Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting information

Metal-doping of nanoplastics enables accurate assessment of uptake and effects on Gammarus pulex

P.E. Redondo-Hasselerharm,[a] *G. Vink,*[b] *D. M. Mitrano,*[c] *A.A. Koelmans*[a*]

[a] Aquatic Ecology and Water Quality Management group, Wageningen University & Research, P.O. Box 47, 6700 AA Wageningen, The Netherlands

[b] Chemical Biological Soil Laboratory, Wageningen University & Research, P.O. Box 47, 6700 AA Wageningen, The Netherlands

[c] Department of Environmental Systems Science, ETH Zurich, Universitatstrasse 16, 8092 Zurich, Switzerland.

* Corresponding author: bart.koelmans@wur.nl

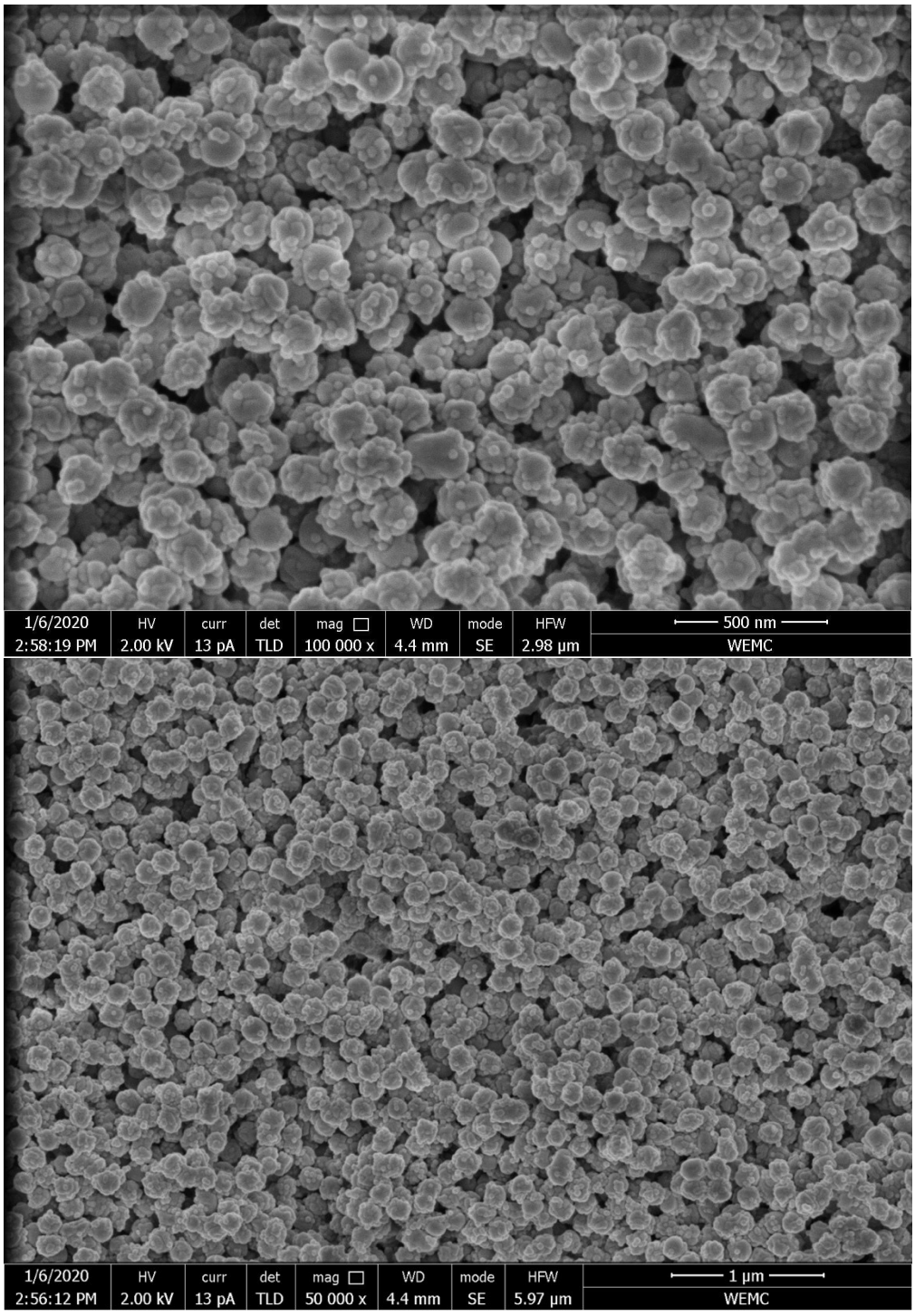

Figure S1*. Images of the Pd-doped NP taken under a FEI Magellan 400 scanning electron spectroscope (50 000x and 100 000 x magnification) illustrating the bumpy surface of the particles.*

