The fate and toxicity of Pb-based perovskite nanoparticles on soil bacterial community: Impacts of pH, humic acid, and divalent cations

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HIGHLIGHTS

- The fate and toxicity of PbPNPs are influenced by pH, HA and divalent cation content.
- Increasing pH promoted PbPNPs dispersion but decreased Pb-ion release and toxicity.
- The surface coating by HA increased PbPNPs stability but decreased bioavailability.
- The divalent cations enhanced the PbPNPs aggregation and increased toxicity.
- The divalent cations concentration drives the fate and toxicity when coexist with HA.

ARTICLE INFO

ABSTRACT

Pb-based perovskite nanoparticles (PbPNPs) are amongst others used within highly efficient solar cells. PbPNPs can be released into the environment during their production, recycling or waste processing. In this study we investigated the fate and toxicity of PbPNPs on soil bacterial community under simulated natural environmental conditions across a range of pH, humic acid, and divalent cation concentrations. Increasing pH decreased PbPNPs-particle aggregation as well as Pb-ion release. The presence of only humic acid (HA) prevented the aggregation of PbPNPs-particles, whereas the presence of only divalent cations promoted the aggregation of PbPNPs-particles. The amount of Pb-ions released from the PbPNPs-particles was reduced in the presence of either HA or the divalent cations. Results of toxicity testing of PbPNPs by determining the metabolic potential of a bacterial community indicated that increasing pH alleviated particle toxicity. The presence of only HA reduced the toxicity of PbPNPs, while the presence of only divalent cations enhanced the particle toxicity. The coexistence of HA and divalent cations enhanced PbPNPs aggregation and reduced toxicity, with both Pb-ions and the interaction between the PbPNPs-particles and bacterial cells contributing to the toxic effects. Our study emphasized that environmental conditions play important roles that influencing the fate and toxicity of PbPNPs.

1. Introduction

Perovskite nanoparticles have become very popular in development of solar cell technology over the last few years due to their high-power conversion efficiency (Lee et al., 2012). The most commonly studied perovskite solar cell materials are methyl-ammonium metal trihalides ($CH_3NH_3AX_3$, where A is a metal atom, and X is a halogen atom such as iodine, bromine or chlorine) (Eames et al., 2015). The most suited metal cation for use in highly efficient perovskite solar cells is lead (Pb), and especially for-mamidinum lead trihalide ($H_2NCHNH_2PbX_3$) has shown great promise (Eperon et al., 2014). Upon disintegration of the unit cell,

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Pb-containing compounds can enter the environment during solar cell production, handling, use, and in the waste phase of the cells (Babayigit et al., 2016). This gives rise to concerns about the potential environmental risk of Pb-based perovskite nanoparticles (PbPNPs) (Hailegnaw et al., 2015).

Once released into the environment, nanoparticles (NPs) can undergo various processes like aggregation, sedimentation, and dissolution, which jointly determine the fate of NPs (Babayigit et al., 2016). These processes are impacted by environmental factors such as pH, ionic strength, and humic acid (Cupi et al., 2015). It is known that pH influences the surface charge of NPs, which subsequently affects the reactivity and stability (Yin et al., 2014). The adsorption of natural compounds like humic acid to the surface of NPs can also affect their aggregation (Omar et al., 2014). Within natural waters, electrolytes are abundant, especially divalent cations like Ca^{2+} and Mg^{2+}, and these too are known to affect the aggregation of NPs (El Badawy et al., 2012). Nonetheless, little is known about how these environmental factors affect the fate of PbPNPs.

