Supplementary Information

Plastic teabags release billions of microparticles and nanoparticles into tea

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The Supplementary Information contains 9 pages and 10 figures.

1. Description of Control Experiments. Several control experiments were conducted to ensure that: (i) the deposition of particles for SEM imaging and counting was uniform; (ii) particles were not released as a result of cutting the teabags; (iii) particle release was enhanced at high temperature, (iv) the experimental apparatus did not introduce particles into the samples, and (v) the plastic particles identified in the leachates did not originate from the tea leaves.

i. Sample preparation for SEM imaging to avoid coffee ring effect

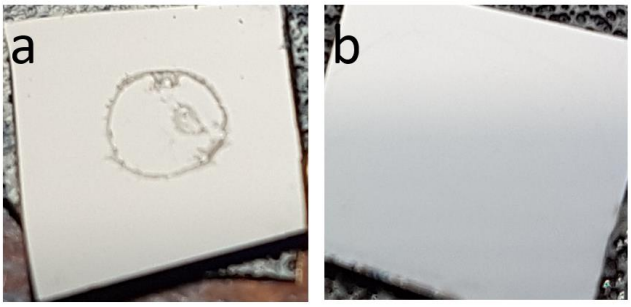

Figure S1. Deposition of particles for SEM imaging. (a) Following deposition of 100 µL of leachate as a single droplet onto an untreated silicon wafer, a coffee ring effect can be observed as a result of the accumulation of particles at the edge of the droplet. **(b)** Following deposition of 100 µL of leachate in ten successive droplets of 10 µL over a silicon wafer washed with ethanol to increase hydrophilicity of the surface by oxidation. No coffee ring effect is observed as the sample dries uniformly over the silicon wafer.

ii-v. Confirming that particles are not released due to cutting or from the apparatus or from tea leaves, and that particle release is enhanced at high temperature

In this study, all experiments were conducted with cut and emptied teabags to ensure that the enumerated particles originated from the teabag material and not from the tea. Control experiments with uncut teabags were conducted to confirm that cutting of the teabag did not cause leaching of particles (i.e., to confirm that particles were released even when the plastic teabag was uncut) (Fig. S2b, c). These samples contain tea and therefore require two additional steps before analysis, as the organics in tea interfere with SEM, FTIR, and XPS characterization. A 0.45 µm EMD Millipore Millex polyethersulfone sterile syringe filter was used to remove small tea leaves and twigs and a 50 mL Amicon stirred cell (UFSC05001 model) with a 30 kDa filter was used to remove dissolved organics (Fig. S2a). These processed leachates were then characterized by SEM, FTIR and XPS (Fig. S2-S4).

Additional controls (Fig. S2) were performed to (i) investigate the effect of heat on the degradation of the plastic teabags (Fig. S2d, e), (ii) to confirm that the experimental apparatus did not introduce particles into the samples (Fig. S2f, h), and (iii) to confirm that the loose leaf tea itself did not release plastic particles into the samples (Fig. S2g).