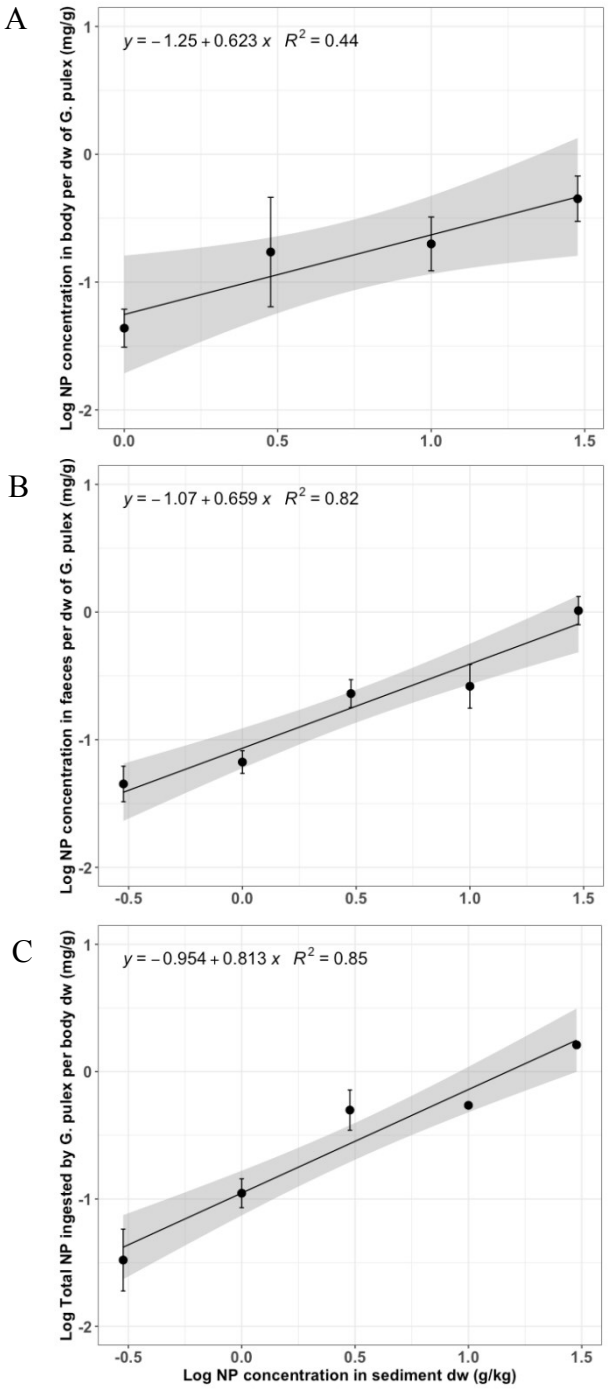

Figure S2. *Log NP concentration (± SE) measured in A) the body of G. pulex (mg/g), B) faeces of G. pulex per body weight (mg/g) and C) total NP ingested by G. pulex (mg/g) per body dw after summing up the concentration of NP in bodies and faeces; after 28 d exposure to Log NP concentrations in sediment dw (g/kg). Linear regresions (p-value$_{body}$ = 1.94×10^{-2}; p-value$_{faeces}$ = 2.87×10^{-6}; p-value$_{Total}$ = 1.02×10^{-6}) are based on 12 individual data points for the body due to the loss of the control and lowest concentration values after log-transforming the data; and 15 individual data points for the faeces and total NP ingested, due to the loss of the control values after log-transforming the data.*

