Development of an Inexpensive and Comparable Microplastic Detection Method Using Fluorescent Staining with Novel Nile Red Derivatives

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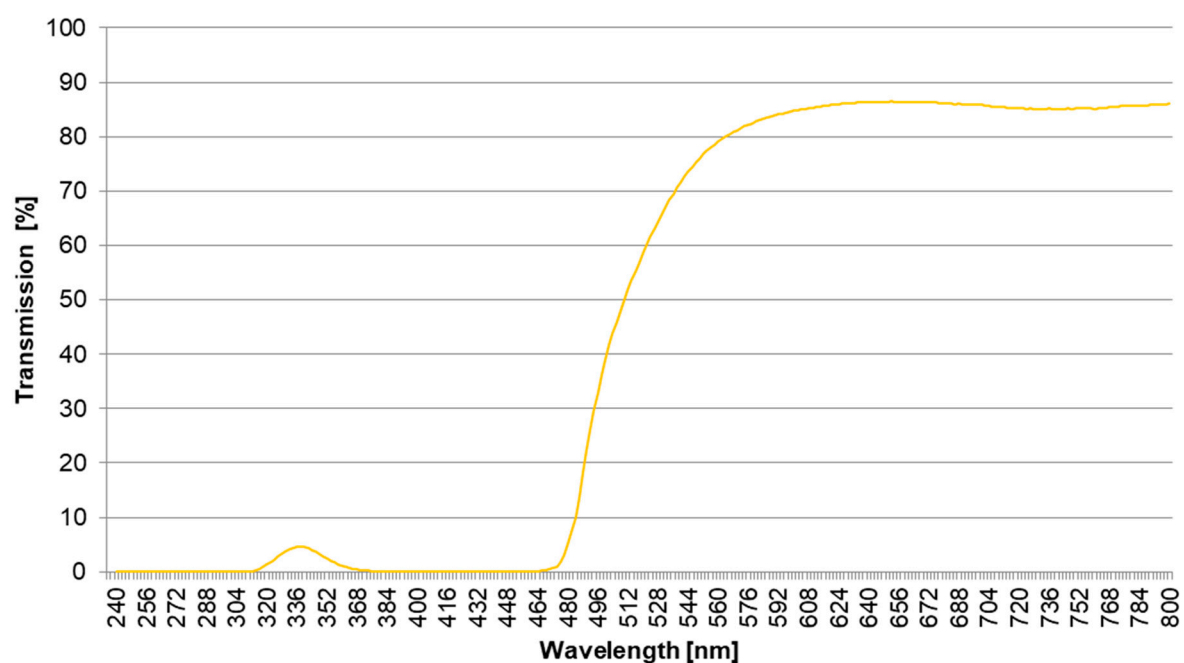[*]   Correspondence: schuhen@wasserdreinull.de; Tel.: +49-(0)-721-15-65-95-93

Figure S1: Transmission spectra of the yellow-colored foil used as emission filter for the modification of the microscope. The spectrum was taken with a Horiba Aqualog® (Horiba Jobin Yvon GmbH, Bensheim, Germany). The range was set to 240-800 nm with 1 nm increment and a integration time of 0.01 s.
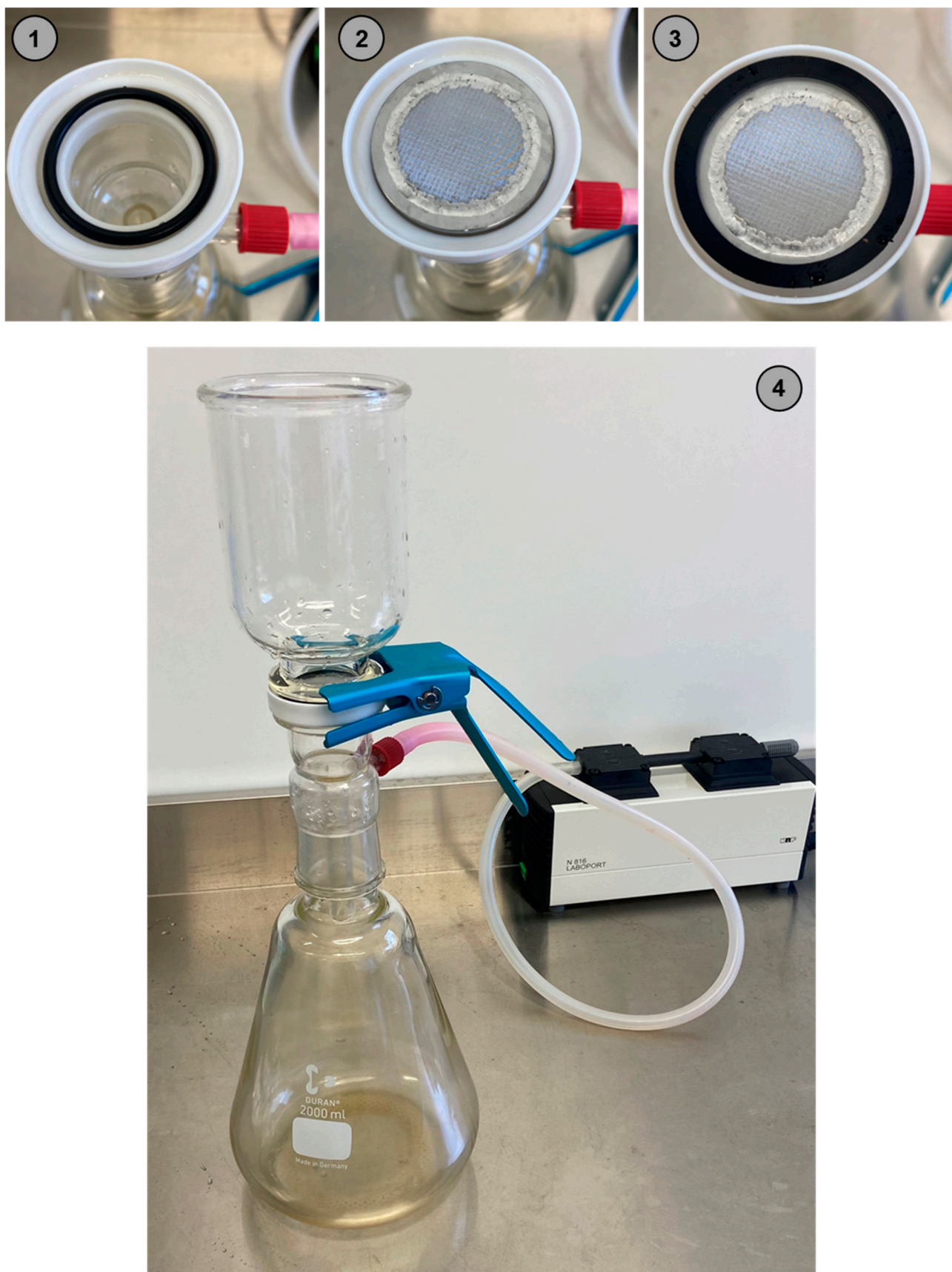
