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RESEARCH ARTICLE

Algae (*Raphidocelis subcapitata*) mitigate combined toxicity of microplastic and lead on *Ceriodaphnia dubia*

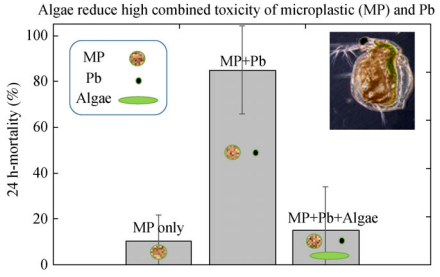
Xuesong Liu[1], Jianmin Wang (✉)[1,2]

1 Department of Civil, Architectural and Environmental Engineering, Missouri University of Science and Technology, Rolla, MO 65409, USA
2 The Center for Research in Energy and Environment (CREE), Missouri University of Science and Technology, Rolla, MO 65409, USA

HIGHLIGHTS

- Micro-plastics (MPs) significantly increase Pb toxicity.
- Algae reduce the combined toxicity of MP and Pb.
- The toxicity increase comes from high soluble Pb and MP-Pb uptake.
- The toxicity reduction might come from energy related pathway.

GRAPHIC ABSTRACT



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ABSTRACT

Microplastics (MPs) have been recognized as a new class of emerging contaminants in recent years. They not only directly impact aquatic organisms, but also indirectly impact these organisms by interacting with background toxins in the environment. Moreover, under realistic environmental conditions, algae, a natural food for aquatic organisms, may alter the toxicity pattern related to MPs. In this research, we first examined the toxicity of MPs alone, and their effect on the toxicity of lead (Pb) on *Ceriodaphnia dubia* (*C. dubia*), a model aquatic organism for toxicity survey. Then, we investigated the effect of algae on the combined toxicity of MPs and Pb. We observed that, MPs significantly increased Pb toxicity, which was related to the increase in soluble Pb concentration and the intake of Pb-loaded MPs, both of which increased the accumulation of Pb in *C. dubia*. The presence of algae mitigated the combined toxicity of MPs and Pb, although algae alone increased Pb accumulation. Therefore, the toxicity mitigation through algae uptake came from mechanisms other than Pb accumulation, which will need further investigation.

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1 Introduction

Plastics have been used in numerous consumer products. In 2017, the global plastic production exceeded 348 million tons (Plastic Europe, 2018). This mass production inevitably increased the release of plastics into the environment, which can break down to smaller sizes under various environmental conditions (Lambert et al., 2013). Microplastics (MPs) are ordinary plastic debris with diameters from 100 nm to 5 mm (Kazmiruk et al., 2018). Kazmiruk et al. (2018) found that most environmental MPs are present in fine sand levels (0.63–250 μm). In recent years, MPs have been recognized as a new class of emerging contaminants. Many studies have revealed that exposure to MPs can result in sub-lethal to lethal effects on organisms in the aquatic food web, such as algae (Zhang et al., 2017), crustacean (Kim et al., 2017), and fish (Chen et al., 2017). Other than lone MP toxicity, MPs have the potential to interact with large amounts of environmental pollutants, and pose additional toxic effect on aquatic organisms. Chen et al. (2017) demonstrated high combined toxicity of MPs and endocrine disrupting compounds (EDCs), also due to enhanced EDC accumulation through

✉ Corresponding author
E-mail: wangjia@mst.edu

MPs; Town et al. (2018) revealed that MPs increased heavy metal toxicity. MPs could also carry heavy metals to higher trophic levels, and pose health threats to humans.

Different levels and types of toxic metals exist in the aquatic environment, which can adversely impact aquatic organisms (Ab Latif Wani and Usmani, 2015). Recent studies have shown that the toxicity of toxic metals can be significantly enhanced by metal oxide-based nanoparticles, although these nanoparticles alone are considered non-toxic (Hu et al., 2012). Collectively, nanoparticles serve as a carrier of toxic metals to facilitate their accumulation and toxicity (Hu et al., 2012; Liu et al., 2019). MPs may act in the same way as conventional nanoparticles and increase the toxicity of background toxic metals. However, because the surface property of MPs is significantly different from that of the conventional metal oxide-based nanoparticles, the toxicity mechanism of MPs will be different from that of nanoparticles. For example, Johansen et al. (2018) illustrated that the accumulation of heavy metals on plastic particle might be dominated by surface biofilm. Town et al. (2018) demonstrated that the adsorption of metal ions on plastic would modify their speciation dynamic.

In the ecosystem, complex environmental conditions will greatly disturb the toxicity output. Hu et al. (2012) demonstrated that the combined toxicity of nano-TiO_2 and Pb was pH dependent. Jaikumar et al. (2018) reported that the thermal stress was altered the organism's sensitivity to toxin. These studies revealed the necessity to include environmental relevant conditions during toxicity evaluation. Algae commonly exist in all aquatic environment and can be ingested by aquatic organisms. Previous research has indicated that algae exhibit different results in toxicity studies (Luo et al., 2018; Liu et al., 2019). The interaction between algae and other toxins may affect the toxicity of these toxins through different pathways, such as accumulation, distribution, and bio-function (Luo et al., 2018). We previously discovered that algae could mitigate the combined toxicity of TiO_2 particles and lead (Pb) on *Ceriodaphnia dubia* (*C. dubia*) (Liu et al., 2019). A similar effect could also occur for the combined toxicity of MPs and toxic metals. The objectives of this research were to investigate the combined toxicity of MPs and Pb and the effect of algae, *Raphidocelis subcapitata,* on the combined toxicity of MPs and Pb.

2 Materials and methods

2.1 MPs, chemicals, and organisms

The carboxylate polystyrene MP (PS-COOH, d = 1.0 μm, density = 1.05 g/cm^3, 2.6% solid) was purchased from Polyscience Inc. (Warrington, PA, USA). The algae (*Raphidocelis subcapitata*) stock solution was purchased from ABS Inc. (Fort Collins, CO, USA) and used as received. The chemicals used in this research, including $CaSO_4 \cdot 2H_2O$ (98%), Na_2SeO_4 (99%), $NaHCO_3$ (100.2%, [Pb] < 5 mg/kg), $MgSO_4$ ([Pb] < 0.001%), KCl (99%), Pb $(NO_3)_2$, and trace metal grade nitric acid, were acquired from Fisher Scientific (Fair Lawn, New Jersey, USA). The Milli-Q (MQ) water (resistivity = 18.2 MΩ·cm) was used to prepare all the solutions.

