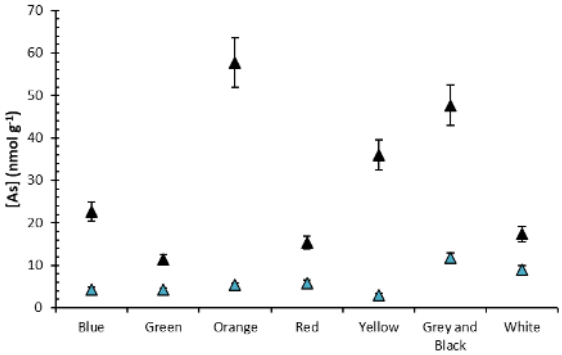

[Zn] (nmol g-1)
0
2000
4000
6000
8000
10000
12000
Blue
Green
Orange
Red
Yellow
Grey and Black
White

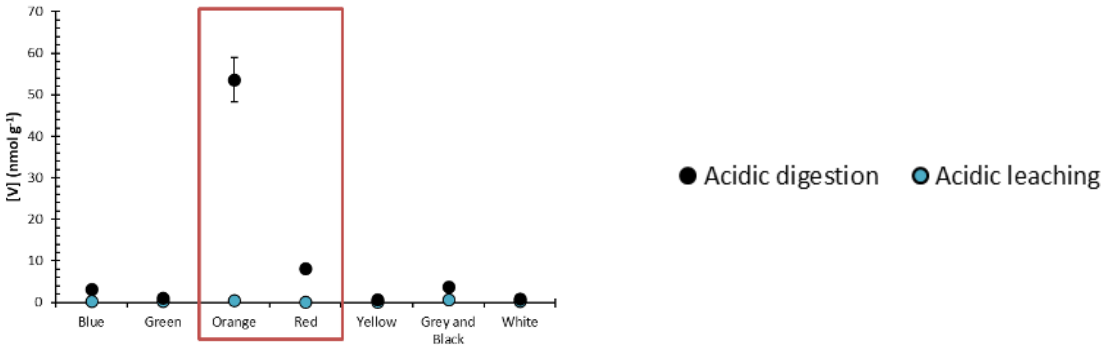

[Cd] (nmol g-1)
0
50
100
150
200
250
300
350
400
450
500
Blue
Green
Orange
Red
Yellow
Grey and Black
White


[Pb] (nmol g-1)
0
100
200
300
400
500
600
700
800
Blue
Green
Orange
Red
Yellow
Grey and Black
White


[Ba] (nmol g-1)
0
500
1000
1500
2000
2500
3000
Blue
Green
Orange
Red
Yellow
Grey and Black
White


[Cr] (nmol g-1)
0
100
200
300
400
500
600
Blue
Green
Orange
Red
Yellow
Grey and Black
White


[As] (nmol g-1)
0
10
20
30
40
50
60
70
Blue
Green
Orange
Red
Yellow
Grey and Black
White



350 *Figure 2: Element concentrations measured in the acidic leachate after total acidic digestion. Red rectangles*

351 *highlight the high differences in extraction concentrations.*

Section S3: Tables

Table 2: Summary of elements that are used as pigments. Most of the data comes from the Internet site https://colourlex.com/pigments/pigments-colour/.

