A FIRST INVENTORY OF PLASTIC LITTER AND SOME ASSOCIATED TOTAL METAL CONTENTS AT THE COASTLINE OF THE BAY OF ASUNCIÓN (PARAGUAY)

Diana Diez-Pérez Núñez[1,2], Deyanira Peralta[1], Dahiana Serafini[1], Derlysa Colmán[1],

José M. Andrade[2], Adrián López-Rosales[2], Soledad Muniategui-Lorenzo[2]

[1] National University of Asunción, Faculty of Chemical Sciences, Department of Physical Chemistry, San Lorenzo, Paraguay

[2] University of A Coruña, Faculty of Sciences, Group of Applied Analytical Chemistry, Campus da Zapateira 15008, A Coruña, Spain

SUPPORTING INFORMATION

The supporting information presented here is composed of:

1. Three chapters that provide additional information and informative data on three corresponding sections of the main text:
    a. 2.4. Validation and quality control
    b. 2.5. Statistical treatment
    c. 3.3.1. Analytical performance parameters
2. Ten tables
3. Seven figures

NOTE: Full details for the references included in the discussions are given in the main text of the paper, to which readers are kindly forwarded.

S1. Supplementary information to section 2.4. Validation and quality control

To validate the metal analyses several studies were done. First, **precision** was evaluated as repeatability (within day, single laboratory), and reported as relative standard deviation, RSDr = $100 \cdot \bar{X} / SD$. For this, a set of eight replicates of spiked laboratory-prepared fragments were prepared adding appropriate volumes of the metal stock solutions to 0.1000 g of a collection of laboratory-prepared fragments so that the following nominal concentrations were obtained: Cd (8 $\mu g \cdot g^{-1}$), Zn (250 $\mu g \cdot g^{-1}$), Cu (200 $\mu g \cdot g^{-1}$), Fe (800 $\mu g \cdot g^{-1}$) and Mn (1500 $\mu g \cdot g^{-1}$). These levels were selected after studying reported concentrations for field samples measured elsewhere (see e.g. Kutralam-Muniasamy et al., 2021).

To study the **analytical recoveries** six replicates of five-metal spikes were prepared, each at three nominal concentration levels: low, medium, and high. The nominal concentrations for each metal and the corresponding recoveries are presented in **Table S6**.

All blanks and spiked laboratory-prepared fragments were transferred to 55 mL Teflon vessels, 10 mL of HNO_3 (65%) were added and the mixtures digested applying the microwave program detailed in the main text (Section 2.3). The resulting solutions were transferred to 25 mL flasks, the volumes made up, and aspirated to the FAAS equipment.

Before measuring each batch of samples, procedural blanks and the slope of the batch-related calibration lines were compared against historical ranges so that obvious biases were not present. The experimental measurements for the blanks and slopes obtained in a batch should be within the following 95% confidence historical ranges (average ± 2*standard deviation):

	Cd	Cu	Fe	Mn	Zn
Blank (A.u.)	0.0030 ± 0.0009	0.0030 ± 0.0027	0.0074 ± 0.0091	0.0026 ± 0.0030	0.0334 ± 0.0126
Slope (A.u./(mg·L^{-1})	0.4060 ± 0.0433	0.1388 ± 0.0355	0.0301 ± 0.0123	0.1181 ± 0.0170	0.4159 ± 0.1512

S2. Supplementary information to section 2.5. Statistical treatment

Calibrations are a cornerstone of the analytical methodology and some studies were done on them. Metals were quantified using five-points calibrations plus a blank (measured by duplicate, at least). Regression statistics were evaluated (**Figure S3**), along with linear ranges and sensitivities (Eurachem, 2019; Eurolab España, 2016). ANOVA studies demonstrated statistical significance of the regressions (goodness-of-fit, p-values < 0.005). Residual plots were used to assess homoscedasticity and the working

linear range was assessed using the classical criterion by which the deviation of sensitivity should be <0.03 when the uppermost standard is deleted (Kateman & Pijpers, 1981).

Further, different ways of calculating the LOD and LOQ were compared. Thus, the classical (IUPAC) (Eurolab España, 2016; Long & Winefordner, 1983) and the ISO/IUPAC/EU updated definitions (Commission Implementing Regulation (EU), 2021; ISO, 2019; IUPAC, 2014) were calculated, along with a simplification posed by Eurachem, which essentially resembles the IUPAC's calculations, using a blank solution. For this, the experimental standard deviation of the 'concentration' obtained for blank samples from various replicates (S_o, n = 10) was used to calculate the $3 \cdot S_o$' and $10 \cdot S_o$' thresholds (where, $S_o' = S_o/\sqrt{n}$). For the IUPAC definition the intercept of the calibration line and its associated standard error were employed.

The most recent harmonized definitions stated by ISO, IUPAC and the European Union for the 'LOD' and 'LOQ' take account of the Type I and Type II errors. Although the exact names do not agree through the institutions, the mathematical equations do. The critical limit or decision limit is denoted by x_c (ISO and IUPAC) or CC_α (EU), and it corresponds to the old LOD definition where only the false positives are controlled. The minimum detectable net concentration or detection capability x_d (ISO, IUPAC) or CC_β (EU) should be considered as the new 'detection limit' (although this term is superseded) as it controls simultaneously the risks of false positives and negatives. The LOQ parameter is kept only by IUPAC, denoted as x_q. Noteworthy, the new definitions proceed from error propagation of the calibration lines according to equations 1, 2, 3, respectively; details can be consulted elsewhere (Andrade-Garda & Gómez-Carracedo, 2017).