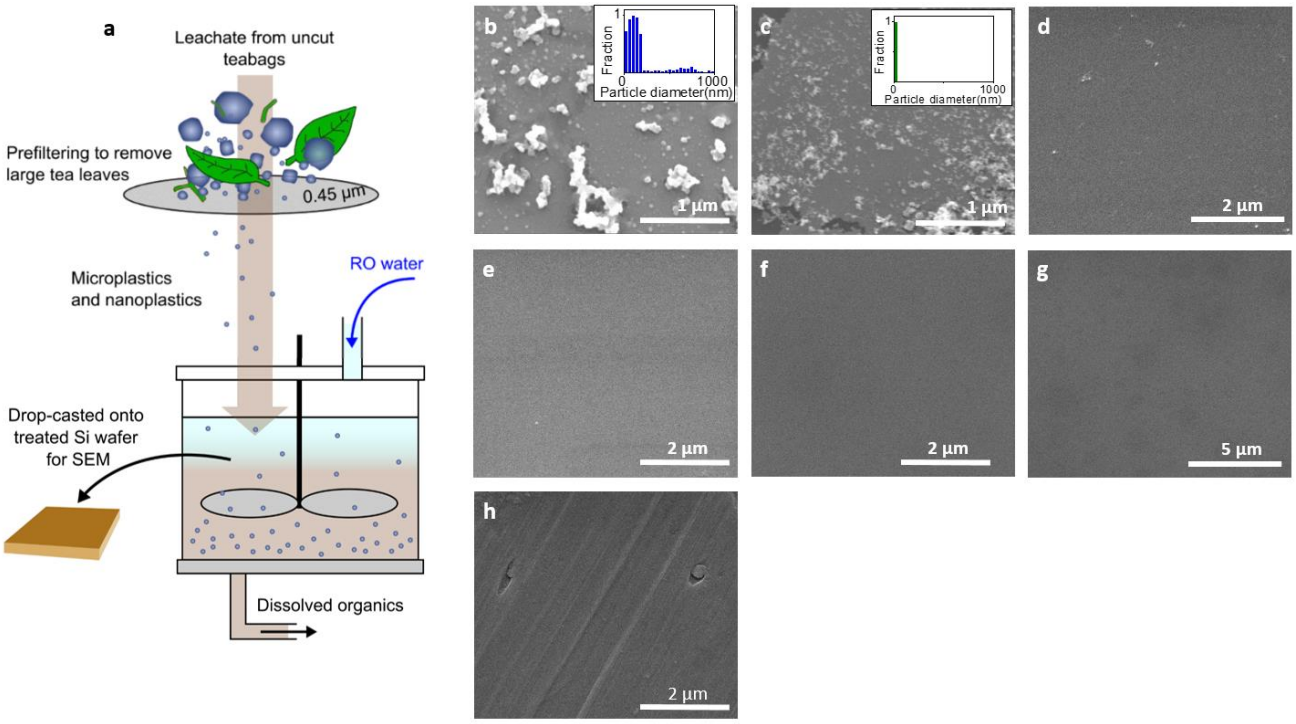

Figure S2. Filtration set-up used to process leachate from the unopened teabag (with loose leaf tea inside) and SEM images from control experiments. (a) This process was necessary to remove tea debris and organics; however, it adds steps to the sample processing. Therefore, counts should not be directly compared with those obtained in the main experiment because samples were not processed in the exact same way. First, leaf and twig fragments are removed using a 0.45 µm EMD Millipore Millex sterile syringe filter. Subsequently, dissolved organics are removed using a 50 mL Amicon stirred cell. This procedure is carried out to remove the dissolved organics in order to adequately characterize the leachate. Imaging at 1,000× of dried leachate from **(b)** control experiment with uncut teabag B and **(c)** control experiment with uncut teabag D. Both samples were prepared by following the process described in Fig. 1a (with the exception of opening and emptying the teabag). Subsequently, the leachates were processed as shown in Fig. S2a to remove dissolved organics. Particulate material is observed in both images, supporting the hypothesis that particles are leaching from teabags even when they are unopened. Imaging at 50,000× of **(d)** unheated leachate B and **(e)** unheated leachate D. Both samples were prepared by following the process described in Fig. 1a with the exception that the temperature of the water in which the teabags were steeped was at 22 °C instead of 95 °C. **(f)** Imaging at 50,000× of filtrate from the control experiment in which RO water was processed through the system described in Fig. 1a. The image shows no micro- or nanoparticles in the filtrate, confirming that the particles are not released from the experimental system (tubing, filters, etc). **(g)** Imaging at 30,000× of the filtrate from the control experiment conducted using a metallic steeper and loose tea leaves (which were not previously packaged in individual teabags). The image shows no micro- or nanoparticles in the filtrate, confirming that the particles are not released from the tea leaves. **(h)** SEM image at 50,000× of negative control experiment where RO water was processed through the system described in Figure S2a. Results show no micro- or nanoparticles in the filtrate, confirming that there are no particles released by the EMD Millipore Millex sterile syringe filter or the Amicon stirred cell.

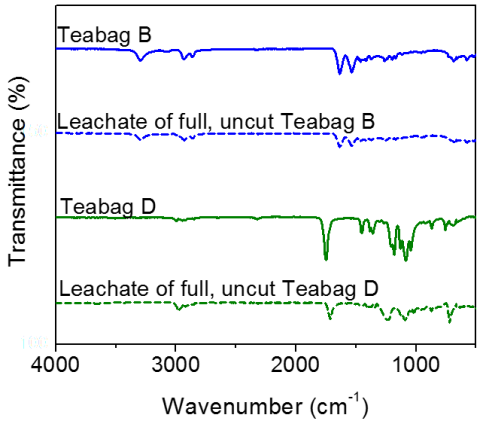

Figure S3. FTIR spectra for control experiments. FTIR spectra for the original (cut) teabag and the corresponding leachates prepared without cutting the teabag and removing the tea. FTIR peaks identified for the dried leachates match with those observed for the original teabag material. This confirms that the particles leaching out of uncut teabags are plastic. This also confirms that teabags leach micro- and nanoplastics even when they are not cut.