Colour	Name of the pigment	Composition
Blue		$YIn_{1-x}Mn_xO_3$
	Han Blue	$BaCuSi_2O_6$
	Egyptian blue	$CaCuSi_4O_{10}$
	Blue Verditer	$2CuCO_3{\cdot}Cu(OH)_2$
	Manganese Blue	$BaMnO_4{\cdot}BaSO_4$
	Vivianite	$Fe_3(PO_4)_2 \cdot 8H_2O$
	Cerulean Blue	$CoSnO_3$
	Prussian Blue	$Fe_4[Fe(CN)_6]_3{\cdot}xH_2O$
	Smalt	contain Co
	Azurite	$2CuCO_3{\cdot}Cu(OH)_2$
	Cobalt Blue	$CoAl_2O_4$
	Ultramarine	$Na_7Al_6Si_6O_{24}S_3$
Red	Pompeiian Red	Iron oxide + clay and quartz
	Chrome Red	PbO $PbCrO_4$
	Cadmium Red	Cd(S,Se)
	Red Lead	Pb_3O_4
	Red Ochre	Hematite (Iron oxide)
	Vermilion	HgS
	Realgar	As_4S_4
Yellow	Raw Sienna	Iron oxide + small amounts of Mn oxides
	Bismuth Vanadate Yellow	$BiVO_4$
	Zinc Yellow	$K_2O\ 4ZnCrO_4(H_2O)_3$
	Lemon Yellow	$BaCrO_4$
	Cobalt Yellow	$K_3[Co(NO_2)_6]$
	Naples Yellow	$Pb_3(SbO_4)_2$
	Cadmium Yellow	CdS
	Yellow Ochre	Iron oxides
	Orpiment	As_2S_3
	Chrome Yellow	$PbCrO_4$

	Lead-Tin Yellow	Pb_2SnO_4
Green	Phthalocyanine Green	Cu organic complex
	Cobalt Titanate Green	Co_2TiO_4
	Verdigris	$Cu(CH_3COO)_2 \cdot H_2O$
	Green Earth	$K[(Al,Fe^{III}),(Fe^{II},Mg](AlSi_3,Si_4)O_{10}(OH)_2$
	Malachite	$CuCO_3 \cdot Cu(OH)_2$
	Viridian	$Cr_2O_3 . 2\ H_2O$
	Emerald Green	$3\ Cu(AsO_2)_2 \cdot Cu(CH_3COO)_2$
	Cadmium Green	mix of CdS and Cr_2O_3
Orange	Antimony Orange	$2\ Sb_2S_3 \bullet Sb_2O_3$
	Cadmium Orange	CdS
	Chrome Orange	$PbO \bullet PbCrO_4$
	Orange Ochre	Iron oxides
	Realgar	AsS, As_2S_2 or As_4S_4
Grey and Black	Spinel black	$MnFe_2O_4$
	Manganese Black	Manganese and Iron oxides
White	Titanium Dioxide White	TiO_2
	Calcite	$CaCO_3$
	Zinc White	ZnO
	Lead White	$2\ PbCO_3 \cdot Pb(OH)_2$

356 *Table 3 : Additive information from Hahladakis et al., 2018.*

Element	Use
Al	- Special effects (such as fluorescence). - Flame retardant
Zn	- Inorganic pigments - Fillers - Flame retardant as zinc borate
As	- Biocides
Fe	- Inorganic pigments
Mn	- Inorganic pigments (cadmium-manganese based possible)
Cu	- Special effect (such as fluorescence)
Cr	- Inorganic pigments
Ba	- Fillers
Pb	- Stabilisers, Antioxidants and UV stabilisers - Heat stabilisers - Inorganic pigments - Special effect (such as fluorescence)
Cd	- Stabilisers, Antioxidants and UV stabilisers - Heat stabilisers

	- Inorganic pigments
Ca	- Fillers

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359 *Table 4: Summary of the results obtained by El Hadri (2020) from microplastics collected at the same sampling*

360 *site measured by LC-ICP-MS. Two behaviours were identified in the samples: additive (Add) and sorbed (Sor)*

Colour	Orange	White	Yellow	Blue	Beige	Green	Grey
Cd	Add	Add/Sor	Add/Sor	Sor	Add	Add	Sor
As	Sor	Sor	Sor	Sor	Sor	Sor	Sor
Zn	Sor	Sor	Add	Add	Add	Add	Sor
Pb	S	Sor	S	Sor	Sor	Add	Add

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Table 5: Element concentrations measured after acid leaching and the lowest observed effect concentration (LOEC) determined for each element in this study. LOEC data is from the Internet database: https://cfpub.epa.gov/ecotox/