The starter *C. dubia* was purchased from MBL Aquaculture (Sarasota, FL, USA) and continuously cultured in a laminar flow hood (SVC-6AX, Streamline® laboratory products, Fort Myers, FL, USA) in a temperature-controlled chamber at 25°C and a light to dark cycle of 16 h: 8 h. A moderate hardness water was prepared by following the EPA standard method and used as culture medium (EPA, 2002). The pH and hardness of the culture medium were 7.8±0.2 and 85±5 mg/L as $CaCO_3$, respectively. The laminar flow hood was used to eliminate any airborne particles that could negatively impact *C. dubia* during culturing, and an illuminating light was used to simulate the natural light cycles. The mass culture of *C. dubia* was carried out by following the EPA protocol (EPA, 2002).

2.2 Test solution preparation

The 1,000 mg/L Pb stock solution was prepared by dissolving $Pb(NO_3)_2$ into MQ water, which was then acidified to pH less than 4. A total of 10 series of test solutions were prepared for toxicity tests. The soluble Pb concentrations in some of these solutions were also measured, to determine the solubility of Pb under selected conditions (Pb alone, in the presence of algae and/or MP). Table 1 shows the composition of the solution matrices for all test solutions used in this research. All test solutions were contained in 125 mL glass bottles. They were prepared by diluting different volumes of stock solutions of Pb, algae, and/or MP, respectively, using the culture medium buffer, and mixed at 250 r/min and 25°C for 24 h in an incubator shaker. The algae concentration used in test solutions was 1.8×10^5 cell/mL, as recommended by EPA (EPA, 2002). The final volume of each solution was 70 mL. Glass bottles were used in this research to avoid any potential interference between MPs and plastic containers during the test (Zhang et al., 2017; Kim et al., 2017). All glass bottles were washed and soaked in 3% nitric acid for 12 h before use. After mixing, all test solutions, except the series #10, were directly used for toxicity tests. The series #10 test solutions were first filtered through a 0.22 μm nylon membrane filter to obtain particle-free filtrates, which were then used for the toxicity test. This test was used to determine the effect of the soluble fraction of the test solution on the toxicity, without the interference of any particles. For all tests, the final pH (after mixing for 24 h) was determined to be 7.8±0.2.

Table 1 Solution matrices for toxicity and solubility tests

Test type	Solution series	Solution composition
Toxicity	1	MP (0–200 mg/L)
	2	Pb (0–2,500 μg/L)
	3	Pb (0–2,500 μg/L)+ Algae (1.8×10^5 cell/mL)
	4	Pb (0–2,500 μg/L)+ MP (5 mg/L)
	5	Pb (0–2,500 μg/L)+ MP (10 mg/L)
	6	Pb (0–2,500 μg/L)+ MP (20 mg/L)
	7	Pb (0–2,500 μg/L)+ Algae (1.8×10^5 cell/mL)+ MP (5 mg/L)
	8	Pb (0–2,500 μg/L)+ Algae (1.8×10^5 cell/mL)+ MP (10 mg/L)
	9	Pb (0–2,500 μg/L)+ Algae (1.8×10^5 cell/mL)+ MP (20 mg/L)
	10	Pb (1,500–2,500 μg/L)+ MP (20 mg/L); filtrate
Solubility	2	Pb (0–2,500 μg/L)
	3	Pb (0–2,500 μg/L)+ Algae (1.8×10^5 cell/mL)
	6	Pb (0–2,500 μg/L)+ MP (20 mg/L)
	9	Pb (0–2,500 μg/L)+ Algae (1.8×10^5 cell/mL)+ MP (20 mg/L)

2.3 Toxicity test

The 24 h acute toxicity of Pb alone, MP alone, Pb + algae, Pb + MP, Pb + algae + MP, and the soluble fraction of the Pb + MP were determined, respectively, by following the EPA standard method (EPA, 2002). In brief, four replicate tests were conducted for each test solution. For each replicate test, five healthy neonates (aged 24 h or less) were transferred to a 25 mL glass reactor that contained 15 mL of test solution, and the 24 h mortality of these neonates was monitored. Before transferring to the test reactor, neonates were washed three times with the fresh culture medium buffer to remove food residual on the neonate surfaces. During the transfer, the fresh culture medium buffer carried to each test reactor was approximately 0.2 mL, which was negligible as compared to the 15 mL of the test solution. No additional foods or particles were added to the reactor during the test. After a 24 h testing period, the mortality of neonates was visually inspected. Control tests were also conducted using the culture medium, and the survival rate for all controls exceeded 90%.

The effect of algae and/or MP on Pb solubility was also determined by measuring the soluble Pb concentrations in selected test solutions, shown in Table 1. Ten milliliter of each test solution, after 24 h mixing, were collected and filtered through a 0.22 μm nylon membrane filter. The filtrate was acidified and determined for soluble Pb.

2.4 Accumulation test

Accumulation of Pb in *C. dubia* is a key factor on Pb toxicity. Ideally, the same *C. dubia* neonates used for a toxicity text should be used to determine Pb accumulation. However, because the fragile natural of neonates, *C. dubia* adults (7 day) were used for Pb accumulation test. This method was used in previous research (Liu et al., 2019). In brief, approximately 50 pre-washed adult *C. dubia* were used to determine the Pb accumulation for each exposure time in each type of test solutions. Two types of test solutions, Pb + MP and Pb + MP + algae, were prepared by following procedures in Section 2.2. The concentrations of Pb, MP, and algae, in corresponding test solutions, were 2,500 μg/L, 20 mg/L, and 1.8×10^5 cell/mL, respectively. The total accumulation time was 24 h, which was the same as toxicity test. After pre-selected exposure time, the post-treatment of *C. dubia*, e.g., pick and digest, followed the method in our previous publication (Liu et al., 2019). All Pb accumulation experiments were performed in duplicate.