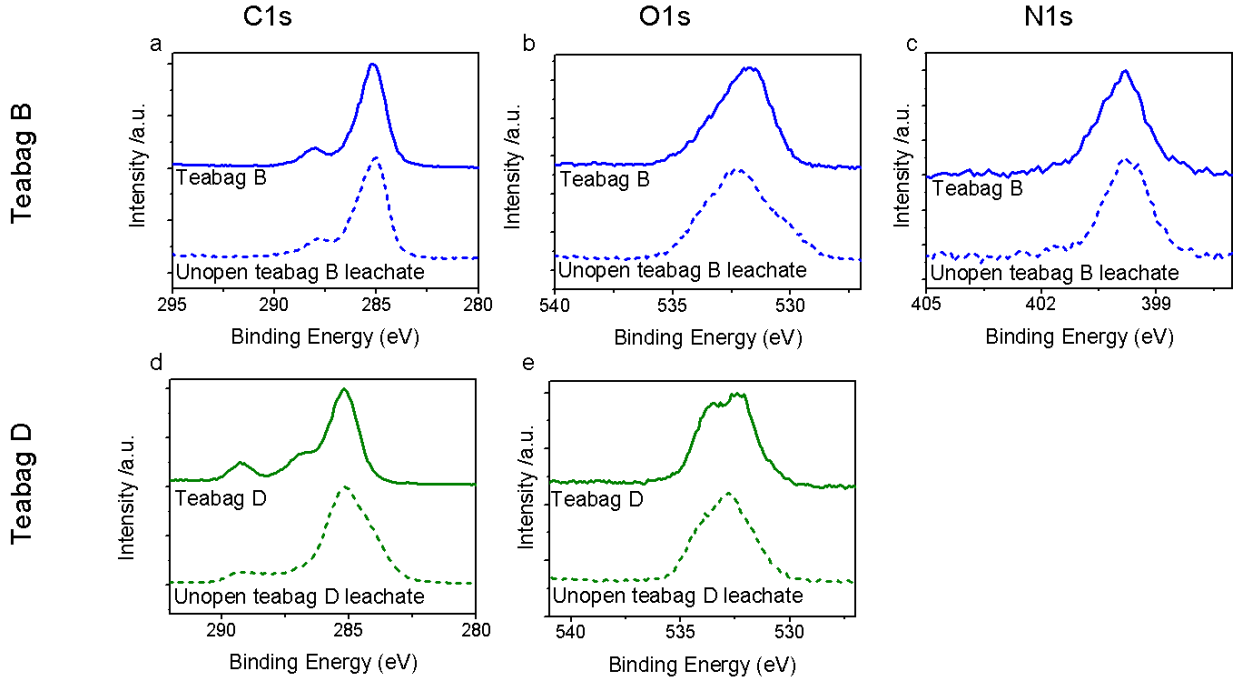

Figure S4. XPS analysis of the original (cut and emptied) teabag and leachates prepared without cutting the teabag and removing the tea. (a,b,c) C1s, O1s and N1s spectra of the original teabag B and the leachate obtained by steeping an unopened teabag B (with tea inside) and further processing the leachate as shown in Fig. S2a to remove dissolved organics. The measurements confirm that the particles in the leachate are nylon. **(d,e)** C1s and O1s spectra of the original teabag D and the leachate obtained by steeping an unopened teabag D (with tea inside) and further processing the leachate as shown in Fig. S2a to remove dissolved organics. The signals confirm that the particles in the leachate are PET.