Table S1. *Detection limit values for the different sample types calculated based on the controls analysed during the digestion and corrected for the dilution factor or the weight, depending on the sample type.*

Sample type	Detection limit
Water	0.007 µg/l
Faeces	0.004 µg/l
Biota	21.6 µg/kg
Sediment	0.71 µg/kg

Table S2. *Mean* ± SD *nanoplastic concentration per body dw of Gammarus pulex (mg/g) in body, faeces and the sum of both (grey); and nanoplastic concentration in body and faeces with respect to the total ingested nanoplastics (%) (blue) at nanoplastic concentrations in sediment of 0.3, 1, 3, 10 and 30 g/kg of sediment.*

	NP concentration per body dw of *Gammarus pulex* (mg/g)			**% of NP in body and faeces of the total ingested NP**	
NP concentration in sediment (g/kg)	**BODY** Mean ± SD	**FAECES** Mean ± SD	**TOTAL** Mean ± SD	**BODY** Mean	**FAECES** Mean
0.3	<Detection limit	0.034 ± 0.026	0.030 ± 0.030	-	-
1	0.053 ± 0.031	0.054 ± 0.026	0.107 ± 0.057	39.6	60.4
3	0.328 ± 0.300	0.228 ± 0.098	0.556 ± 0.351	47.8	52.2
10	0.246 ± 0.160	0.285 ± 0.173	0.532 ± 0.020	45.2	54.8
30	0.541 ± 0.415	1.073 ± 0.423	1.614 ± 0.138	33.0	67.0
Average %				**41.4 ± 23.2**	**58.6 ± 23.2**

Assessment of the likeliness of effects caused by chemical residues from nanoplastic synthesis

The concentrations of the chemicals used in the synthesis of Pd-doped NPs may change due to (incomplete) polymerization, dilution upon transfer of the spiked volume to the bioassay systems, sorption to sediment, and volatilization due to purging, either prior to the experiment, or during acclimatization prior to exposure. The concentrations during all of these steps are summarized in Table S3. Ultimately, margins of exposure (MOE) were larger than 1 for all chemicals.

***Table S3**. Numbers calculated for the different steps in the assessment of the likeliness of effects from the background chemicals originating from NP synthesis.*

1	2	3	4	5	6	7	8	9	10
Chemical	**Weight**	**Yield**	**C_{total}**	**$C_{residual}$**	**$C_{bioassay,TOT}$**	**Kp**	**$C_{bioassay,total}$**	**C_{crit}**	**MOE**
	g	%	g/L	g/L	g/L	L/kg	mg/L	mg/L	(-)
Water	627,43								
Acrylonitrile	50,00	95,00	79,69	3,98	9,42E-02	5,0	73,6	2,00	913,5
Styrene	22,50	95,00	35,86	1,79	4,24E-02	50,0	11,1	1,90	1×10^{18}
DVB	1,18	95,00	1,88	0,09	2,22E-03	778,1	4,98E-02	0,69	13,85
SDS	2,46	90,00	3,92	0,39	9,27E-03	2700,0	6,08E-02	1.8	29,60
KPS	3,30	95,00	5,26	0,26	6,22E-03	1,0	5,89	92	15,63
KPE	1,50	90,00	2,39	0,24	5,65E-03	2700,0	3,71E-02	0,18*	4.85
K_2PdCl_4	0,76	99,00	1,21	0,01	2,86E-04	1000,0	5,02E-03	0.063	12,56