$$x_c = t_{(0.95,1-tail,\nu)} \cdot \frac{s_{y/x}}{b} \cdot \sqrt{\frac{1}{q} + \frac{1}{N} + \frac{\bar{x}^2}{\sum_{i=1}^{N}(x_i - \bar{x})^2}} \qquad \text{Equation 1}$$

$$x_d = \delta_{(\alpha=0.95,\beta=0.95,\nu)} \cdot \frac{s_{y/x}}{b} \cdot \sqrt{\frac{1}{q} + \frac{1}{N} + \frac{\bar{x}^2}{\sum_{i=1}^{N}(x_i - \bar{x})^2}} \qquad \text{Equation 2}$$

$$LOQ \equiv x_q \approx 10 \cdot \frac{s_{y/x}}{b} \cdot \sqrt{\frac{1}{q} + \frac{1}{N} + \frac{(x_q - \bar{x})^2}{\sum_{i=1}^{N}(x_i - \bar{x})^2}} \qquad \text{Equation 3}$$

Where x_c critical limit or decision limit; t: critical Student's t value; $s_{y/x}$: standard error of the regression; b: slope of the calibration line; q: number of replicate measurements of the sample; N:

number of calibration points; $\bar{x}$: mean of the calibration concentrations; x_i: individual calibration concentration; x_d: minimum detectable net concentration or detection capability; δ: a non-centred t distribution for ν degrees of freedom, and stated risks of false positives and negatives; in this case, both were set at 5%).

S3. Supplementary information to section 3.3.1. Analytical performance parameters

So far, very few studies intended to quantify metals on MPs reported on quality assurance tests. One of them evaluated the recoveries on spiked samples in field measurements, being 102 ± 4% for Cu, and 123 ± 50% for Cd (Ottander, 2022). Another report showed recoveries of 102 and 111% for Cd depending on the acidic digestion (Hildebrandt et al., 2020). The recoveries we obtained agree with those studies. Also, the linear range and sensitivities were similar to other studies using inductively coupled plasma (ICP) (Lee et al., 2021; Nakashima et al., 2012).

The other performance parameters studied here were the instrumental LOD and LOQ, whose results can be seen in **Table 2**. Cd was the metal with the lowest LOD and LOQ, whereas Fe had the highest ones. They were higher than those obtained for MS-ICP and AAS using graphite furnace, as expected because they are more sensitive techniques, as shown in **Table S8**. Nevertheless, as the metal concentrations reported previously for these metals in MPs collected in a relatively close geographical region (Kutralam-Muniasamy et al., 2021; Munari et al., 2021) are higher than the LOD and LOQ found in this study, these performance parameters were considered appropriate. In Uruguay, a nearby country, concentrations of heavy metals exhibited a wide range of values, from 261 $\mu g \cdot g^{-1}$ to 6,273 $\mu g \cdot g^{-1}$ (Rodríguez et al., 2020), whereas the maximum concentrations reported by Kutralam-Muniasamy et al. (2021) were Fe: 28,822 $\mu g \cdot g^{-1}$, Mn: 513.8 $\mu g \cdot g^{-1}$, Cu: 1,611.8 $\mu g \cdot g^{-1}$, Zn: 4,303.21 $\mu g \cdot g^{-1}$, and Cd: 211.7 $\mu g \cdot g^{-1}$.

As expected, CC_β (modern 'LOD') values are higher than those calculated by the classical procedure (**Table 2**). This is due to the simultaneous control of false positives and negatives (95% confidence level) exerted when calculating it and because of error propagation from the Hotelling's confidence region around the regression lines. On the contrary, classical LODs are lower but have an approximate 50% risk of false negatives (5% risk of false positives). It is worth noting the closeness between CC_α and the classical LOD (as expected, as per their definition) when the latter is calculated using the intercept of the calibration line. On the contrary, huge differences occur when the classical approach is calculated from replicates of a blank solution; most often, pure water. In this case the day-to-day variability and the so low absorbances –arguably, close to zero- yield negative quantifications for the blanks that lead to unrealistically low LOD and LOQ figures and, worst, vary hugely among working sessions. Therefore,

this approach is not recommended here. Noteworthy, the Eurachem guideline (Eurolab España, 2016) points out this problem when indicating explicitly that the signal associated to the blank should be 'measurable' (i.e., not close to zero) or, otherwise, use samples with very low concentration levels as 'blanks'. In this study, the values obtained by the classical approach were considered for comparison with other previous studies that employed it (although in some cases the calculation procedure was not disclosed at all), see **Table S8**. However, one should be advised that the use of CC_α and CC_β is mandatory within the EU for specific fields (food, pharmacological residues in food, animal health, etc.) (2002/657/CE, 2002; Commission Implementing Regulation (EU), 2021; Hildebrandt et al., 2020; Maršić-Lučić et al., 2018).

Table S1. Geographical coordinates for each sampling point and associated area.

Location	**Geographical coordinates**		**Area (m^2)**
	Start	**End**	
1	Latitude: 25º 16' 01.70 " S Longitude: 57º 37' 42.96" W	Latitude: 25º 16' 04.55" S Longitude: 57º 37' 41.53" W	300
2	Latitude: 25º 15' 58.54" S Longitude: 57º 37' 37.42" W	Latitude: 25º 15' 56.19" S Longitude: 57º 37' 34.87" W	300
3	Latitude: 25º 16' 18.08" S Longitude: 57º 37' 07.16" W	Latitude: 25º 16' 16.92 " S Longitude: 57º 37' 06.47" W	120
4	Latitude: 25º 16' 26.67" S Longitude: 57º 37' 41.42" W	Latitude: 25º 16' 16.92 " S Longitude: 57º 37' 4' 0.62" W	120

Table S2. Operating conditions of the atomic absorption spectrometer.