2. Description of *D. magna* Swimming Assay.

At 48 hours, the mobile *Daphnia magna* from each treated group and control group were removed from the beakers and placed in glass bottom culture dishes with 14 mm microwell (MatTek, Ashland, US) filled with 2 mL of MHRW under ambient light conditions. This allowed for lateral movement with restricted vertical movement. The daphnids were allowed 2 minutes to acclimate prior to being recorded. The video was taken from above for 1 minute using a stereomicroscope (Fisher Stereomaster) mounted with a digital camera set at 30 frames per second and a resolution of 1920×1080 pixels. The 14 mm diameter of the microwell was used as the scale in each recording. The swimming movement in each video was identified by Kinovea-0.8.26 (https://www.kinovea.org/) and the swimming track density was determined by the image analysis of the trails left by daphnids during a 1-minute video recording. The tracks in each image were transformed to black pixels and the background to white, and the percentage of black pixels from each image was calculated using ImageJ 1.8.0. Statistical analyses on swimming movement were performed using Prism 5 version 5.01. The normality of data and homogeneity of variances was evaluated by Kolmogorov-Smirnov test and Bartlett's test, respectively. Comparisons between means among the experimental groups were achieved by one-way analysis of variance (ANOVA) followed by the *post-hoc* Tukey's multiple comparison test or Kruskal-Wallis test followed by Dunn's multiple comparison test when data did not meet the assumptions of ANOVA. The level of significance was set as $p \leq 0.05$.

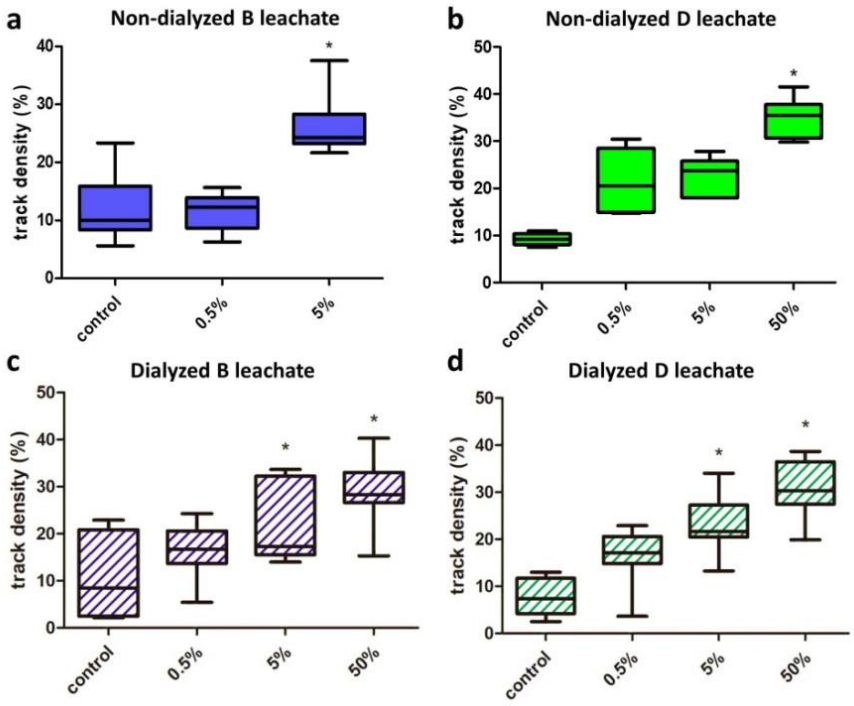

Figure S5. Swimming track density of *Daphnia magna* after exposure to different concentrations of (a) non-dialyzed teabag B leachate and (b) teabag D leachate, and (c) dialyzed teabag B leachate and (d) teabag D leachate. Swimming track density was determined by the image analysis of the trails left by *D. magna* during one-minute video recording. The tracks in each image were transformed to black pixels and background as white, and then the percentage of black pixels from each image was calculated using ImageJ 1.8.0. Asterisk indicates that the measurement is significantly different (p-value <0.05) from the control; n=9 for dialyzed teabag B, dialyzed leachate D, and non-dialyzed leachate B, and n=6-9 for non-dialyzed teabag D leachate.