Figure S2: Setup of the vacuum filtration device for the filtration of the microplastics in the laboratory. 1) Insertion of Viton sealing ring, 2) insertion of stainless-steel filter, 3) placing of the 2nd Viton sealing ring, 4) placing the funnel and securing it with a clamp.

Image J-Script for automated particle counting. Marked Yellow are the values for the color threshold, light blue for the saturation threshold and green for the brightness threshold.

```
min=newArray(3);
max=newArray(3);
filter=newArray(3);
a=getTitle();
run("HSB Stack");
run("Convert Stack to Images");
selectWindow("Hue");
rename("0");
selectWindow("Saturation");
rename("1");
selectWindow("Brightness");
rename("2");
min[0]=60;
max[0]=90;
filter[0]="pass";
min[1]=0;
max[1]=255;
filter[1]="pass";
min[2]=20;
max[2]=255;
filter[2]="pass";
for (i=0;i<3;i++){
  selectWindow(""+i);
  setThreshold(min[i], max[i]);
  run("Convert to Mask");
  if (filter[i]=="stop")  run("Invert");
}
imageCalculator("AND create", "0","1");
imageCalculator("AND create", "Result of 0","2");
for (i=0;i<3;i++){
  selectWindow(""+i);
  close();
}
selectWindow("Result of 0");
close();
selectWindow("Result of Result of 0");
rename(a);
// Colour Thresholding------------