2.5 Analytical method

A graphite furnace atomic absorbance spectrometer (GFAA) (Perking Elmer AAnalyst 600) was used to determine the soluble Pb concentration from different tests. The detection limit of the GFAA was 0.5 μg/L. The Pb adsorption on MPs was verified by X-ray photoelectron spectroscopy (XPS) (Kratos Axis 165). Dried particles of MPs, after 24 h of mixing with Pb, were analyzed through a survey scan with hybrid lens mode. To maintain an appropriate Pb peak strength, 5,000 μg/L of Pb were used in the XPS sample preparation. The images of *C. dubia* in different conditions were taken using phase contrast microscopy (Olympus CKX41SF). For each condition, at least 10 *C. dubia* were visually checked and the image of the most representative one was captured.

2.6 Statistical analysis

Data from toxicity tests were presented as mean±standard deviations. To determine whether the difference between various treatment groups was significant or not, a two-way analysis of variance (ANOVA) was conducted. The "initial Pb concentration" and "type of particle" were used as factors to analyze mortality outcomes between different treatment groups. Statistical significance was set at $p < 0.05$.

3 Results and discussion

3.1 Microplastic toxicity

C. dubia can ingest particles that range in size from 100 to 5,000 nm (Geller and Müller, 1981). Figure 1 shows that the test MPs (d = 1.0 μm) could be accumulated in the gut of *C. dubia* and caused 25% of the mortality at concentrations between 100 and 200 mg/L. No significant mortality was observed at MP concentrations of 50 mg/L and less. This concentration-depended MP toxicity was also observed by Ziajahromi et al. (2017) and Jaikumar et al. (2019) in both acute and chronic toxicity tests. Other than the concentration, the size of the plastic particles could also impact the toxicity, and this kind of impact was related to the distribution of particles within the organisms (Lee et al., 2019). In this research, we used plastic particles with a uniform size of 1.0 μm, which could also well disperse in the culture medium (Fig. S1 and Fig. S2). Although MPs of this size have low toxicity, they are the most commonly seen in the environment (Kazmiruk et al., 2018). Therefore, they were selected for this research. In addition to the concentration and size distribution, the surface property of plastic particles could also affect the toxicity pattern (Haegerbaeumer et al., 2019). Fig. S1b shows the surface morphology of the MPs used in this research. They have a smooth surface and non-porous structure. MPs could cause histological changes (e.g., abscission, disintegration, and thickening) that damage the tissue through physical contact with cell membrane (Wang et al., 2019). Consequently, these MPs have a small specific surface area to cause toxicity (Braakhuis et al., 2016). Furthermore, functional groups on the MPs affect the toxicity pattern. Kim et al. (2017) reported that plastic particles with carboxylate groups exhibited a higher toxicity than plain plastic, presumably due to the presence of functional groups that affect the binding capacity of MP in organisms. Therefore, the carboxylate groups on the surface of the MPs could contribute to the death of *C. dubia*. Collectively, the concentration, size distribution, and surface properties of plastic particles should be considered when evaluating their adverse effect on aquatic organisms.

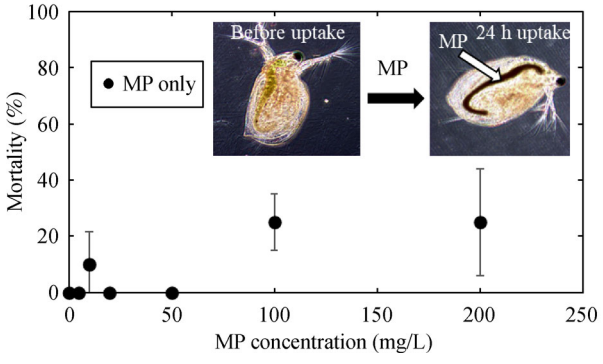

Fig. 1 The 24 h mortality of *C. dubia* in the presence of MPs. Photo was *C. dubia* from 24 h uptake with MP (20 mg/L). Standard deviation is represented by an error bar attached to each point (N = 4). The control test (without MP addition) exhibited a survival rate of greater than 90% (data not shown).

3.2 Effect of MPs on Pb toxicity

Figure 2 shows the effect of MPs on the toxicity of Pb. It was found that Pb alone up to 2,500 μg/L did not show significant toxicity on *C. dubia*, which is consistent with our previous research (Liu et al., 2019). This is because the Pb toxicity is related to its solubility in water (Erten-Unal et al., 1998). In the culture medium (moderate hardness water) or the natural water, Pb forms precipitation with different anions, such as hydroxide, carbonate, and sulfate (Escudero-García et al., 2013), resulting in a very low solubility.

Remarkably, Figure 2 shows that MP significantly increased Pb toxicity for all three MP concentrations used in this study (5, 10, and 20 mg/L) ($p < 0.05$), causing more than 85% mortality at the initial Pb concentration of 2,500 μg/L. In addition, a higher MP concentration resulted in a greater Pb toxicity (between any two MP concentrations, $p < 0.05$). As indicated in Fig. 1, MPs alone had negligible toxicity at these three concentrations, but they enhanced Pb toxicity by several times. The same phenomenon was also observed in MP with different heavy metals (Kim et al., 2017; Lee et al., 2019). Previously, we found that particles served as adsorbents to carry excessive amounts of toxic elements to organisms, resulting in an increase in toxicity (Liu et al., 2019). By the same token, MPs may also carry excessive amounts of Pb to *C. dubia* in this research. Other than excessive Pb accumulation, the deposition or adsorption of Pb on the MP surface could alter the surface property (Zhang et al., 2006), thereby elevating MP toxicity (Haegerbaeumer et al., 2019). As a result, the increase in the toxicity could come from the excessive Pb uptake and the change in MP surface property.

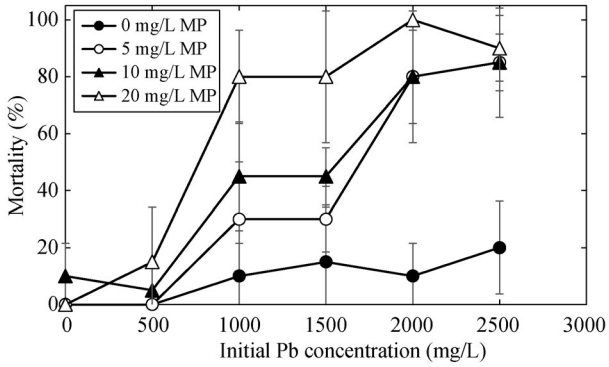

Fig. 2 The effect of MPs on the toxicity of Pb as indicated by the 24 h mortality of *C. dubia*. Standard deviation is represented by an error bar attached to each point ($N = 4$).