Metal	Fe	Mn	Zn	Cu	Cd
Radiation source			Hollow cathode lamp		
Wavelength (nm)	248.33	279.48	213.86	324.75	228.80
Lamp current low (mA)	10	10	7	10	5
Fuel			Acetylene		
Oxidant			Air		
Acetylene flow rate (L/min)			2.0		
Burner height (mm)			16		

Table S3. Polymers collected and identified in meso- and macroplastics according to their original use and sampling location

	Location 1									Location 2										Location 3						Location 4						**Total items**	**%**
	T	Bot	B	FWs	CWs	S	C	FWh	F	Bot	CWh	T	FWs	S	C	FWh	B	CWs	Bc	FWh	B	T	FWs	Bot	S	Bc	T	FWs	FWh	CWs	B		
PE			8	6	5			1	1		6	1	5			1	4	4	6		9		4			1	1	1			1	8	73	31
PP	2		4	7	5		7	19	10		2	10	6		4	6		1	9	1		1						2		1		97	42
PS	1					5	2	2				1		6		2				2					3								24	10
PET		6			1					7	2		3						3					5			1					28	12
PVC								3				2								2												7	3
PMMA											3									2												5	2
Total					95										94								29						16			234	

T: Toys; Bot: Bottles; B: Bags; FWs: soft Food wrappers; CWs: soft Cleaning wrappers; S: Styrofoam; C: Cups; FWh: hard Food wrappers; F: Films; Bc: Bottle caps; CWh: hard Cleaning wrappers.

NOTE: For statistical analyses, soft and hard wrappers were included in one category “food wrappers” and “cleaning wrappers”, with no statistical differences between both groups at each sampling point (U-Mann Whitney, $p>0.05$)

Table S4. Number of items of each type of polymer classified in each category 'intended original use'.

	PE	%	**PP**	%	**PS**	%	**PET**	%	**PVC**	%	**PMMA**	%
Bags	29	39.7	4	4.12								
Food wrappers, soft	16	21.9	13	13.4			4	14.3				
Food wrappers, hard	2	2.74	28	28.9	6	25.0			5	71.4	2	40.0
Cleaning wrappers, soft	10	13.7	7	7.22			4	14.3				
Cleaning wrappers, hard	6	8.22	2	2.06			2	7.14			3	60.0
Films	1	1.37	10	10.3								
Toys	2	2.74	13	13.4	2	8.33			2	28.6		
Bottle caps	7	9.59	9	9.28								
Cups			11	11.3	2	8.33						
Styrofoam					14	58.3						
Bottles							18	64.3				
Total	73		97		24		28		7		5	

Table S5. Quality control of FAAS measurements. Absorbances for procedural blanks (prepared as indicated in section 2.4) and for 1 ppm standards, along with recovery assays for the latter.

Metal	Absorbance for Procedural blanks	Absorbance for 1 ppm standards	Recovery for 1 ppm (%)
Cd	0.0033	0.3921	84
Mn	0.0027	0.1198	93
Zn	0.0382	0.4051	103
Cu	0.0042	0.1282	79
Fe	0.0085	0.0523	95

Table S6. Recoveries (given as %, mean ± standard deviation, n=6) for the five studied metals at three nominal concentration levels (shown between brackets, µg·g-1).

Metal	Low	Medium	High
Fe	[300] 106 ± 2	[800] 119 ± 2	[2500] 101 ± 2
Mn	[20] 93 ± 19	[100] 86 ± 4	[800] 93 ± 1
Zn	[40] 98 ± 6	[100] 104 ± 2	[250] 102 ± 2
Cu	[12] 117 ±3	[40] 91 ± 8	[200] 91 ± 1
Cd	[2.5] 107 ± 7	[4.0] 105 ± 7	[7.5] 119 ± 3

D. Diez-Pérez Núñez[1,2], D. Peralta[1], D. Serafini[1], D. Colmán[1],
J.M. Andrade[2], A. López-Rosales[2], S. Muniategui-Lorenzo[2]

Table S7. Pairwise comparisons (Dunn-Bonferroni test) among pairs of intended original use of the plastics.

Sample 1- Sample 2	Fe	Mn	Zn	Cu	Cd
Cups – Bottle caps	1.000	1.000	1.000	1.000	1.000
Films – Bags	1.000	1.000	1.000	0.305	1.000
Films – Food wrappers	1.000	1.000	1.000	0.609	1.000
Cups – Cleaning wrappers	1.000	1.000	1.000	0.007*	0.972
Cups – Styrofoam	1.000	1.000	0.262	1.000	1.000
Films – Toys	1.000	1.000	1.000	1.000	1.000
Cups – Bags	1.000	1.000	1.000	0.024*	0.650
Cups – Toys	1.000	1.000	0.649	1.000	0.026*
Films – Cups	1.000	1.000	0.517	1.000	1.000
Films – Cleaning wrappers	1.000	1.000	1.000	0.142	1.000
Films – Bottles	0.447	1.000	0.405	1.000	1.000
Films – Bottle caps	1.000	1.000	1.000	1.000	1.000
Cups – Food wrappers	1.000	1.000	1.000	0.049*	0.008*
Films – Styrofoam	1.000	1.000	1.000	1.000	1.000
Cups – Bottles	1.000	1.000	1.000	1.000	1.000
Cleaning wrappers – Bottles	1.000	1.000	1.000	1.000	1.000
Cleaning wrappers – Styrofoam	0.042*	0.074	1.000	1.000	1.000
Cleaning wrappers – Bags	0.281	0.028*	1.000	1.000	1.000
Cleaning wrappers – Bottles caps	0.779	1.000	1.000	1.000	1.000
Cleaning wrappers – Toys	1.000	0.425	1.000	1.000	1.000
Cleaning wrappers – Food wrappers	1.000	1.000	1.000	1.000	1.000
Bottles – Styrofoam	0.000*	0.094	0.129	1.000	1.000
Bottles – Bags	0.001*	0.042*	1.000	1.000	1.000
Bottles – Bottle caps	0.008*	1.000	1.000	1.000	1.000
Bottles – Toys	0.019*	0.489	0.348	1.000	1.000
Bottles – Food wrappers	1.000	1.000	1.000	1.000	1.000
Styrofoam – Bags	1.000	1.000	1.000	1.000	1.000
Styrofoam – Bottle caps	1.000	1.000	1.000	1.000	1.000
Styrofoam – Toys	1.000	1.000	1.000	1.000	1.000
Styrofoam – Food wrappers	0.003*	0.305	1.000	1.000	1.000
Bags – Bottle caps	1.000	1.000	1.000	1.000	1.000
Bags – Toys	1.000	1.000	1.000	1.000	1.000
Bags – Food wrappers	0.029*	0.117	1.000	1.000	1.000
Bottle caps – Toys	1.000	1.000	1.000	1.000	1.000
Bottle caps – Food wrappers	0.226	1.000	1.000	1.000	1.000
Toys – Food wrappers	0.717	1.000	1.000	1.000	1.000