3. Description of ICP-MS analysis of leachates.

Dialyzed and non-dialyzed leachates as well as the empty and full teabags were digested and analyzed by ICP-MS to determine the concentrations of four metal(loid)s. The closed digestion of all the samples was performed in Teflon tubes that were pre-rinsed five times with RO water. A two-step digestion was performed to reduce the explosion risk due to nitration of organic carbon. The samples to be digested were weighed directly in the Teflon tubes. Subsequently, 5 mL of trace metal grade hydrochloric acid (Fisher) was added to each sample. The Teflon tubes were closed and placed in a Mars 6 OneTouch microwave to complete a cycle that consisted of 20 minutes ramping from 20 °C to 150 °C, 20 minutes holding the temperature at 150 °C, and 20 minutes of cool down. After carefully opening the Teflon tubes, 3 mL of trace metal grade nitric acid (Fisher) was added to each sample. The tubes were closed and placed in the microwave for the same cycle. After the microwave cycle, the tubes were left on the bench to cool overnight. The following day, the digested contents were transferred into DigiPREP tubes. To minimize the loss of metal(loid)s in the Teflon tubes, they were rinsed at least five times with RO water that was added into the DigiPREP tubes. The final volumes of the diluted digested samples were brought to 50 mL with RO water. For the ICP-MS analysis, the samples were further diluted (10 times) to achieve final acid (HNO_3/HCl) concentrations below the 3% v/v level.

To account for solution matrix effects in the ICP-MS measurements, the internal standards of Sc (for Al and Cr), Y (for As), and Bi (for Pb) were used. A calibration curve was done at the beginning of the run and after 20 samples to correct possible signal variations throughout the measurement period. For each metal(loid), NIST standard reference material (Millipore ICP standards, Al #170301, Pb #170328, As #170303, Cr #170312) was used to verify the quality of the measurements at the start of the run, after every 10 samples, and at the end of the run. These quality control measurements confirmed that the measurements were performed within an acceptable error range (±3%).

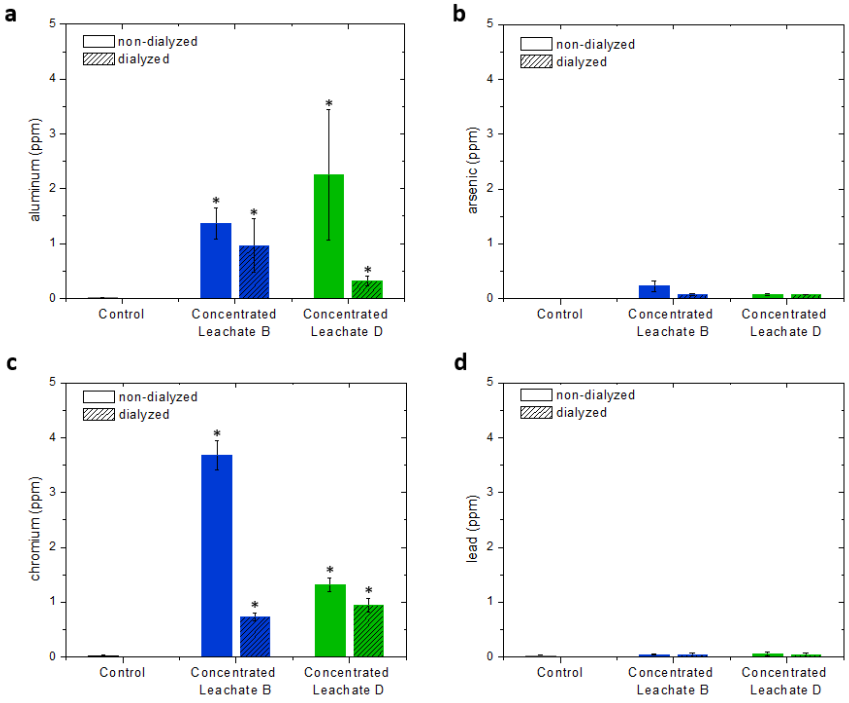

Figure S6. ICP-MS measurements of concentrations of (a) aluminum, (b) arsenic, (c) chromium and (d) lead found in the concentrated (100%) leachate before and after dialysis. The negative control was prepared by performing the complete digestion process without any leachate sample (adding acids and performing the temperature ramps). Asterisk indicates that the measurement is significantly (p-value <0.05) different from the control using a t-test. Statistics were not performed on the arsenic measurements as n=2 for those samples. Al and Cr are the most abundant metal(loid)s detected, with concentrations up to 2.3 and 3.7 ppm, respectively. In contrast, the concentrations of As and Pb are more than one order of magnitude lower (0.05-0.3 ppm).