3.3 MP and Pb interaction: Soluble Pb concentration

Previous research has indicated that the soluble heavy metal concentration played a key role in metal toxicity (Erten-Unal et al., 1998; Hu et al., 2012). Figure 3(a) shows the effects of MPs on a soluble Pb concentration in the culture medium. It shows that the soluble Pb concentration was low when only Pb present in culture medium. The corresponding toxicity was also low (Fig. 2).

Figure 3(a) also indicates that MP significantly increased the soluble Pb concentration, contradicting to our initial assumption that MPs could serve as a Pb adsorbent to reduce the soluble Pb concentration. For example, when the initial Pb concentration is 2,500 μg/L, the presence of 20 mg/L MPs increased the soluble Pb concentration from 208 to 568 μg/L. Korshin et al. (2005) found that the natural organic matter in the solution could form a complex with Pb to increase the soluble Pb concentration. In this study, MPs significantly increased the total organic carbon (TOC) in the culture medium (Table S1). MPs could release carbon content when induced to the natural environment, due to the light-driven photochemistry and photo-dissolve (Yu et al., 2019). The TOC could form complexes with Pb and increase the soluble Pb concentration, similar to that reported by Turner and Holmes (2015). Unlike the inorganic Pb ion, organic Pb can easily dissolve in the lipid bilayer, and then pass through cell membrane of *C. dubia*, resulting in a greater toxicity (Ab Latif Wani and Usmani, 2015). Therefore, both the increased soluble Pb concentration and the formation of organic Pb complex could contribute to the increased toxicity.

3.4 MP and Pb interaction: Pb adsorption

Small particles can serve as transport vector for toxic metals (Liu et al., 2019). As a consequence, the adsorption capacity of toxic metals on particles is always the focus in nanoparticle toxicity study. Because Pb could form precipitates and MPs increased soluble Pb concentration in this study, we did not use the conventional subtraction method (the soluble concentration difference between the initial and final) to determine the adsorbed Pb on MPs. Instead, we used XPS to directly examine if the Pb was adsorbed on MPs. Figure 3(b) shows the XPS spectra of the MP surface after contacting with the Pb solution. The spectrum confirmed that Pb was present on the surface of the MP. The binding energy positions of ~18, ~137, ~142, ~412, and ~434 eV were assigned to a major Pb compound, according to the NIST X-ray Photoelectron Spectroscopy Database (NIST, 2012). Therefore, through MP and Pb interaction, MPs adsorbed some Pb on the surface. Apparently, the adsorbed Pb could be delivered to *C. dubia* through MP ingestion, resulting in a greater Pb accumulation. The binding between MPs and heavy metals are highly depended on the type of plastic and metals, but any complexes of metals will tend to dissociate in the acidic media (Town et al., 2018). Because the gut pH of *C. dubia* is typically in the range of 6.0–6.8, lower than culture medium (pH = 7.8) (Ebert, 2005), the adsorbed Pb could desorb from the MP surface within the gut, making more Pb available for tissue uptake. Pb could increase reactive oxidative species (ROS) production and/or replace

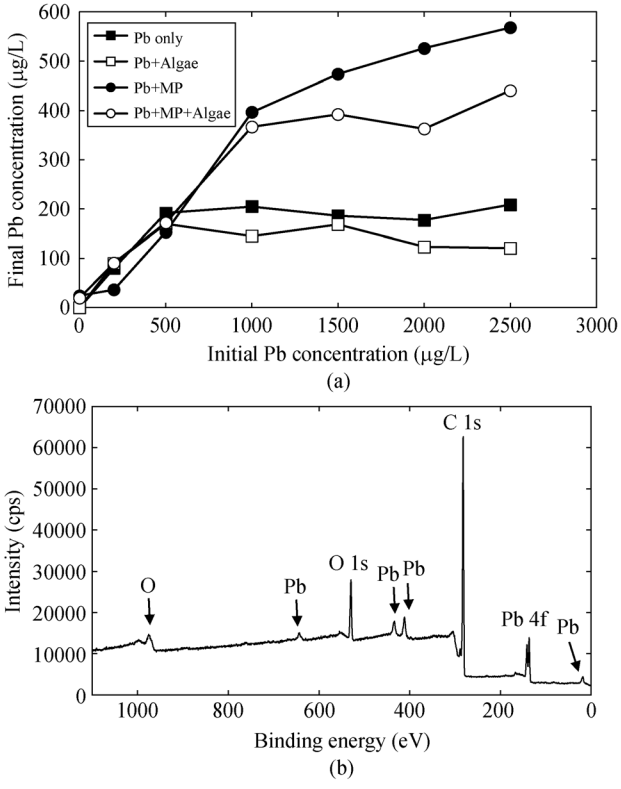

Fig. 3 MP and Pb interaction in the culture medium. (a) Soluble Pb concentration with and without different particles (MP = 20 mg/L; Algae = 1.8×10^5 cell/mL); (b) XPS survey spectra of MP surface after Pb adsorption (MP = 20 mg/L, Pb = 5,000 μg/L).

essential elements in enzyme to cause the death of organisms (Bray and Bettger, 1990; Ercal et al., 2001). In addition, as indicated earlier, toxic metal adsorption could change the surface properties of MPs and, therefore, induce toxicity. As a result, through the view of Pb adsorption, the excessive Pb uptake and the change in MP surface property led to the increase in the toxicity on *C. dubia*.

3.5 Soluble Pb toxicity

As indicated earlier, through the interaction between MP and Pb, Pb could be divided into MP adsorbed Pb and soluble Pb (Figs. 3(a) and 3(b)). The presence of MPs increased soluble Pb concentration. Obviously, this increased soluble Pb concentration could elevate the toxicity. In order to examine the toxicity contribution from this soluble Pb after contacting with MPs, we filtered the MP + Pb test solution through a 0.22 μm filter and used the filtrate to conduct the toxicity test. Figure 4 shows a comparison of the 24 h mortality of the original MP + Pb test solution and the filtrate that contains only soluble Pb. It appears that the soluble Pb contributed to less than 40% of the total mortality. For example, in the presence of 20 mg/L MP and 2,500 μg/L Pb, the overall toxicity resulted in 90% of the mortality. However, the filtrate of the test solution only resulted in 35% of the mortality. Apparently, MPs with the adsorbed Pb contributed 60% of the toxicity to the *C. dubia*. In addition, the synergistic effect of soluble Pb and MP might have also contributed to the overall toxicity.