1. Chemical abbreviations: DVB = divinylbenzene. SDS = sodiumdodecylsulphate, KPS = potassium persulphate, KPE = poly(ethyleneglycol)4-nonlpheyl 3-sulfopropylether potassium salt. K_2PdCl_4 = Potassium tetrachloropalladate(II).
2. Weight used in the synthesis of Pd-doped NP.
3. The polymerization and encapsulation of monomer, intitiator and surfactants is virtually complete. Still conservative yields < 100% were used in order to obtain a worst case assessment of chemical effects.
4. Original aqueous concentration prior to polymerization, i.e. at start, the concentration of acrylonitrile is 50/627,43 = 79,69 g/L
5. The residual concentration after polymerization taking the yield into account.
6. Concentration in the bioassay, calculated from the spiked volume of the NP disperson and the water volume in the bioassay.
7. The sediment to water partition coefficient (literature value)
8. The aqueous concentration during the bioassay, assuming equilibration with sediment, calculated from the volume of water, the mass of sediment,m the Kp and the quantity of added chemical: $C_{bioassay,TOT} = \frac{Cresidual}{1 + [SED] * Kp}$ with [SED] is the mass to liquid ration of the sediment (kg/L) concentration of the sediment.
9. The threshold effect concentreation for chemical toxicity, based on literature values. For KPE (*) no threshold effect concentration could be found. For this chemical we used a worst case scenario and set the threeshold value at 10% of that for the other surfactant, SDS.

10. The Margin of exposure (MOE), calculated as MOE = C_{crit} / $C_{bioassay,total}$. An MOE larger than 1 means that no chemical effect can occur. For the volatile chemicals acrylonitrile and styrene, the value for $C_{bioassay,total}$ was refined by taking into account the removal due to purging the systems prior to exposure and during the acclimitization period (Figs S3 and S4). The effect of purging was calculated using: $[C] = [Co] * e^{-\frac{FxHxt}{V}}$ with [C] and [C0] are chemical concentrations at start (Co) and during purging (C), F is flow rate (L/h), H is Henry's law constant, t is time (h) and V is water volume.

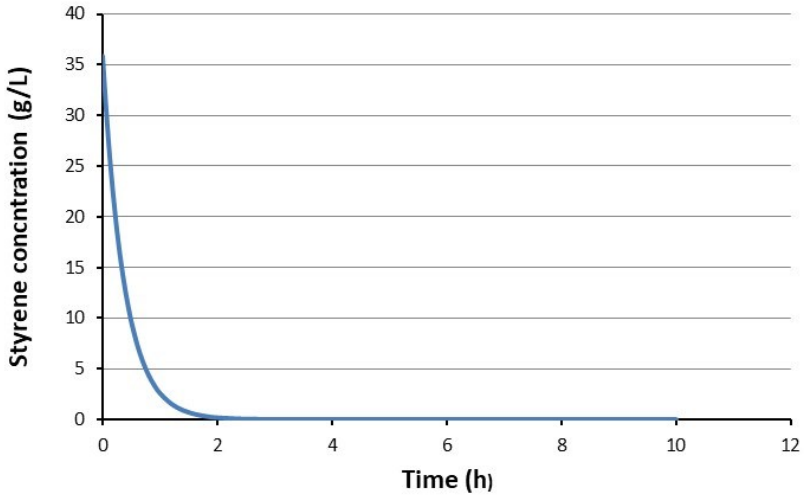

Figure S3: *In 2-3 hours of gas purging, the styrene concentration (Table S3; 35.86 g/L) is reduced to neglible concentration.*