//setThreshold(255, 255);
run("Convert to Mask");
run("Analyze Particles...", "size=400-Infinity pixel show=Overlay display exclude clear include summarize in_situ");
```
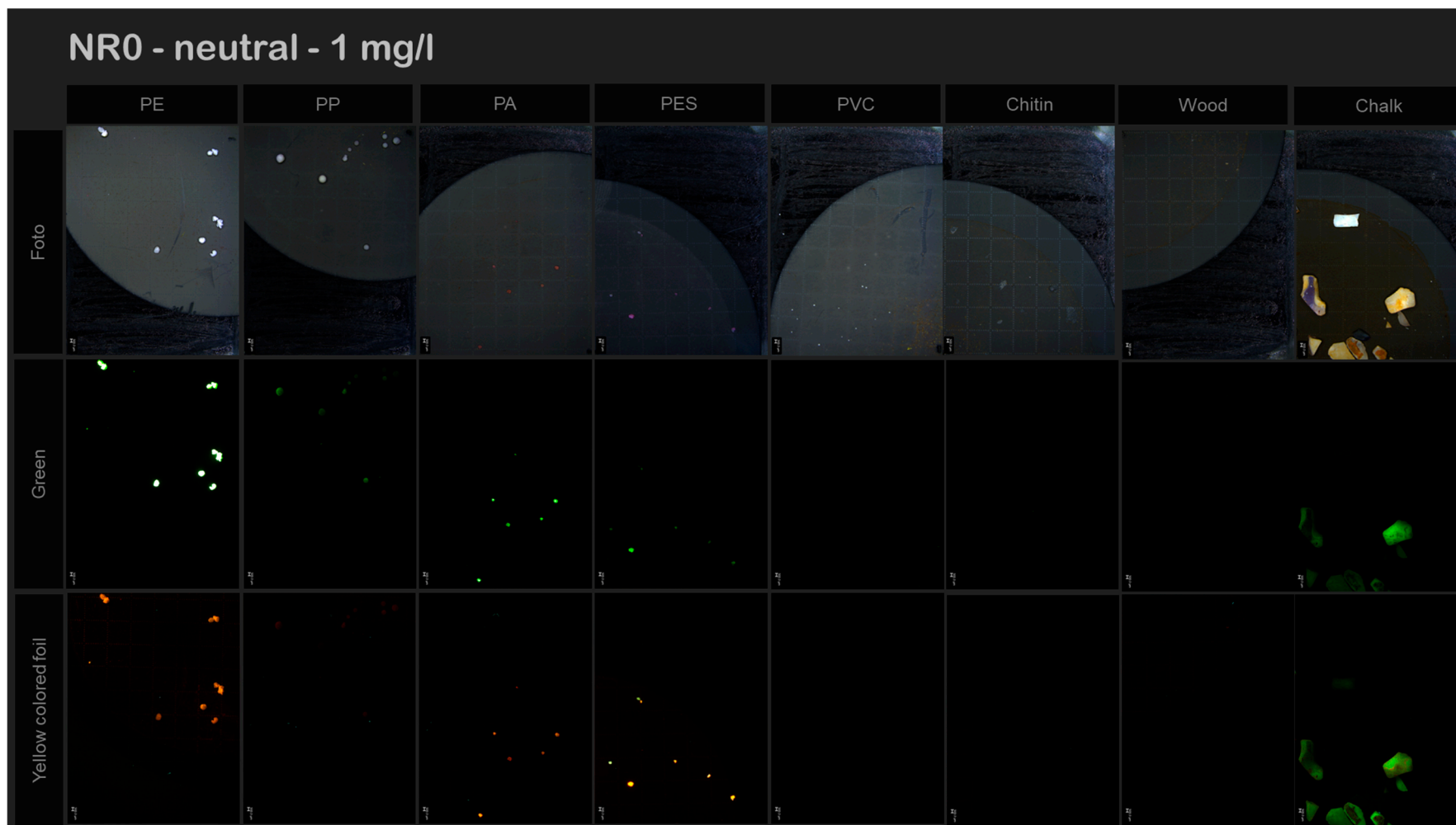
Figure S3: Images taken to determine the recovery rate of microplastic, and natural particles stained with Nile red (NR0). Green fluorescence: Ex: 420-470 nm;

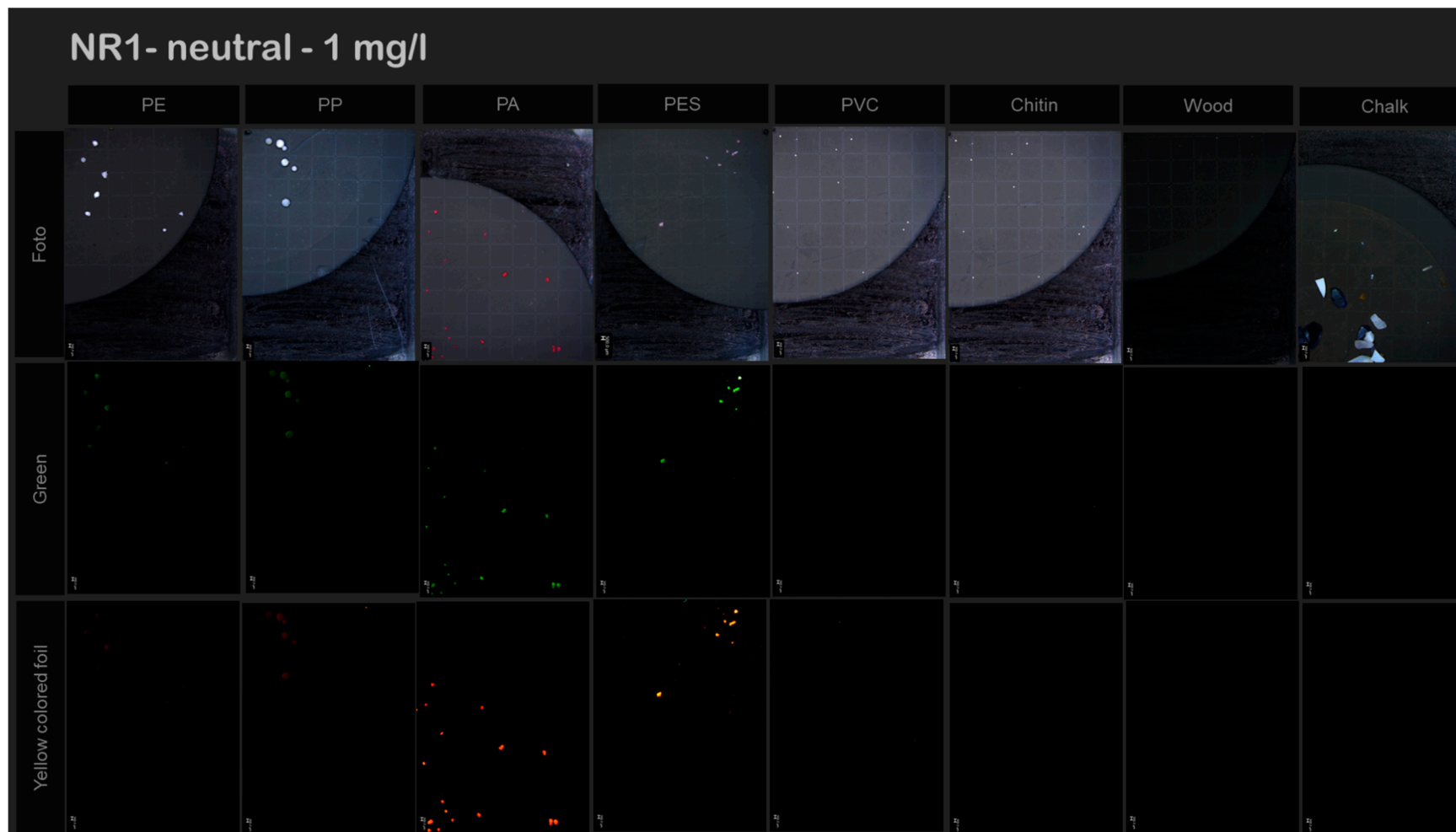*Em: 500-570 nm; yellow colored foil: Ex: UV-Lamp, Peak 365 nm; Em: Fig. S1*

Figure S4 Images taken to determine the recovery rate of microplastic, and natural particles stained with Nile red derivatives NR1. Green fluorescence: Ex: 420-470 nm; Em: 500-570 nm; yellow colored foil: Ex: UV-Lamp, Peak 365 nm; Em: Fig. S1
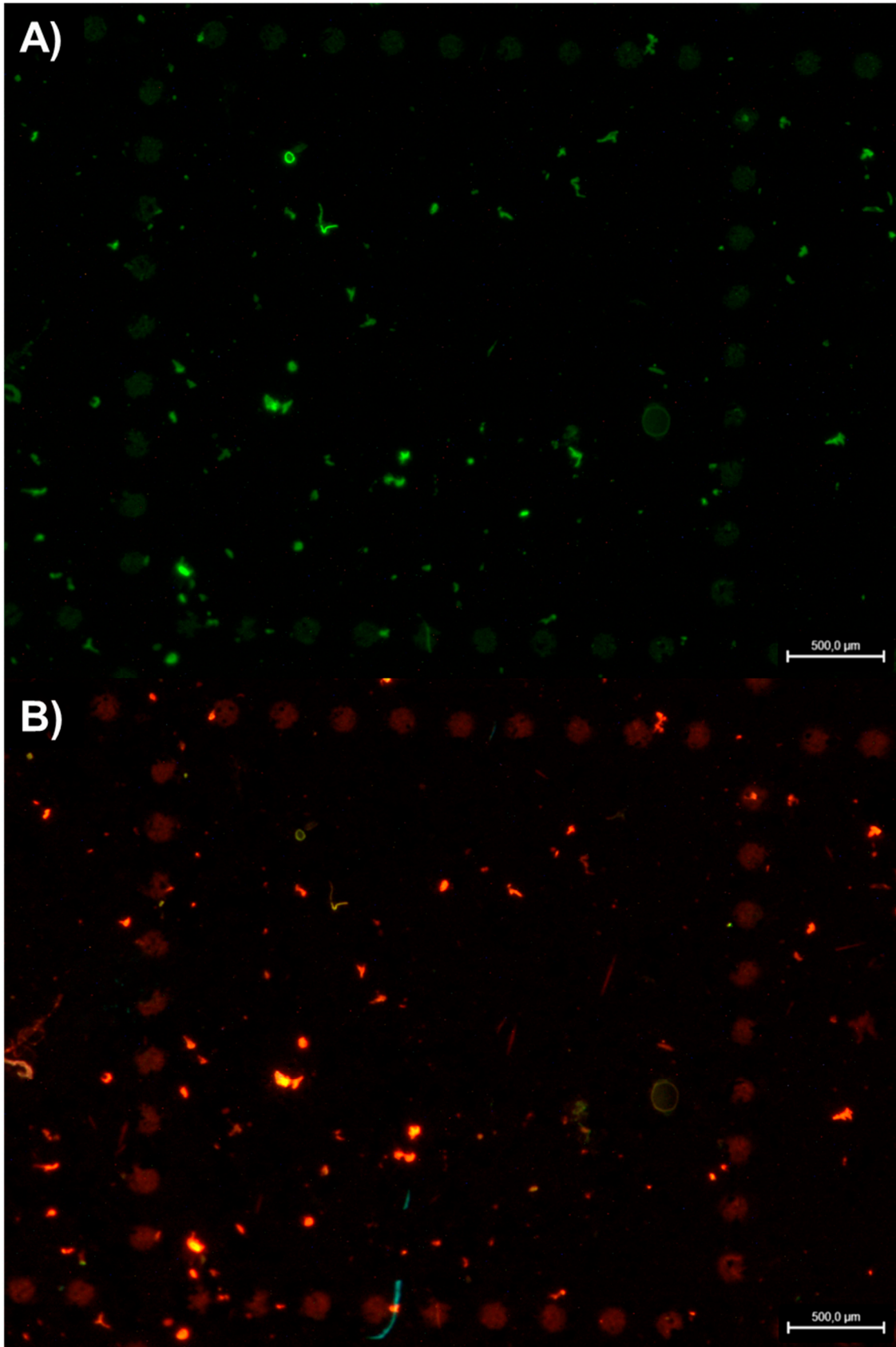
Figure S5: Comparison of the photos taken of processed wastewater samples stained with Nile red (NR0). A) Green fluorescence: Ex: 420-470 nm; Em: 500-570 nm; B) yellow colored foil: Ex: UV-Lamp, Peak 365 nm; Em: Fig. SXX. The difference between the fluorescent particles seen with both options is low.
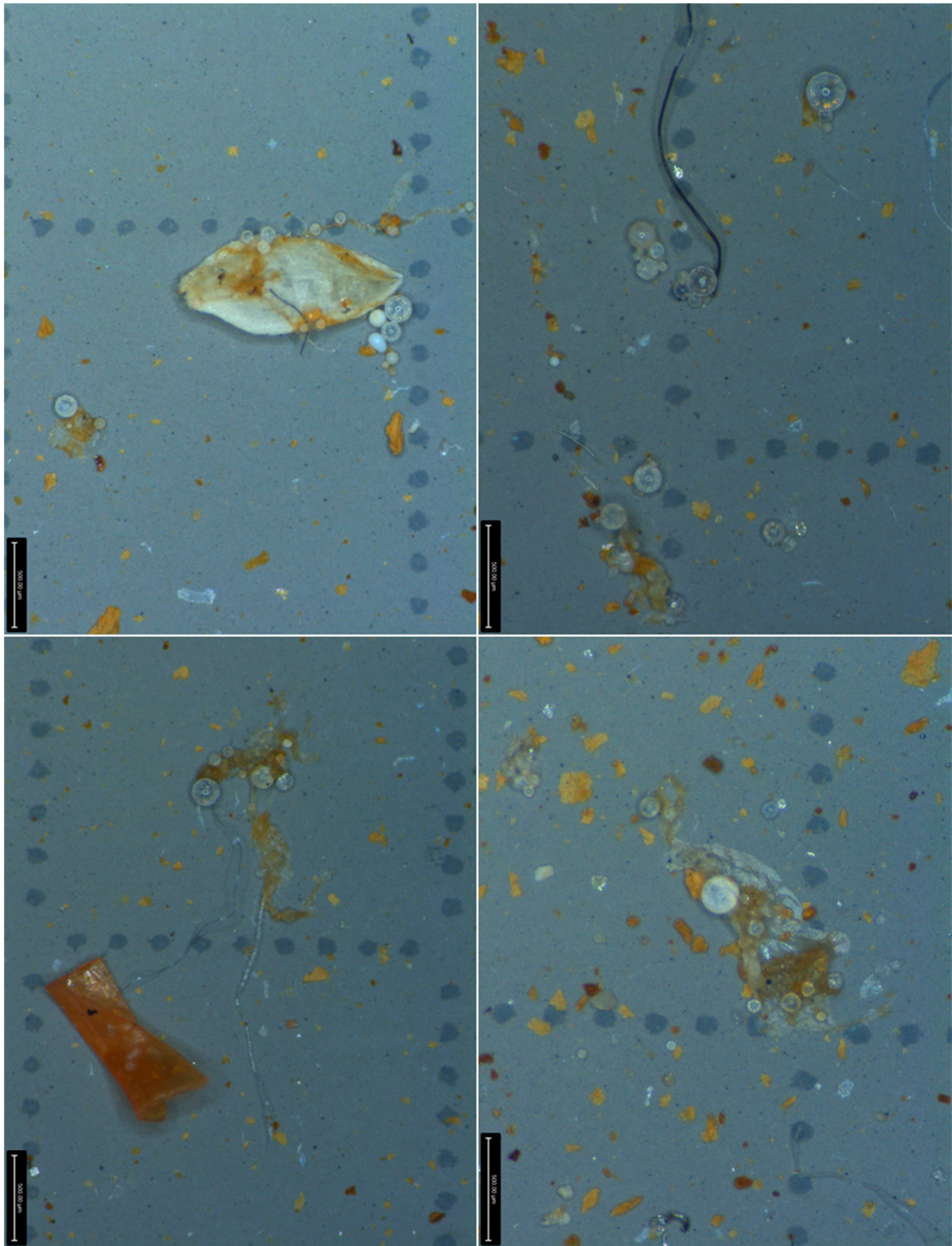
Figure S6: Microscope images of the sample of the WWTP effluent from 10.11.2021. This sample was highly contaminated with different sized microbeads.