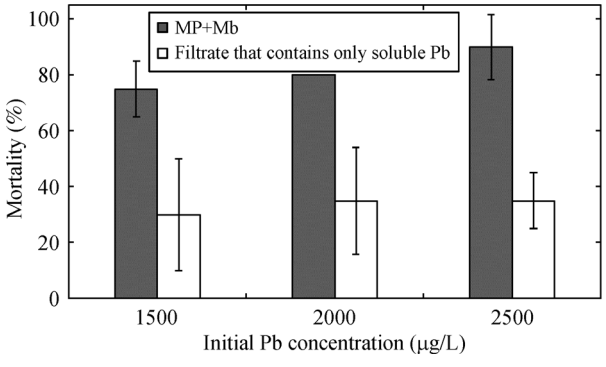

Fig. 4 The toxicity of original MP + Pb test solution and the filtrate from the original test solution. The MP = 20 mg/L in the original test solution. Filtrate was collected by passing through 0.22 μm filter. Standard deviation is represented by an error bar attached to each point (N = 4).

It is also interesting to note that the soluble Pb after MP and Pb interaction exhibited greater toxicity than Pb alone. For example, when total 2,500 μg/L of Pb in the culture medium, it resulted 20% mortality of *C. dubia* (Fig. 2). However, after contacting with MP (20 mg/L), the total soluble Pb concentration was 568 μg/L (Fig. 3a), and it resulted in 35% mortality. As discussed earlier, the form of metals, organic/inorganic and soluble/particulate, significantly affected the toxicity output (Erten-Unal et al., 1998; Ab Latif Wani and Usmani, 2015). Therefore, MP-induced change in the form of metals needs to be evaluated in the toxicity assessment.

3.6 Effect of algae on the combined toxicity of MP and Pb

As one of the most prevalent aquatic organisms, algae inevitably participate in the toxic process. Many studies have shown that algae could impact the toxicity of other contaminants through different mechanisms (Luo et al., 2018; Liu et al., 2019). Figure 5 indicates that algae significantly reduced the combined toxicity of MPs and Pb (for all three MP concentrations, $p < 0.05$). For instance, in the presence of 2,500 μg/L of Pb and 20 mg/L of MP, 1.8×10^5 cells/mL of algae reduced the mortality of *C. dubia* from 90% to 45%. Algae also reduced the mortality of *C. dubia* to less than 20% for the other two lower MP concentrations.

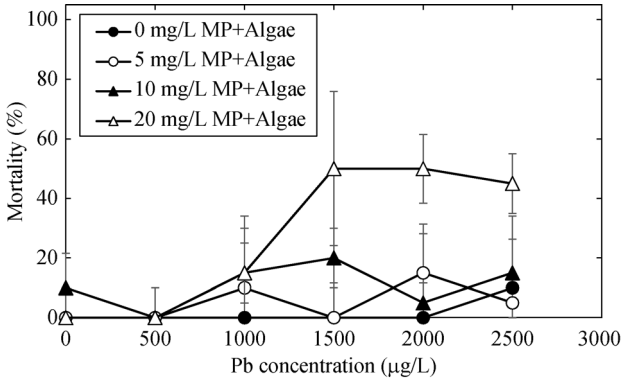

Fig. 5 The effect of 1.8 10^5 cells/mL algae on the combined toxicity of MP and Pb indicated by the 24 h mortality of *C. dubia*. Standard deviation is represented by an error bar attached to each point (N = 4).

As indicated in Fig. 3(a), algae could adsorb Pb and reduce the soluble Pb concentration, in the absence and presence of MPs. Our previous research found that, if only algae present with Pb, the ingestion of algae could increase metal accumulation in *C. dubia*, but the energy produced from the consumption of algae could mitigate this toxicity (Liu et al., 2019). Our previous research also revealed that algae actually reduced the combined toxicity of nano-TiO_2 and Pb on *C. dubia* (Liu et al., 2019). This is because that, as a food source, algae could provide energy for organisms to boost antioxidant synthesis or directly serve as an antioxidant to mitigate the toxicity (Romay et al., 1998; Poljsak et al., 2013). On the other hand, because algae exhibited lower metal adsorption capacity than nanoparticles, they could occupy some gut space and reduce the

uptake of nanoparticles that are loaded with toxic metals in *C. dubia* (Liu et al., 2019). Both factors could contribute to the combined toxicity reduction in this research.

3.7 MP accumulation in *C. dubia*

The accumulation of particles in organisms is one focus when investigating particle related toxicity. Prior to MP accumulation test, the *C. dubia* were fed with algae. They were then placed into the test solution (MP + Pb) that did not contain algae. Figure 6 shows photos taken at different time periods showing MP accumulation results. It shows that MPs filled up the entire gut of *C. dubia* in 0.25–0.5 h. Within this time period, *C. dubia* could actively uptake particles through the mouthparts, and accumulate them in the gut. However, it is interesting to note that MP accumulation in the gut of *C. dubia* was significantly reduced after 0.5 h (Fig. 7), while most algae accumulated at the beginning of this test still stayed in the gut. This test suggests that, after 0.5 h of culturing, the *C. dubia* reduces the uptake of MPs from the test solution.

The MP accumulation pattern in this study may be caused by a change in the feeding behavior of *C. dubia*. As a filter feeder organism, *C. dubia* use an appendage to filter water and particles (Geller and Müller, 1981). The appendage beat frequency could be significantly affected by toxic element exposure (Porter, 1977). Therefore, *C. dubia* could selectively ingest some particles, on the basis of size, shape, and texture (Porter, 1977), and also reject particles, even food, from their mouthparts based on the taste and/or toxicity (Porter, 1977). Figure S3 shows that *C. dubia* exhibited a solid accumulation of MPs in the gut through the entire exposure period, if there is no Pb present in the test solution. As a result, the interaction between MP and Pb reduced MP ingestion by *C. dubia*.