(*) Significant difference, $p < 0.05$

Table S8. Limits of detection and quantitation found in previous studies for the metals considered in this study

Region of study	Matrix	Analytical method	LOD ($\mu g \cdot g^{-1}$)	LOQ ($\mu g \cdot g^{-1}$)	Reference
Brazil	Plastic material from waste electronic	GFAAS	Cd: 0.1	Cd: 0.4	Duarte et al. (2010)
Brazil	Sandy beach	ICP-OES	-	Cu 0.14* Fe 0.48* Mn 2.72* Zn 0.87*	Vedolin et al. (2017)
Croatia	CRM (IAEA 158)	GFAAS (FAAS for Fe)	Cu 0.03 Cd 0.001 Fe 10.1 Mn 0.04 Zn 0.08	-	Maršić-Lučić et al. (2018)
Portugal	Sandy beach	ICP-OES	-	Cd 0.005 Cu 0.15 Fe 0.5 Mn 0.05 Zn 0.05	Martins et al. (2020)
Germany	ERM-EC CRMs	ICP-MS/MS	-	Cu 0.14	Hildebrandt et al. (2020)
China	Commercial MPs	ICP-MS	-	Cu 0.008* Mn 0.011*	Shi et al. (2023)

ICP-OES: Inductively coupled plasma optical emission spectrometry
ICP-MS/MS: Inductively coupled plasma—tandem mass spectrometry
ICP-MS: Inductively coupled plasma-mass spectrometry
CRM: Certified reference material
GFAAS: Graphite furnace AAS
(*)method quantitation limit

Table S9. Statistical comparison (Kruskal-Wallis test) of the plastics classified by intended original use.

Grouping variable	Fe	Mn	Zn	Cu	Cd
	Significance (p-value)				
Intended original use	0.000*	0.001*	0.030*	0.003*	0.013*

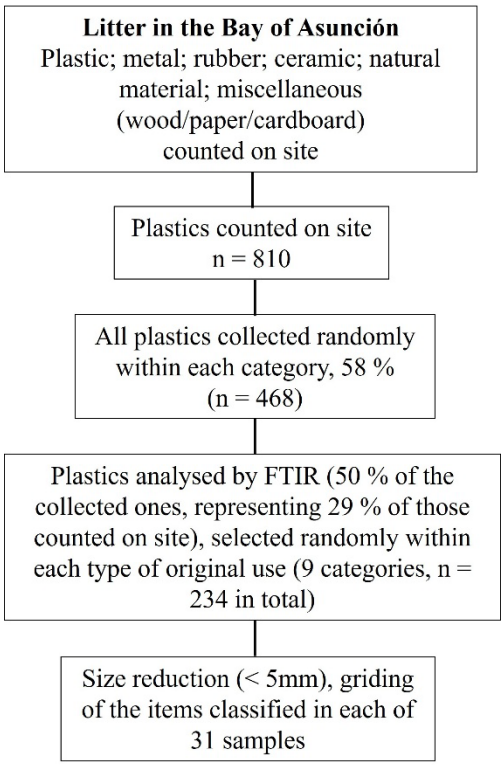
(*) Significant difference, $p < 0.05$

Table S10. Some common polyolefinic additives containing Zn and Cu (ECHA, 2024)

CAS number	Substance name	Function in plastics	Typical concentration (%)
1314-98-3	Zinc sulphide	Pigments	2.0 – 10.0
12239-87-1	Copper chlorophthalocyanine	Pigments	2.0
27614-71-7	Copper (tetrachloro-29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32	Pigments	0.05
68186-91-4	Copper chromite black spinel	Pigments	0.5
68186-88-9	Zinc iron chromite brown spinel	Pigments	5.0
68187-51-9	Zinc ferrite brown spinel	Pigments	5.0
68512-13-0	Copper, [29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]-, brominated chlorinated	Pigments	2.0
68987-63-3	Copper, [29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]-, chlorinated	Pigments	2.0
81457-65-0	Copper, [29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]-, [[3-(1-methylethoxy)propyl]amino]sulfonyl derivs.	Pigments	2.0
1314-13-2	Zinc oxide	Antistatic	5.0

Figure S1. Sampling locations in the Bay of Asunción, Paraguay. Each point was sampled according to the River-OSPAR protocol (Schone Rivieren, 2017). Notice the presence of vegetation at Locations 3 4.

Figure S2. Flow diagram showing how the items to be analyzed were selected. Selection of items was random within each type or 'category' of plastic (i.e., intended use of the original item: caps, wrappers, etc.) so that metals were measured in all types.



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Note: The final amount of 31 samples correspond to nine categories at the four sampling locations, but for those categories not present in the sites. The categories identified in the sampling locations are detailed in **Table S3** and are resumed next (the numbers indicate the number of sampling locations where that kind of litter was found: bags (4), soft food wrappers (4), hard food wrappers (4), bottles (3), films (1), Styrofoam (3), toys (4), cups (2), soft cleaning wrappers (3), hard cleaning wrappers (1), bottle caps (2). From each sample two aliquots were analyzed by separate.