The increasing application of PbPNPs runs a high risk of release into the soil and sediments through wastewater effluents, runoff and deposition (Hailegnaw et al., 2015). Therefore, the natural water chemistry could affect the transport of PbPNPs as well as the toxicity to soil microorganisms that play key roles in soil biogeochemical processes. The toxicity of NPs can be changed by the fate modifying processes in the natural environment, as dependent on a plethora of variables (i.e. pH, natural organic matter, electrolyte concentration) (Xiao et al., 2018). In toxicity testing of PbPNP suspensions it is important to explicitly consider the key attribute of PbPNPs-particles as well as the released Pb-ions contributing to the overall toxicity of PbPNPs suspensions (Babayigit et al., 2016). Our previous study found that PbPNPs significantly affect the metabolic functioning of a soil bacterial community with Pb-ions mainly driving the toxicity (Zhai et al., 2017). However, the toxicity of NPs on bacterial communities was found to strongly depend on environmental conditions such as organic matter content (Simonin et al., 2015), pH (Read et al., 2016) and ionic strength (De Souza et al., 2019). Whether different environmental exposure conditions could change the toxicity as well as the mode of action of PbPNPs on a soil bacterial community remains unclear.

The aim of this study was to investigate: 1) the influence of pH, humic acid content and divalent cations concentrations on the fate of PbPNPs, 2) the bacterial toxicity of PbPNPs under these different exposure conditions. The hypothesis of our study is that the fate and toxicity of PbPNPs depend on different exposure conditions. To characterize the PbPNPs fate, the zeta potential and the hydrodynamic diameter of PbPNPs-particles, as well as the Pb ion release profiles were quantified at different exposure conditions. Responses were assessed by determining the metabolic potential of the bacterial community upon changing exposure conditions.

2. Materials and methods

2.1. Perovskites

A Pb-based perovskite ($CHNHNH_3PbI_3$, PbPNP) was obtained from Ecole Polytechnique Federale de Lausanne (Lausanne, Switzerland, 99.5% purity nanopowder, hexagon-shape). The characteristics of this PbPNP have been provided in our previous study (Zhai et al., 2017). The preparation of PbPNPs suspensions was conducted following the Risk Assessment of Engineered Nanoparticles protocol (Jacobsen et al., 2010). Suspensions of the PbPNP (1 mg/L) were prepared by dispersing PbPNPs in MilliQ water, after which the suspensions were sonicated at 4 °C at 38 ± 10 KHz for 16 min (Zhai et al., 2017).

2.2. Soil extracts characterization

A bacterial community was extracted from a non-polluted soil, which was collected from the top 15 cm of a soil dominated by deciduous trees (52°07′06.7″N 5°11′23.1″E, The Netherlands). Soils were sieved through an 8 mm sieve and stored at 4 °C (Zhai et al., 2016, 2017). The soil used had a loamy texture with pH of 6.2 and water holding capacity of 18.4% of the dry soil weight. Soil samples were first diluted 10 times with Bis-Tris buffer (Sigma-Aldrich B9754), centrifuged at 1500 rpm for 10 min. Afterwards the supernatant was filtered through a syringe filter with 0.02 μm pore diameter (Anotop 25, Whatman), and further diluted 5 times to obtain the soil extract that contained the bacterial community (Rutgers et al., 2016). The soil bacterial community was mainly dominated by *Burkholderia*, *Pseudomonas*, *Ochrobactrum*, *Staphylococcus*, and *Pseudomonas*. The dissolved organic carbon (DOC) content in the soil extract was 8.2 mg C/L, measured by means of a TOC analyzer (TOC-VCPH, Shimadzu Corporation). The concentrations of Ca^{2+} and Mg^{2+} in the soil extract were 3.6 and 0.8 mg/L, respectively, measured using Atomic Absorption Spectroscopy (AAS; PerkinElmer1100 B).

2.3. Experimental design

Eighteen suspensions of 1 mg/L of PbPNPs were prepared in triplicate in the bacterial community extract in polycarbonate bottles (54 bottles in total) in order to systematically evaluate changes in the properties of the PbPNPs under different levels of pH, humic acid content and divalent cations concentrations as a function of time (Table S1).

2.4. Fate assessment of PbPNPs

To investigate the effect of pH on the fate of PbPNPs, 3 groups of suspensions of 1 mg/L of PbPNPs were prepared in triplicate at different pH values: pH = 5.0, pH = 7.0 and pH = 8.5, which were pH-adjusted by 0.1 M NaOH or 0.1 M HCl. The pH of each suspension was monitored constantly throughout the experiment.