Figure S4 shows the MP accumulation results in the medium that contained MPs, Pb, and algae. Although *C. dubia* may ingest some MPs which attach on the surface of

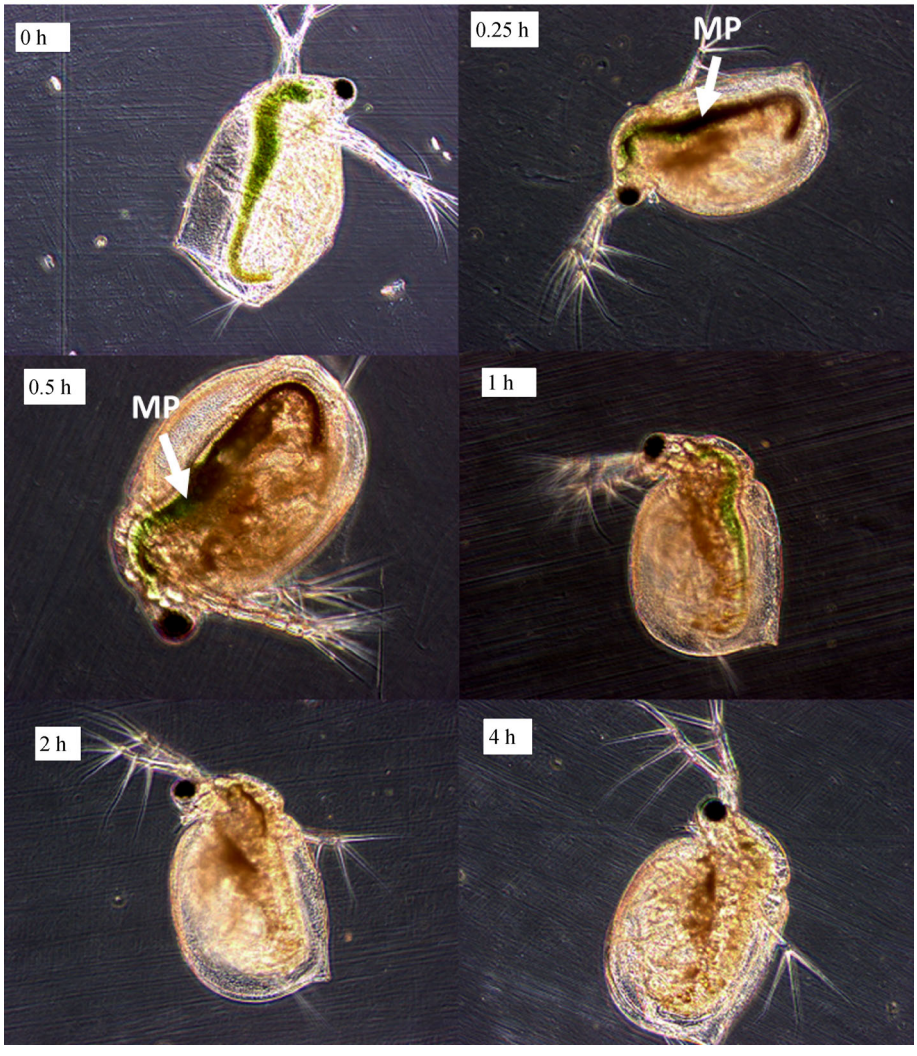

Fig. 6 MP accumulation in *C. dubia* in Pb solution. Conditions of the exposure medium: [Pb] = 2,500 μg/L; MP = 20 mg/L. Photos were *C. dubia* from 0, 0.25, 0.5, 1, 2, 4 h of exposure.

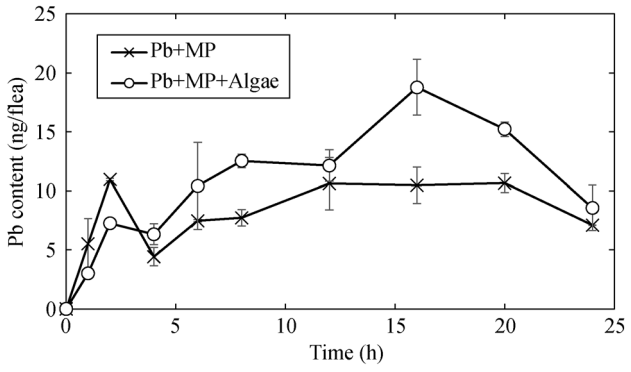

Fig. 7 Pb accumulation in *C. dubia* body as effects of MP, with and without algae. Conditions of the exposure medium: [Pb] = 2,500 μg/L; MP = 20 mg/L; Algae = 1.8×10^5 cell/mL. * $p < 0.05$ compared to Pb content at 2 h and 4 h for both Pb + MP and Pb + MP + Algae groups. Each point represents the average value of data ($N = 2$), error bar attached to each point represents the range of data.

algae, the MP accumulation pattern was similar to *C. dubia* exposed to MP + Pb, which was significantly reduced after 0.5 h. As discussed earlier, MP gut accumulation is the most important aspect in toxicity studies. Because the presence of algae did not change the MP accumulation pattern in *C. dubia* (Fig. 6 and Fig. S4), the presence of algae might not reduce the combined toxicity of MP and Pb through altering the MP accumulation. We also observed that, in the presence of algae, *C. dubia* continuously accumulated algae in the gut, although MPs ingestion was reduced.

3.8 Pb accumulation in *C. dubia*

The accumulation of Pb in *C. dubia* directly impacts its toxicity. In the presence of MPs, Pb has been divided into two portions: the adsorbed Pb and the soluble Pb (Figs. 3 (a) and 3(b)). Therefore, the accumulation of MPs would increase the accumulation of the adsorbed Pb in *C. dubia*. As shown in Fig. 6, *C. dubia* could uptake MPs at the beginning of exposure, while the accumulation of MP was significantly reduced after 0.5 h of exposure. Therefore, the corresponding accumulation of MP-adsorbed Pb would initially increase and then decrease after 0.5 h. The soluble Pb accumulation would consistently increase, due to the relatively stable soluble Pb concentration. Collectively, the overall Pb accumulation in *C. dubia* would increase at the beginning of the exposure to MP + Pb. After this initial increase, the Pb accumulation in *C. dubia* might decrease, due to the depuration of MPs, and then gradually increase, caused by a consistent soluble Pb concentration.