Figure S3. Calibration lines for Cd, Cu, Fe, Mn and Zn, goodness-of-fit ANOVA studies for all of them were significant (p-values < 0.0005) (for other associated studies see main text, and chapter S2 above). A Student's *t*-test was applied to every intercept (Null hypothesis: intercept=0) in order to check whether it was statistically zero. In all cases the null hypothesis could not be rejected ($t_{exp} < t_{tab,\ 95\ \%\ confidence\ level,\ dof=4}$). Therefore, negative (or positive) values should be considered only as mathematical parameters without chemical significance, hence systematic errors cannot be envisioned.

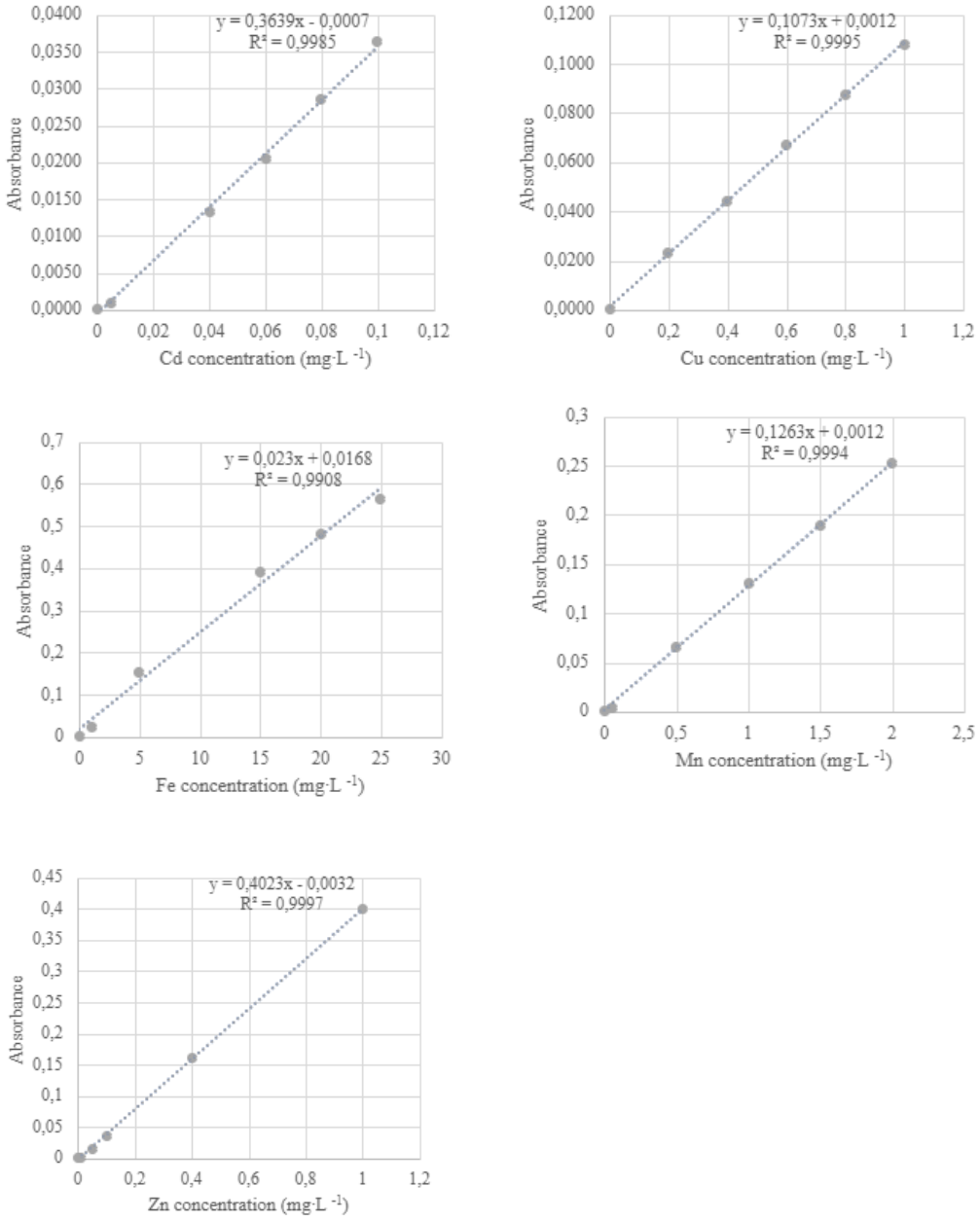


Figure S4. Separation of layers of different composition in an aged mesoplastic item. The spectra show the different polymers present in the layers.

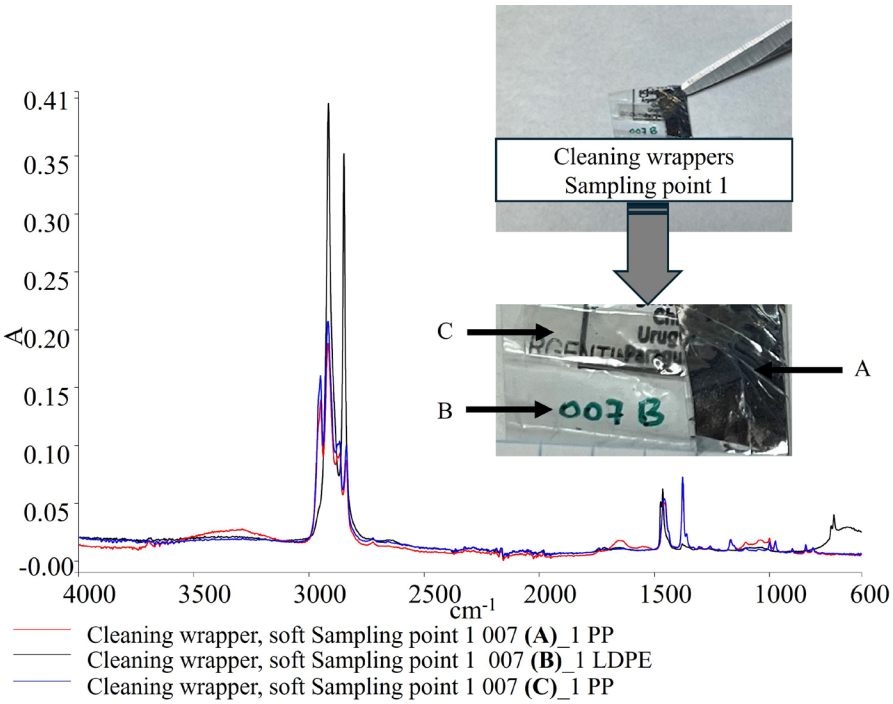


Figure S5. Spectra of PMMA and HDPE identified in the same macroplastic piece.