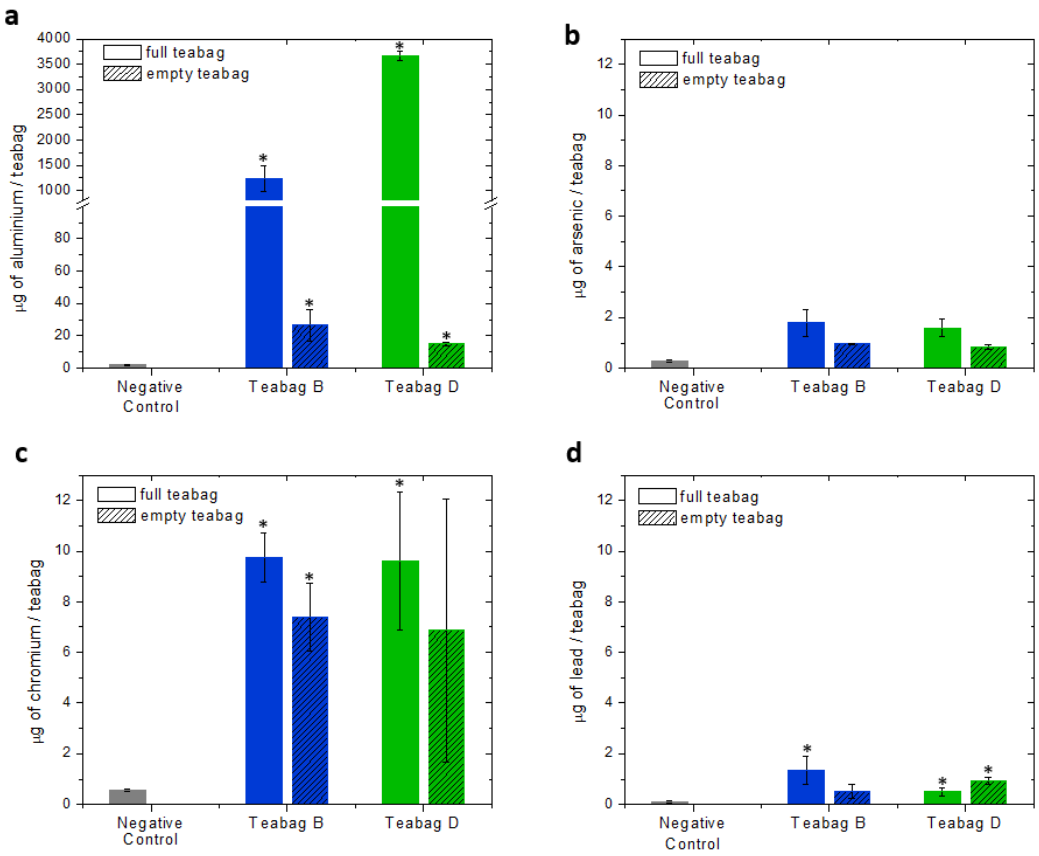

Figure S7. ICP-MS measurements of total mass of (a) aluminum, (b) arsenic, (c) chromium and (d) lead detected in a single empty plastic teabag (cleaned with RO water) and one full plastic teabag (containing loose leaf tea) for brands B and D. The negative control was prepared by performing the complete digestion without any leachate sample (i.e., acids were added to empty tubes and temperature ramps were performed). Asterisk indicates that the measurement is significantly (p-value <0.05) different from the control using a t-test. Statistics were not performed on the arsenic measurements as n=2 for those samples. Note that the lead content of full teabag D and empty teabag D are not significantly (p-value <0.05) different from each other using a t-test. The presence of tea leaves significantly increases the levels of Al and As. The concentration of Al is two orders of magnitude higher (18-25 µg per empty teabag compared to 1200-3700 µg per cup of tea), whereas the concentration of As is two-fold greater (from 0.7-1.0 µg per teabag compared to 1.6-1.7 µg per cup of tea). The levels of Pb and Cr are comparable for the empty and full teabags; therefore, the source of these metal(loid)s could be either the tea leaves or the plastic teabags.

4. Immobility assessment, body size, X-ray computed tomography scanning, and optical imaging of *D. magna* exposed to non-dialyzed teabag leachate.