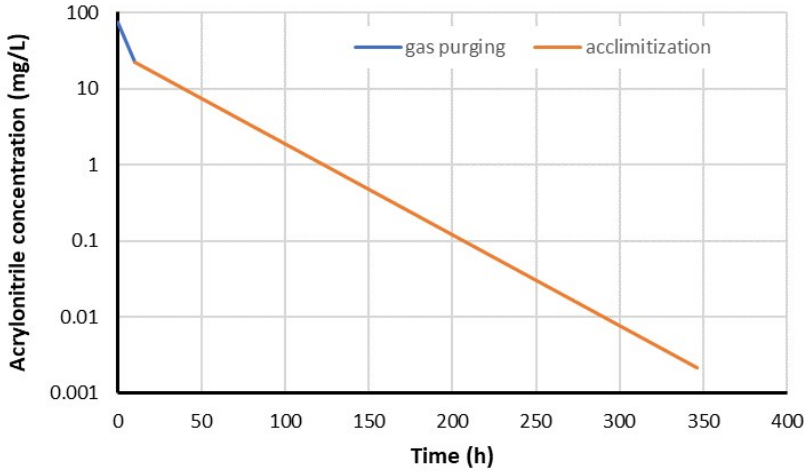

Figure S4*. In 8 hours of gas purging (blue line), followed by two weeks of acclimatization under continuous aeration prior to the experiment (orange line), the bioassay acrylonitrile concentration (Table S3; 73.6 mg/L) is reduced to a marginal concentration of 0.002 mg/L.*

Biodynamic modelling of the bioaccumulation of microplastic by *Gammarus pulex*

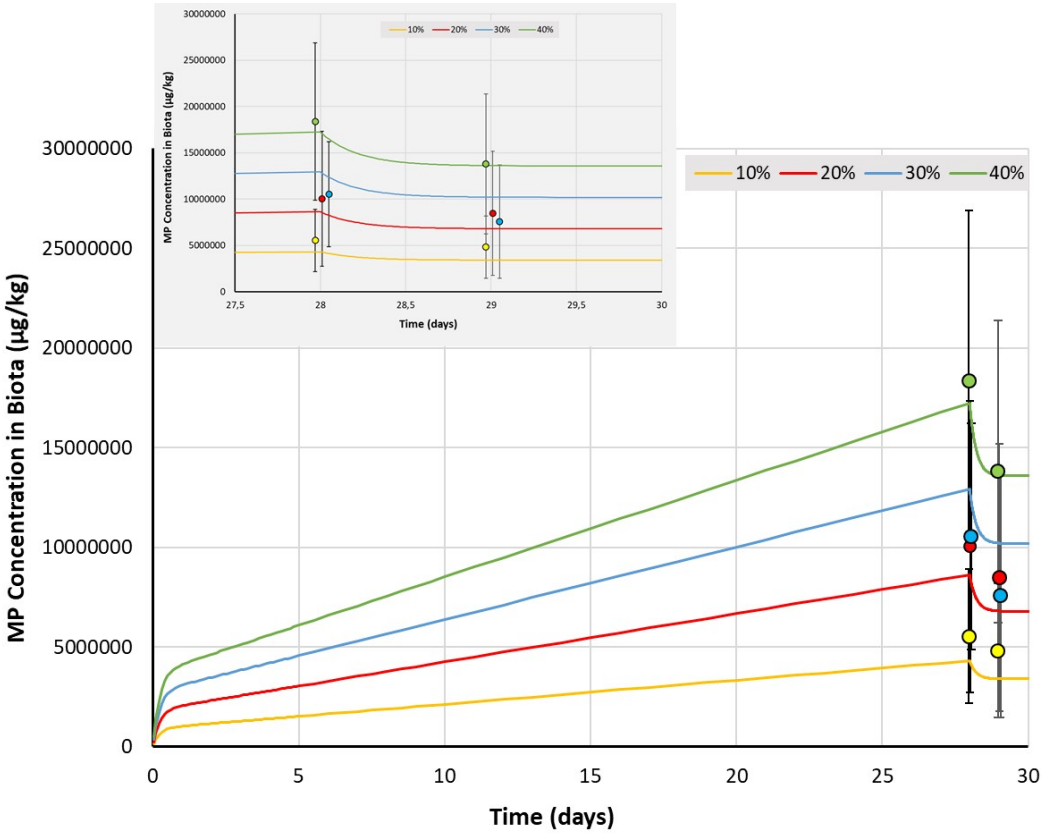

Figure S5. *Measured and modelled (Eqs. 1 and 2) uptake of MP by G. pulex over 28 d of exposure to sediment amended with MP, followed by 1 d of depuration in clean medium (insert). Data on measured NP concentrations (± 1 SD) after depuration (see insert) after 29 days were set apart for 0.05 day for better visibility of the datapoints on the x-axis. Data from Redondo-Hasselerharm et al, 2018. The model was highly significant ($p=4.4\times10^{-99}$).*