A sodium salt of humic acid (HA) was obtained from Sigma-Aldrich (Zwijndrecht, Netherlands) and used as a model natural organic matter in this study. A stock solution (400 mg C/L) was prepared by dissolving HA in milliQ water for 24 h and stored at 4 °C prior to experimental use. HA solutions were prepared by diluting the ready-made stock solution with milli-Q water and stirring overnight (12 h). The entire volumetric flask was covered up with aluminum foil to prevent exposure to light. The experiment was continued by adding HA at concentrations of 10, 25 and 50 mg C/L at pH = 7.0 into 3 groups of suspensions of 1 mg/L of PbPNPs with three replicates, representing low, medium and high DOC levels (Xiao et al., 2018).

$CaCl_2$ and $MgSO_4$ (Sigma-Aldrich) were selected to evaluate the effect of divalent cations concentrations on the aggregation and dissolution of PbPNPs. The experiment was continued by adding Ca^{2+}/Mg^{2+} at a molar ratio of 4:1. This ratio was chosen to stimulate natural conditions (Abdolahpur Monikh et al., 2018), with total cation concentrations of 1, 2.5 and 10 mM at each HA concentration (0, 10, 25 and 50 mg C/L) at pH = 7 into 12 groups of suspensions of 1 mg/L of PbPNPs with three replicates, representing low, medium and high ionic strength (Arenas-Lago et al., 2019).

In each of the experiments in which either pH or of HA content or divalent cations concentrations were varied, the exposure time was fixed at 300 min. During the exposure, 5 mL of the supernatant of the PbPNPs suspensions were sampled at 5, 20, 40, 60, 120 and 300 min to determine the total Pb concentration in suspension. To quantify the amount of Pb-ions present in the water column due to

release from PbPNPs during exposure, 10 mL of the PbPNPs suspensions were pipetted from the water column and centrifuged at 5000 rpm for 30 min at 4 °C (Zhai et al., 2017), Sorvall RC5Bplus centrifuge, Fiberlite F21-8). After centrifugation, the supernatant was filtered through a syringe filter with 0.02 μm pore diameter (Anotop 25, Whatman) to obtain the released ions (free Pb-ion in the water column). The concentrations of PbPNPs suspension and released Pb-ion were determined after digestion in a 65% HNO$_3$ solution using the AAS. The size distribution and zeta potential of the PbPNPs were analyzed by dynamic light scattering (DLS) on a zetasizer Nano-ZS instrument (Malvern, Instruments Ltd., UK).

2.5. Toxicity assessment of PbPNPs

The exposures were performed in triplicate. Biolog Ecoplates (Biolog, Hayward, USA) were used to determine the metabolic potential of the bacterial community in response to different treatments of PbPNPs. The Ecoplate consists of 96-well microplates containing 3 times a blank well plus 31 ecologically relevant and structurally diverse carbon sources that could be utilized by the inoculated bacteria (Garland and Mills, 1991). Each well of the Ecoplate contains a single carbon substrate and a tetrazolium-containing compound that turns purple as dependent on the extent of bacterial metabolism through the reduction of a tetrazolium violet redox dye (Pohland and Owen, 2009). Each well of the Ecoplate was inoculated with 100 μl of the prepared bacterial community extract and the PbPNPs suspension mixture with different pH, HA content and divalent cations concentrations for 96 at 20 °C. Optical density was measured at 590 nm (OD$_{590}$) each 24 h until OD$_{590}$ values show a turnover to saturation. The final calculations were analyzed based on the 96-h measurement.

2.6. Statistical analysis

The aggregation and dissolution kinetics of PbPNPs were derived by means of a nonlinear fit (supplementary data). The data from the Ecoplates were further used to evaluate the impact of environmental factors on the toxicity of PbPNPs. Community level physiological profiling (CLPP) was used to represent the average bacterial metabolic activity (Insam and Goberna, 2004). The metabolic capacity of the bacterial community was quantified by the average well color development (AWCD) of the 31 substrates (Garland and Mills, 1991). The color development of substrates that was greater than 0.25 was chosen to reflect substrate richness (S) (Sillen et al., 2015). Substrate diversity (H) and evenness (E) were also calculated based on Gryta et al. (2014).