The *D. magna* Acute Immobilization Test was conducted in triplicates for each leachate concentration and the control. All test beakers were placed in a random order at light: dark conditions of 16 hours: 8 hours. The temperature (21-22 ℃), pH (7-8), and dissolved oxygen (9.9-10.6) of the beakers was recorded. The immobile neonates were counted and removed at 24 and 48 hours (Fig. S8a,b). At 48 hours, the swimming *D. magna* from each treated group and control group were removed from the beakers and placed in glass bottom culture dishes with 14 mm microwell (MatTek, Ashland, US) filled with 2 mL of MHRW under ambient light conditions. *D. magna* was imaged using a stereomicroscope (Fisher Stereomaster) mounted with a digital camera, and the body size (from head to the base of the tail spine) was measured with ImageJ 1.8.0. (Fig. S8c,d).

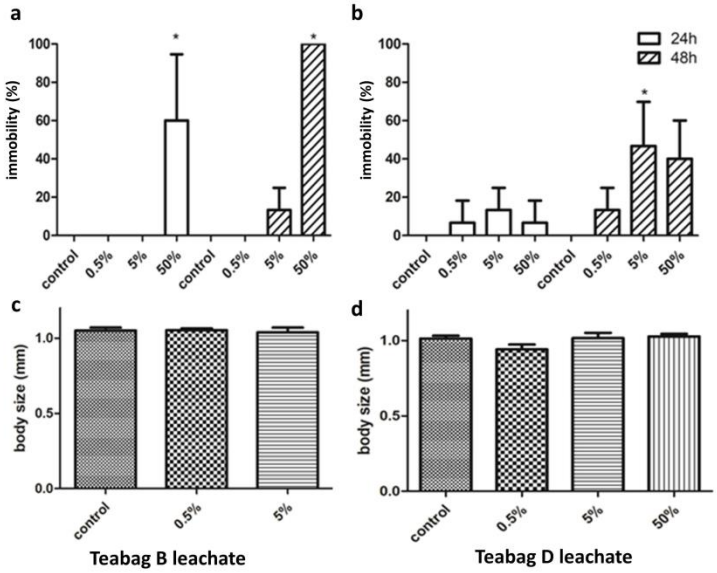

Figure S8. Mean immobility and body size of *Daphnia magna*. *D. magna* was exposed to different concentrations (0.5%, 5%, and 50%) of non-dialyzed **(a, c)** teabag B and **(b, d)** D leachate for 24 h and 48 h. Asterisk indicates that the measurement is significantly different (p-value <0.05) from the control.

After swimming assessment, a subsample of *D. magna* was randomly selected for X-ray computed tomography scan (CT scan) and optical microscopy. For CT scanning, *D. magna* was stained with 1% phosphotungstic acid dissolved in 70% ethanol for 3 days. Then, samples were quickly washed with 70% ethanol and placed in a pipette tip in 70% ethanol for CT imaging. Samples were scanned at 1.5 µm resolution with phase contrast using a Zeiss Xradia 520 Versa (Carl Zeiss Canada Ltd., ON, Canada). Scan parameters were as follows: 60 kV, 82 uA, 4× objective lens, no filter, 3201 projections over 360-degree scan, 3.4-second exposure, and 2×2 pixel binning yielding 1.5 mm^2 field of view. These parameters resulted in 4.5 hours of scanning per specimen. All raw projection data were reconstructed in Zeiss reconstructor software. The CT images were then analyzed with Dragonfly 3.5 (Object Research System Inc, Montreal, Canada, http://www.theobjects.com/dragonfly) in order to visualize the 3D reconstructions of daphnids. The sectioned CT images were reproduced in Dragonfly. For optical imaging, a subsample of *D. magna* was randomly selected and fixed in 70% ethanol overnight, and then

carefully rinsed 6 times with DI water to remove particles attached externally as much as possible before imaging. Images were taken using an Olympus DP80 microscope digital camera at 60× magnification focusing on the intestine region of *D. magna*.