		Fe	Cu	Zn	As	Cd	Pb
Blue	**[mg (kg pl.)$^{-1}$]**	0.87	0.09	0.51	0.25	0.05	0.18
Green	**[mg (kg pl.)$^{-1}$]**	1.02	0.06	0.56	0.24	**0.10**	0.27
Orange	**[mg (kg pl.)$^{-1}$]**	1.94	0.06	0.57	0.24	**0.33**	0.04
Red	**[mg (kg pl.)$^{-1}$]**	0.22	0.04	0.32	0.37	**0.42**	0.00
Yellow	**[mg (kg pl.)$^{-1}$]**	0.52	0.04	0.38	0.12	**0.29**	0.04
Grey and Black	**[mg (kg pl.)$^{-1}$]**	3.29	0.08	7.41	0.68	0.03	0.15
White	**[mg (kg pl.)$^{-1}$]**	0.38	0.07	0.43	0.57	**0.10**	0.00
LOEC min	**[mg (kg food)$^{-1}$]**	560	0.28	100	28	0.07	7.20
LOEC max	**[mg (kg food)$^{-1}$]**	560	1780	5926	732	615	802.92

Figures:

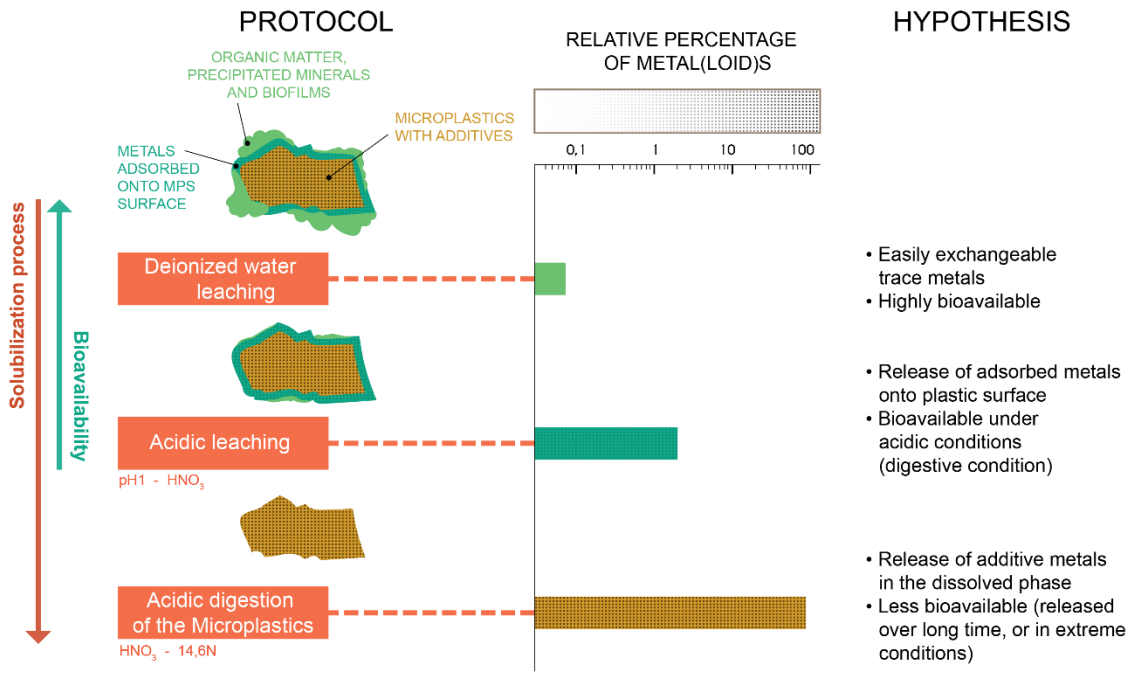
Metals in microplastics: determining which are additive, adsorbed, and bioavailable

Charlotte Catrouillet[a*], Mélanie Davranche[a] Imane Khatib[a], Corentin Fauny[a], Aurélie Wahl[a], Julien Gigault[a,b*].