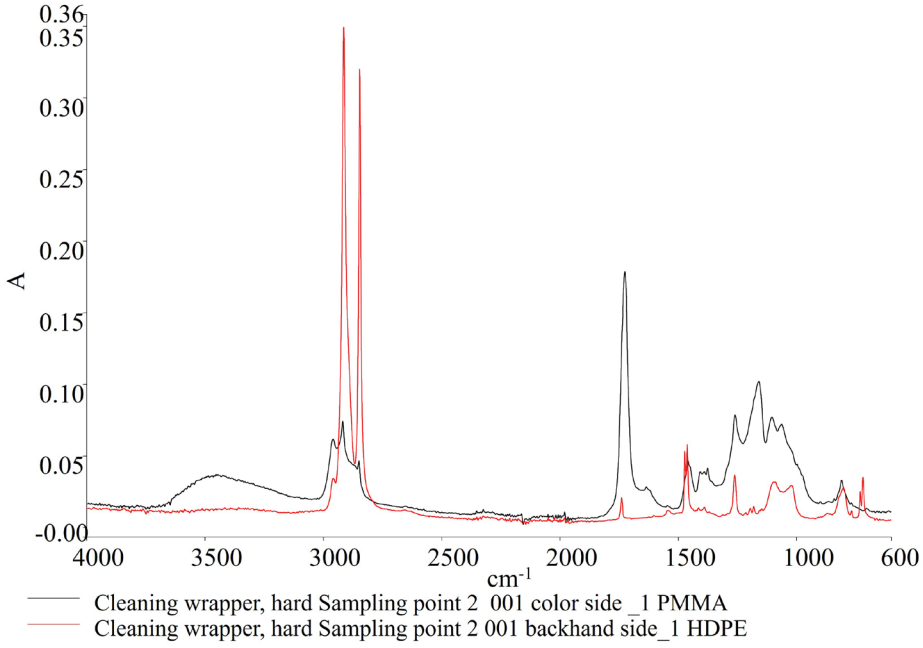
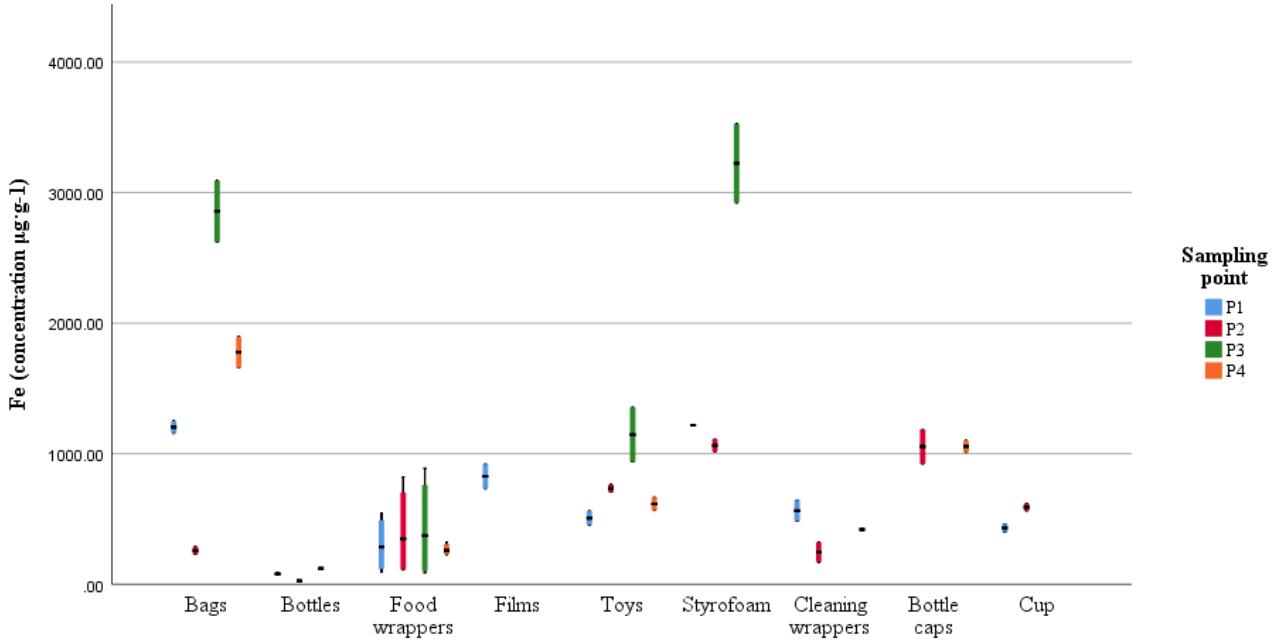
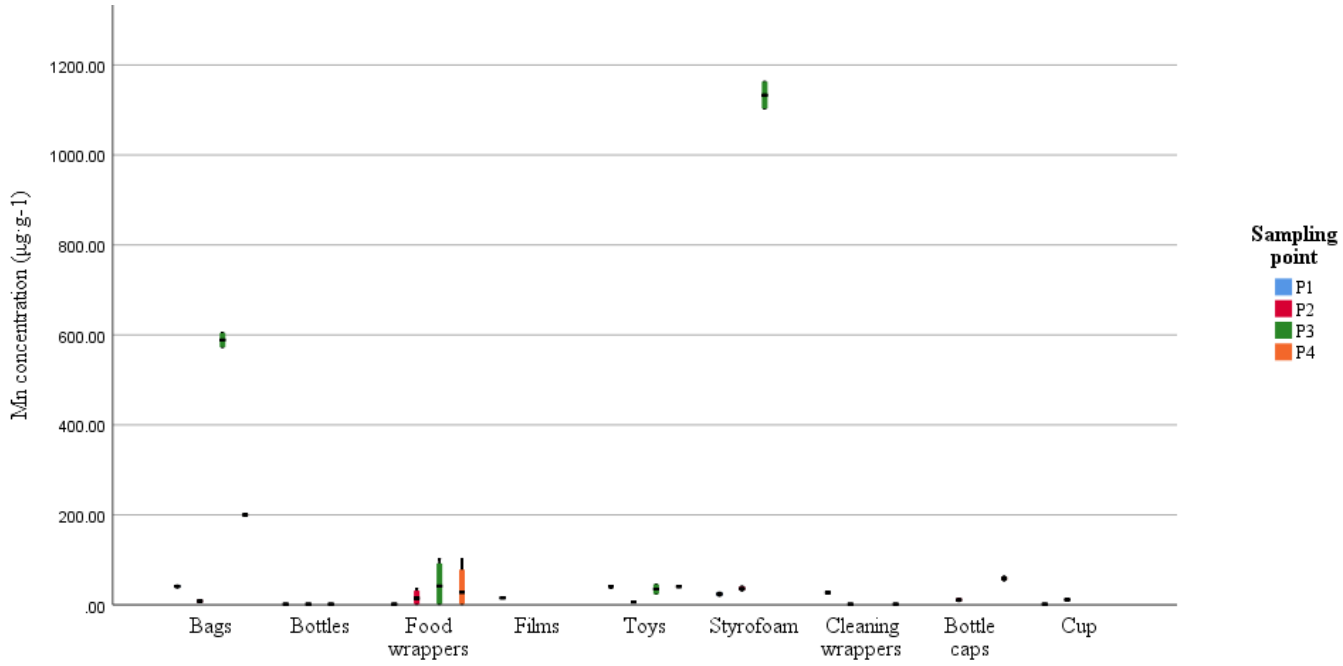