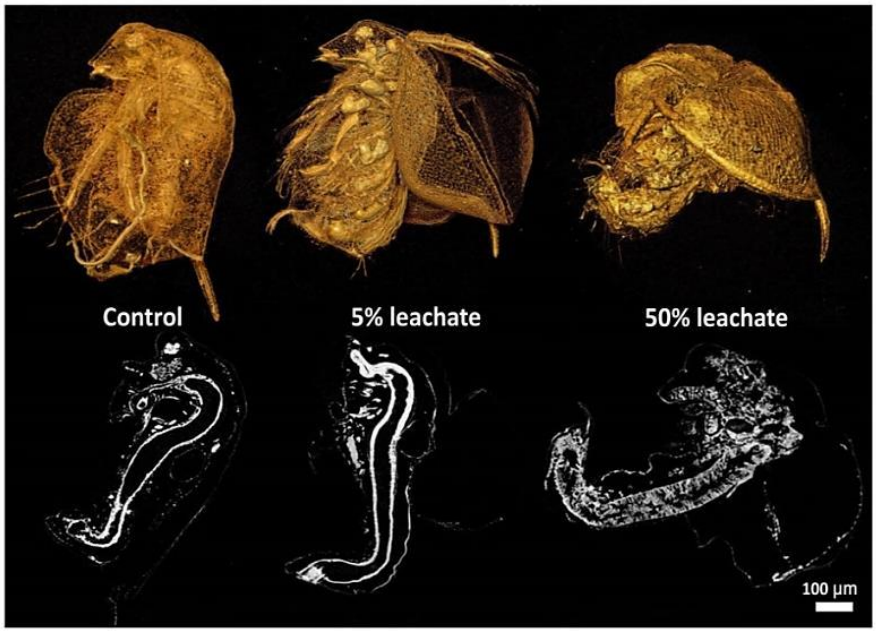

Figure S9. CT images of *Daphnia magna* exposed to 5% and 50% non-dialyzed teabag D leachate. Random samples were stained with phosphotungstic acid and scanned using a Zeiss Xradia 520 Versa. Scale bar, 100 µm.

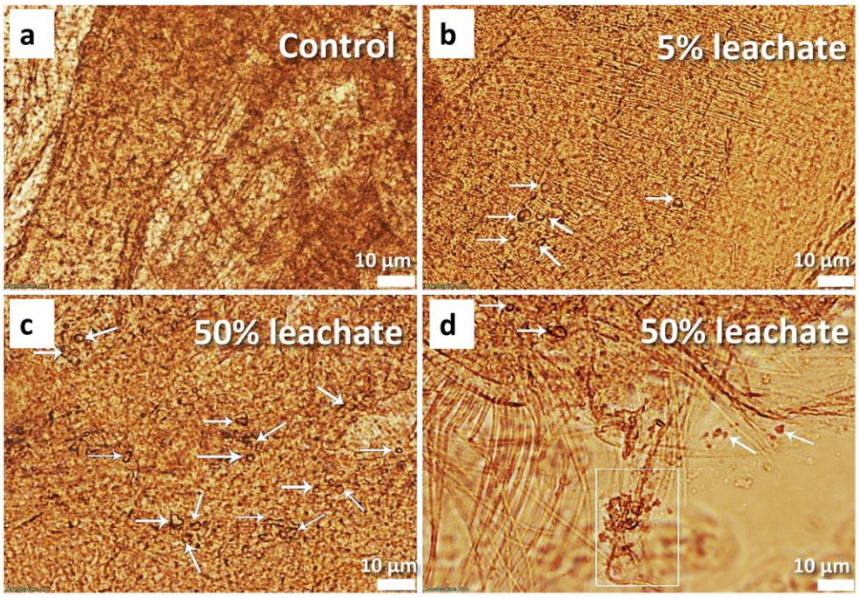

Figure S10. The presence of micro-particles in *Daphnia magna* exposed to non-dialyzed teabag D leachate. The particles are indicated with white arrows in intestine **(a-c)** and abdominal setae **(d)**. Following 48 h of exposure to non-dialyzed leachates, and after the swimming assessment, a subsample of *D. magna* was randomly selected and fixed in 70% ethanol overnight. *D. magna* preserved in ethanol is carefully rinsed 6 times with DI water before imaging. Representative images are taken using an Olympus DP80 microscope digital camera at 60× magnification. Scale bar, 10 µm. Intestine is focused for imaging, as shown in a-c. Foreign microparticles are only found in the leachate-treated *D. magna* (b, c) but not in the control (a). The microparticles are also found on the hair-like abdominal setae close to anus (d). The observed microparticles in (d) may be egested from anus and attach onto the abdominal setae. The size and shape of these microparticles are similar to those of particles we observed in the leachate.