[a]*Univ. Rennes, CNRS, Géosciences Rennes - UMR 6118, F-35000 Rennes, France*

[b]*TAKUVIK CNRS/ULaval, UMI3376, Université Laval, Quebec City, QC, Canada*

*Corresponding authors: julien.gigault@takuvik.ulaval.ca and charlotte.catrouillet@univ-rennes1.fr



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394 *Figure 3: Protocol developed in our study and linked hypothesis*

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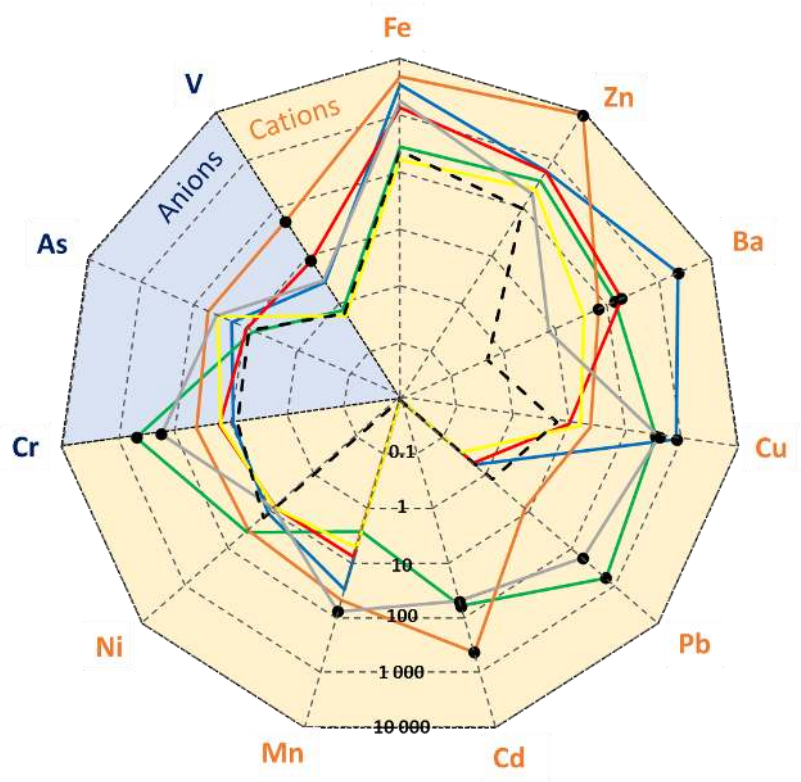