Figure S6. Metal concentrations in plastic waste, classified by sampling point and intended original use (a) Fe; (b) Mn; (c) Zn; (d) Cu; (e) Cd

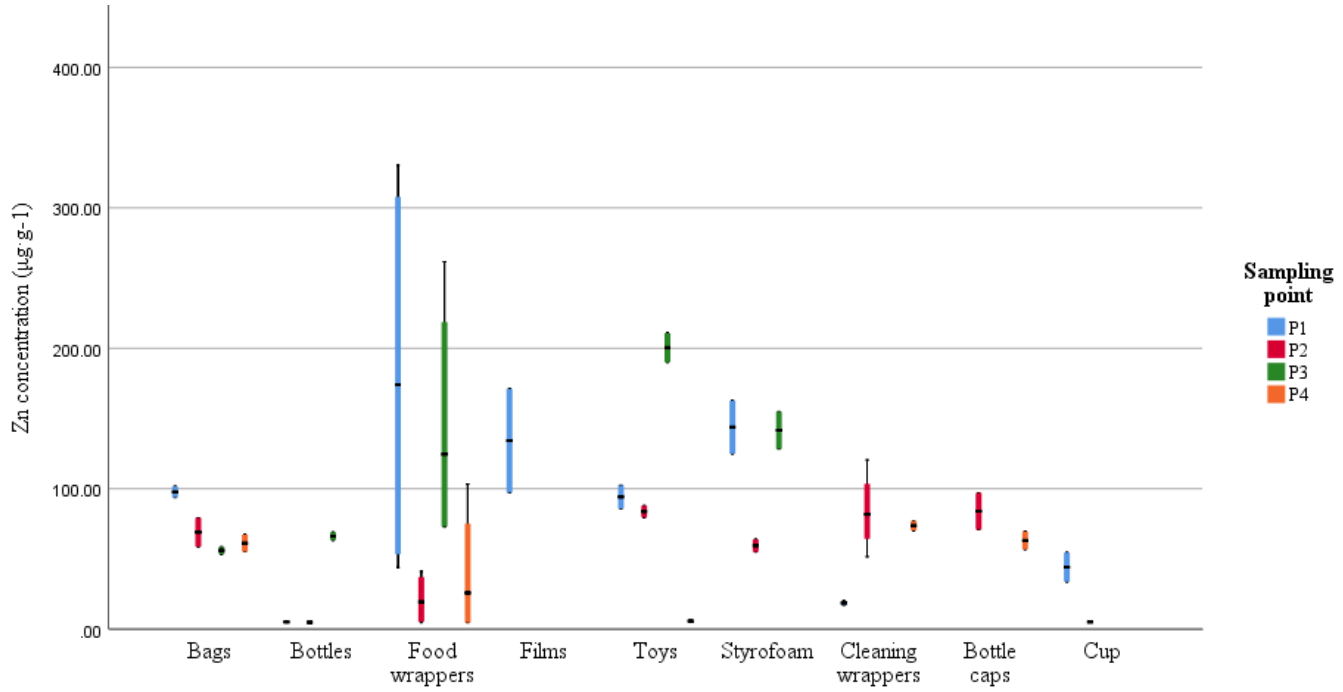
a)