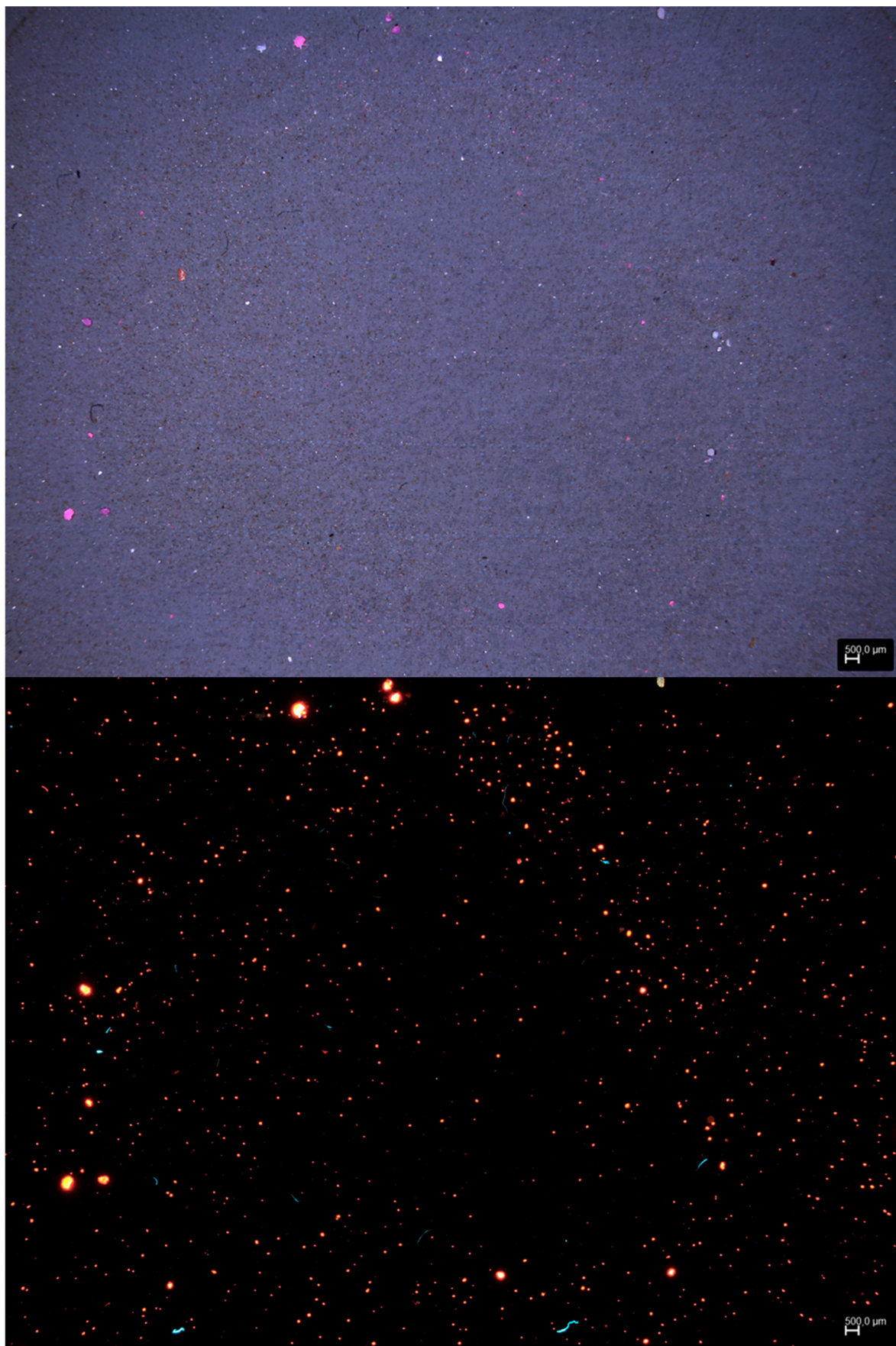
Figure S7: Microscope images of the sample of the WWTP effluent from 10.02.2022. Orange amd yellow fluorescent particles are microplastics, blue particles are cotton or cellulose based contamination enterning the sample in the laboratory. This sample was relatively highly contaminated with 102 microplastics / l
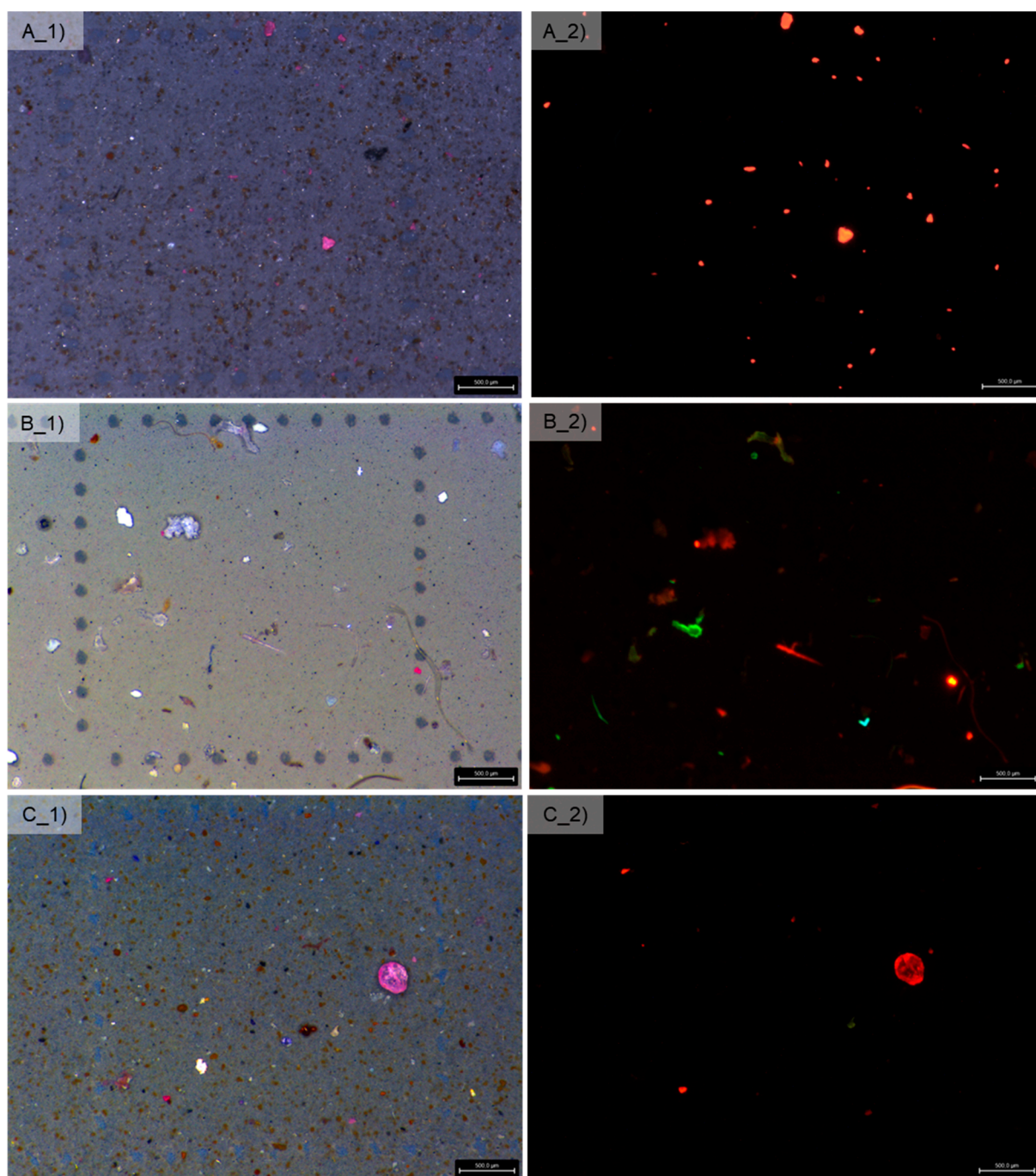
Figure S8: Microscope images of the processed and stained wastewater samples; A -10.03.2022 and B - 10.02.2022, C -03.03.2022; 1 - normal photos, 2 - fluorescent images. Orange amd yellow fluorescent particles are microplastics, blue particles are cotton or cellulose based contamination enterning the sample in the laboratory. The brown residues on the black membrane filters, visible in the fotos, are the residues of the orgnaic matter after the H_2O_2 treatment.