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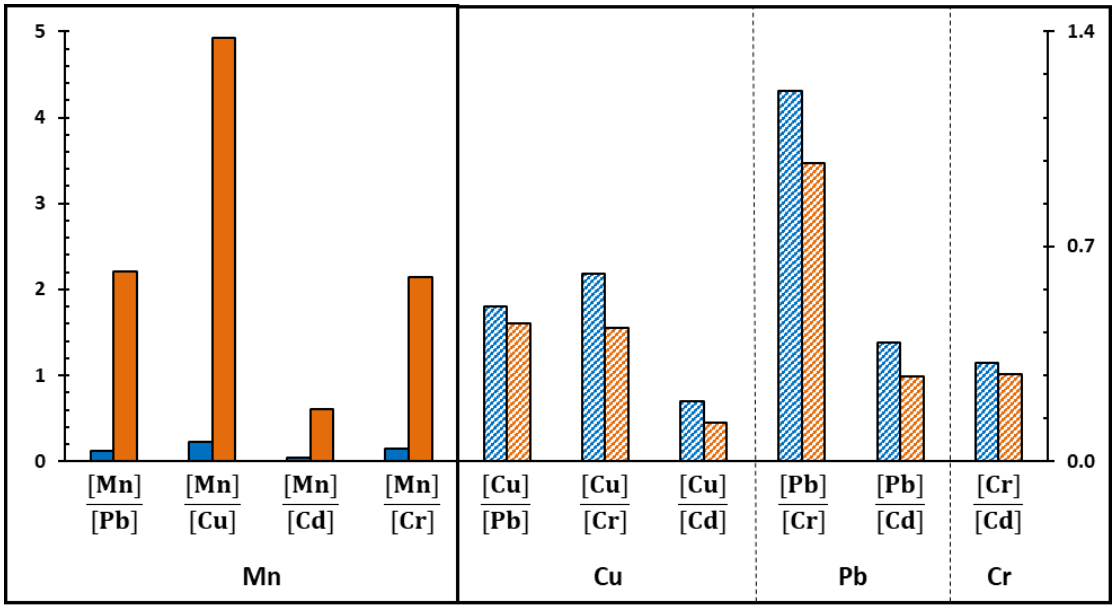
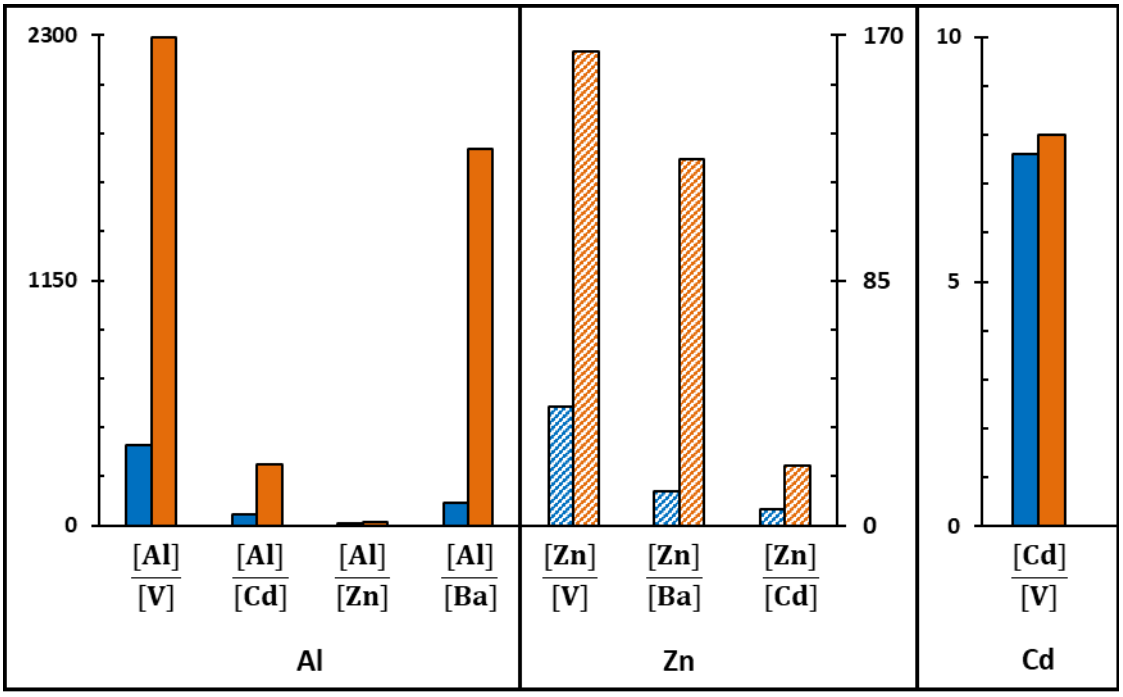
399 *Figure 4: S values obtained from acidic leaching and digestion for Fe, Zn, Ba, Cu, Pb, Cd, Mn, Ni, Cr, As and V.*
400 *For Al, see FIGURE 1 in SI. Metal(loid)s, with low S values (close to the centre) are mainly sorbed onto*
401 *microplastics. Metal(loid)s, with high S values are additives in the microplastics.*

402



a)



b)

Acidic leaching Acidic digestion

Figure 5: Metal/Metal ratio of concentration in the acidic leaching and acidic digestion in the a) grey and black sample and b) orange sample. Cu, Pb, Cr and Cd have similar concentration ratios. Cu, Pb, Cr and Cd adsorption may originate from the alteration of plastics. Note that if one metal is present at higher concentrations in the acidic digestion than in the acidic leaching, this metal imposes a different metal/metal ratio. Thus, Cu/Mn, Pb/Mn, Pb/Cu, Cr/Mn, Cr/Cu and Cr/PB ratios were not represented for part a). Similarly, Zn/Al, Cd/Al and Cd/Zn were not represented for part b).