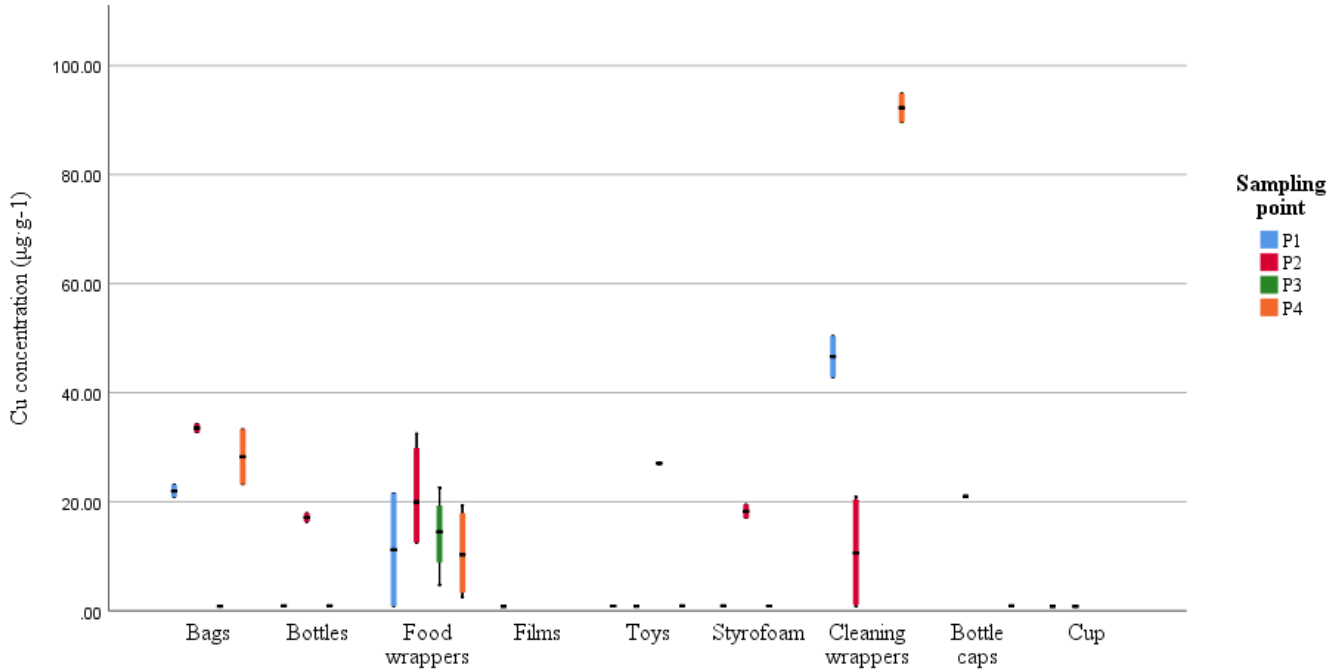
b)



c)


Zn concentration (µg·g-1)
400.00
300.00
200.00
100.00
.00
Bags
Bottles
Food wrappers
Films
Toys
Styrofoam
Cleaning wrappers
Bottle caps
Cup
Sampling point
P1
P2
P3
P4

d)


Cu concentration (µg·g-1)
100.00
80.00
60.00
40.00
20.00
.00
Bags
Bottles
Food wrappers
Films
Toys
Styrofoam
Cleaning wrappers
Bottle caps
Cup
Sampling point
P1
P2
P3
P4

e)

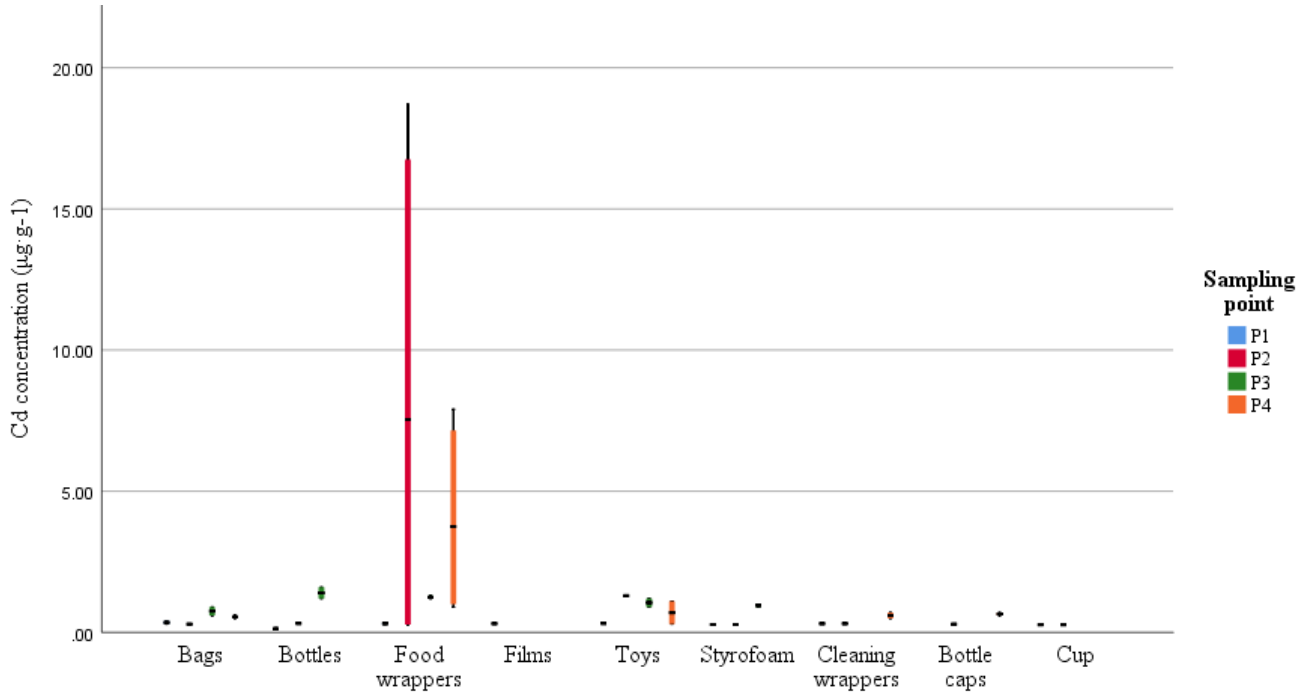


Figure S7. Three samples of Styrofoam (polystyrene) collected at sampling point # 3