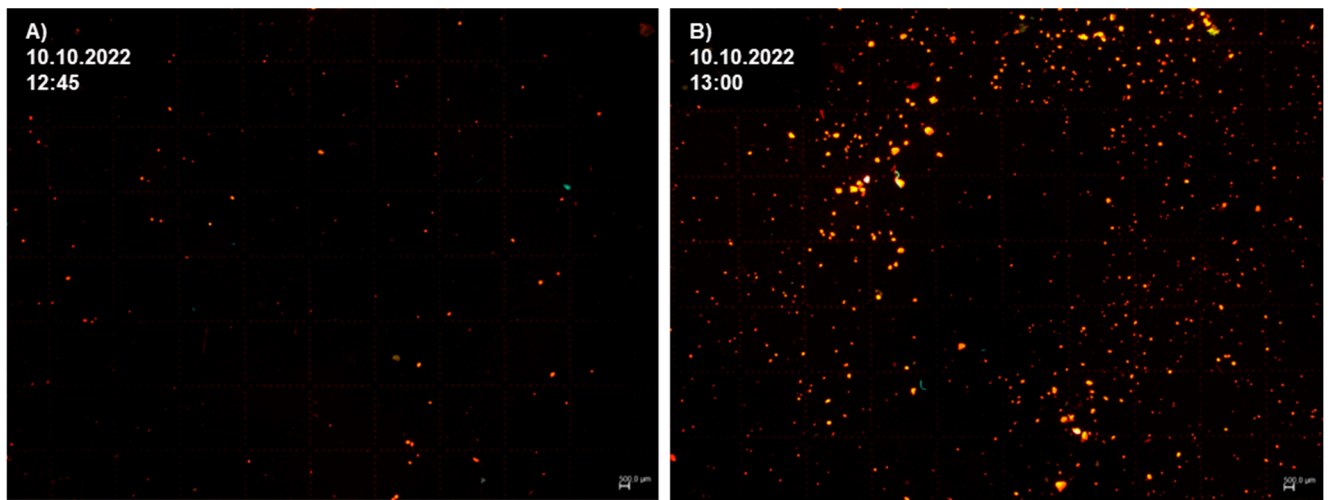
Figure S9: Fluorescent images of the of the processed and stained wastewater samples on 10.10.2022. The time between the two samples is 15 minutes. It can be clearly seen, that withing the 15 minutes, the contamination varies strongly. Sample A (12:45) has a contamination of 13 MP/l and sample B (13:00) 135 MP/l , which is 10x higher.
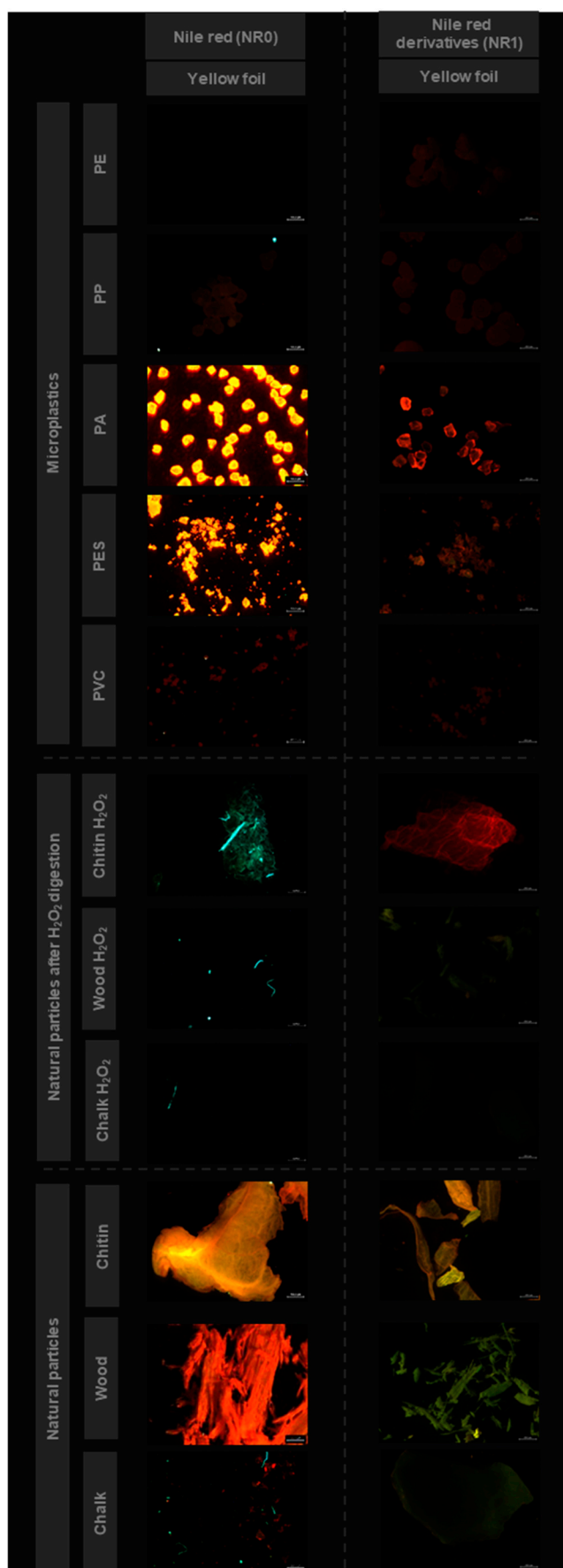
Figure S10: Fluorescent images of microplastics and natural particles stained with Nile red (NR0) and the new derivatives (NR1) taken with the low budget modification of the microscope Additionally, for the natural particles, the effect of the hydrogen peroxide treatment (see Section 2.4) is visualized.