Figure 7 shows the Pb accumulation in the presence of MPs, with and without algae. Our previous research indicated that the net accumulation of Pb in *C. dubia* was approximate 1 ng/flea, if 2,500 μg/L of Pb was present in the culture medium, without any other particles (Liu et al., 2019). This value was significantly lower than that in the presence of MPs. Evidently, MPs significantly increased Pb accumulation in *C. dubia*. Results also indicated that, in the first 2 h, *C. dubia* rapidly accumulated Pb. Then, the Pb content in the treatment group of Pb + MP decreased significantly (for Pb content at 2 h and 4 h, $p < 0.05$). The Pb content gradually increased again after 4 h. The Pb content curve lagged the MP accumulation information (Fig. 6), and this lag could be caused by the retardation effect. MPs carried adsorbed Pb into the gut of *C. dubia*, and released some of it due to the reduced gut pH (Ebert, 2005). Therefore, more soluble Pb was available for tissue uptake. Gillis et al. (2005) found that the removal of toxic metals from the gut of a water flea would take a longer time than the removal of particles. Therefore, we observed that the Pb content decreased after 2 h of exposure, rather than 0.5 h after the maximum amount of MPs was accumulated. At the end of the accumulation process, the Pb content decreased again. At this point we also observed a dead *C. dubia* with a broken body structure. It is possible that the *C. dubia* have lost cell membrane integrity at the end of the test, resulting in the release of cytoplasmic content, include accumulated Pb (Elmore, 2007).

Figure 7 also shows the algae impact on Pb accumulation. The Pb accumulation pattern was similar to that of Pb + MP. It was found that, in the first 2 h, algae reduced Pb accumulation in *C. dubia*. The SEM image shows that algae (*Raphidocelis*) and MPs have a comparable size range (1–10 μm) (Fig. S1c). Therefore, the ingested algae occupied some gut space to reduce MP uptake, thereby reducing MP adsorbed Pb accumulation. Our previous research also observed the same phenomenon when algae were co-existed with nano-TiO_2 and Pb (Liu et al., 2019). However, after 2 h, the Pb content in the algae group exceeded that without algae. This Pb accumulation may be caused by selective uptake algae, which carried adsorbed Pb to the gut of *C. dubia*. Consequently, algae could induce more Pb into *C. dubia* to increase Pb accumulation (Liu et al., 2019). As indicated earlier, *C. dubia* could selectively uptake some particles (Porter, 1977). As indicated in Fig. 6, for the treatment group of Pb + MP, no algae were observed after 2 h of exposure. In contrast, for the treatment group of Pb + MP + algae, *C. dubia* could continuously accumulate algae after 2 h of exposure (Fig. S4). Therefore, in the presence of algae, *C. dubia* rejected some MP after the initial accumulation, but continuously ingested algae alone with the adsorbed Pb during the entire accumulation period, thereby accumulated more Pb than those without algae. Therefore, the reduction of toxicity in the presence of algae was not from the accumulated mass of Pb.

The 24 h mortality of Pb + MP and Pb + MP + algae were 90% and 45%, respectively (Figs. 2 and 5). However, the presence of algae increased Pb content in *C. dubia*. A similar finding was reported in the absence of MPs (Liu

et al., 2019). This could be caused by the biological function of algae. The major toxicity mechanism of toxic metals is ROS production (Ercal et al., 2001). Organisms could produce antioxidants to neutralize ROS, or increase metal binding protein to immobilize metal ions. The production of antioxidants, such as glutathione and superoxide dismutase, and metal binding protein, such as metallothionein, were energy related (Moltedo et al., 2000; Poljsak et al., 2013). Algae could produce needed energy through the metabolic process and, therefore, reduce toxicity. In addition, some algae may also contain a natural antioxidant (Romay et al., 1998). Thus, algae could also directly serve as an antioxidant to reduce toxicity. Collectively, the toxicity mitigation caused by algae was likely from the energy related pathways, rather than the mass of Pb accumulation.

4 Conclusions

In this research we observed that MPs alone had low toxicity on *C. dubia*. However, MPs could interact with Pb and significantly enhance the Pb toxicity through increasing soluble Pb concentration and the uptake of Pb-loaded MPs. Importantly, we found that algae, a natural food for aquatic organisms, could mitigate the combined toxicity of MPs and Pb. In the presence of 20 mg/L of MPs and 2,500 μg/L Pb, 1.8×10^5 cells/mL of algae reduced the mortality of *C. dubia* from 90% to 45%, although algae increased Pb accumulation in *C. dubia*. The reduction in the toxicity might be related to the energy and antioxidant related pathways which need further investigation. Importantly, this research indicated that when evaluating the toxic effect of particles, environmental factors must be included during the investigation.

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References

Ab Latif Wani, Ara A, Usmani J A (2015). Lead toxicity: A review. Interdisciplinary Toxicology, 8(2): 55–64

Braakhuis H M, Cassee F R, Fokkens P H, de La Fonteyne L J, Oomen A G, Krystek P, De Jong W H, Van Loveren H, Park M V (2016). Identification of the appropriate dose metric for pulmonary inflammation of silver nanoparticles in an inhalation toxicity study. Nanotoxicology, 10(1): 63–73

Bray T M, Bettger W J (1990). The physiological role of zinc as an antioxidant. Free Radical Biology & Medicine, 8(3): 281–291

Chen Q, Gundlach M, Yang S, Jiang J, Velki M, Yin D, Hollert H (2017). Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. Science of the Total Environment, 584–585: 1022–1031

Ebert D P (2005). Ecology, epidemiology, and evolution of parasitism in Daphnia. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information, [2005] ©2005

Elmore S (2007). Apoptosis: A review of programmed cell death. Toxicologic Pathology, 35(4): 495–516

EPA (2002). Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Washington DC: U.S. Environmental Protection Agency

Ercal N, Gurer-Orhan H, Aykin-Burns N (2001). Toxic metals and oxidative stress part I: Mechanisms involved in metal-induced oxidative damage. Current Topics in Medicinal Chemistry, 1(6): 529–539

Erten-Unal M, Wixson B G, Gale N, Pitt J L (1998). Evaluation of toxicity, bioavailability and speciation of lead, zinc and cadmium in mine/mill wastewaters. Chemical Speciation and Bioavailability, 10 (2): 37–46

Escudero-García R, Espinoza-Estrada E, Tavera F (2013). Precipitation of lead species in a Pb–H_2O system. Research Journal of Recent Sciences, 9(4): 1–8

Europe P (2018). Plastics-the facts 2018: An analysis of European latest plastics production, demand and waste data. Plastic Europe: 1–60

Geller W, Müller H (1981). The filtration apparatus of *Cladocera*: Filter mesh-sizes and their implications on food selectivity. Oecologia, 49 (3): 316–321

Gillis P, Chow-Fraser P, Ranville J, Ross P, Wood C (2005).Daphnia need to be gut-cleared too: The effect of exposure to and ingestion of metal-contaminated sediment on the gut-clearance patterns of *D. magna*. Aquatic Toxicology (Amsterdam, Netherlands), 71(2): 143–154

Haegerbaeumer A, Mueller M T, Fueser H, Traunspurger W (2019). Impacts of micro-and nano-sized plastic particles on benthic invertebrates: A literature review and gap analysis. Frontiers in Environmental Science, 7: e17

Hu J, Wang D, Wang J, Wang J (2012). Toxicity of lead on *Ceriodaphnia dubia* in the presence of nano-CeO_2 and nano-TiO_2. Chemosphere, 89(5): 536–541

Jaikumar G, Baas J, Brun N R, Vijver M G, Bosker T (2018). Acute sensitivity of three *Cladoceran* species to different types of microplastics in combination with thermal stress. Environmental Pollution, 239: 733–740

Jaikumar G, Brun N R, Vijver M G, Bosker T (2019). Reproductive toxicity of primary and secondary microplastics to three *Cladocerans* during chronic exposure. Environmental Pollution, 249: 638–646

Johansen M P, Prentice E, Cresswell T, Howell N (2018). Initial data on adsorption of Cs and Sr to the surfaces of microplastics with biofilm. Journal of Environmental Radioactivity, 190–191: 130–133

Kazmiruk T, Kazmiruk V, Bendell L (2018). Abundance and distribution of microplastics within surface sediments of a key shellfish growing region of Canada. PLoS One, 13(5): e0196005

Kim D, Chae Y, An Y J (2017). Mixture toxicity of nickel and microplastics with different functional groups on *Daphnia magna*. Environmental Science & Technology, 51(21): 12852–12858

Korshin G V, Ferguson J F, Lancaster A N (2005). Influence of natural organic matter on the morphology of corroding lead surfaces and behavior of lead-containing particles. Water Research, 39(5): 811–818

Lambert S, Sinclair C J, Bradley E L, Boxall A B A (2013). Effects of environmental conditions on latex degradation in aquatic systems. Science of the Total Environment, 447: 225–234

Lee W S, Cho H J, Kim E, Huh Y H, Kim H J, Kim B, Kang T, Lee J S, Jeong J (2019). Bioaccumulation of polystyrene nanoplastics and their effect on the toxicity of Au ions in zebrafish embryos. Nanoscale, 11(7): 3173–3185

Liu X, Wang J, Huang Y W, Kong T (2019). Algae (*Raphidocelis*) reduce combined toxicity of nano-TiO_2 and lead on *C. dubia.* Science of the Total Environment, 686: 246–253

Luo Z, Wang Z, Yan Y, Li J, Yan C, Xing B (2018). Titanium dioxide nanoparticles enhance inorganic arsenic bioavailability and methylation in two freshwater algae species. Environmental Pollution, 238: 631–637

Moltedo O, Verde C, Capasso A, Parisi E, Remondelli P, Bonatti S, Alvarez-Hernandez X, Glass J, Alvino C G, Leone A (2000). Zinc transport and metallothionein secretion in the intestinal human cell line Caco-2. Journal of Biological Chemistry, 275(41): 31819–31825

NIST (2012). NIST X-ray Photoelectron Spectroscopy Database, Version 4.1. Gaithersburg: National Institute of Standards and Technology

Poljsak B, Šuput D, Milisav I (2013). Achieving the balance between ROS and antioxidants: When to use the synthetic antioxidants. Oxidative Medicine and Cellular Longevity, 2013: e956792

Porter K G (1977). The plant-animal interface in freshwater ecosystems: Microscopic grazers feed differentially on planktonic algae and can influence their community structure and succession in ways that are analogous to the effects of herbivores on terrestrial plant communities. American Scientist, 65(2): 159–170

Romay C, Armesto J, Remirez D, Gonzalez R, Ledon N, Garcia I (1998). Antioxidant and anti-inflammatory properties of C-phycocyanin from blue-green algae. Inflammation Research, 47(1): 36–41

Town R M, Van Leeuwen H P, Blust R (2018). Biochemodynamic features of metal ions bound by micro-and nanoplastics in aquatic media. Frontiers in Chemistry, 6: e627

Turner A, Holmes L A (2015). Adsorption of trace metals by microplastic pellets in fresh water. Environmental Chemistry, 12 (5): 600–610

Wang J, Li Y, Lu L, Zheng M, Zhang X, Tian H, Wang W, Ru S (2019). Polystyrene microplastics cause tissue damages, sex-specific reproductive disruption and transgenerational effects in marine medaka (*Oryzias melastigma*). Environmental Pollution, 254: 113024

Yu F, Yang C, Zhu Z, Bai X, Ma J (2019). Adsorption behavior of organic pollutants and metals on micro/nanoplastics in the aquatic environment. Science of the Total Environment, 694: 133643

Zhang C, Chen X, Wang J, Tan L (2017). Toxic effects of microplastic on marine microalgae *Skeletonema costatum*: Interactions between microplastic and algae. Environmental Pollution, 220: 1282–1288

Zhang R, Zhang D, Mao H, Song W, Gao G, Liu F (2006). Preparation and characterization of Ag/AgO nanoshells on carboxylated polystyrene latex particles. Journal of Materials Research, 21(2): 349–354

Ziajahromi S, Kumar A, Neale P A, Leusch F D (2017). Impact of microplastic beads and fibers on waterflea (*Ceriodaphnia dubia*) survival, growth, and reproduction: Implications of single and mixture exposures. Environmental Science & Technology, 51(22), 13397–13406