To analyze the speciation of the dissolved Pb species at pH 5.0, 7.0 and 8.5, the Visual MINTEQ 3.1 software was used, which was downloaded from http://vminteq.lwr. kth. se/and used without modification. Statistical analyses were conducted using a univariate general linear model with Tukey's post Hoc (SPSS 16.0, SPSS Inc., Chicago, IL, USA) to analyze the significance and contribution (%) of HA content, divalent cations concentrations and their interaction and their interactions on the fate and toxicity of PbPNPs. One-way analysis of variance (ANOVA) with Tukey's post-hoc test was used to compare the impact of different levels of pH, HA content and divalent cations concentrations on the CLPP of the bacterial community. The significance level in all calculations was set at p < 0.05. The relationship between the Pb-ions release and the bacterial metabolic capacity was correlated using linear regression (Graphpad Prism 8.0). The bacterial community similarity based on the carbon substrates utilizations in response to different treatments was compared using a principal component analysis (PCA) and a one-way analysis of similarities (ANOSIM) to determine the community functional composition using paleontological statistics software (PAST 3.0).

3. Results

3.1. Fate of PbPNPs

3.1.1. Aggregation of PbPNPs

The influence of pH, HA and divalent cations on the aggregation of PbPNPs as a function of time is presented in Fig. 1. The parameters of the nonlinear fit of aggregation kinetics for hydrodynamic diameters and zeta potential are given in Table S1. Fig. 1A shows the effect of pH (5.0–8.5) on the aggregation kinetics of PbPNPs. The hydrodynamic diameter as well as the zeta potential of PbPNPs increased as a function of time, which indicated that the suspension of PbPNPs was unstable and the particles tended to aggregate along with time. After 5 h, the stabilized hydrodynamic diameter and the zeta potential at pH 5.0 were found to be 429.8 nm and −14.5 mV, respectively. The hydrodynamic diameters and the zeta potential decreased with a rise in pH value. When the pH value increased to 8.5, the hydrodynamic diameters and zeta potential of PbPNPs decreased to 321.1 nm and −22.7 mV, respectively.

The impact of HA on the aggregation kinetics of PbPNPs is presented in Fig. 1B. In general, the presence of HA decreased the surface charge of PbPNPs and reduced the hydrodynamic diameters. After 5 h, the increase of the concentration of HA from 0 to 50 mg C/L decreased the zeta potential from −17.7 to −33.3 mV and decreased the hydrodynamic diameter from 371.1 to 176.6 nm. These findings indicated that the stability of the PbPNPs increased in the presence of HA. Fig. 1C shows the aggregation kinetics of PbPNPs at different concentrations of divalent cations. It was found that the increase of the concentration of divalent cations from 0 to 10 mM significantly increased the zeta potential as well as the hydrodynamic diameters of PbPNPs from −17.7 to −5.0 mV and 371.1–1416.0 nm, respectively, after 5-h incubation. This implied that PbPNPs tended to aggregate in the presence of divalent cations.

The combined effects of HA content and divalent cations concentrations on the aggregation kinetics of PbPNPs are shown in Fig. 1D–F and Table S3. The concentration of divalent cations was the most sensitive factor with regard to aggregate size and zeta potential, explaining 88.9% and 65.5% of the variation of the hydrodynamic diameter and zeta potential, respectively. The stability of PbPNPs increased with increasing concentrations of HA (from 10 to 50 mg C/L) in the presence of low levels of divalent cations (1 and 2.5 mM) in the dispersion medium (Fig. 1D and E). However, when the concentration of divalent cations increased to 10 mM, the extent of aggregation of PbPNPs increased along with increasing HA concentration. This indicated that the coexistence of HA and a high concentration of divalent cations reduced the stability of PbPNPs in the medium.

3.1.2. Dissolution of PbPNPs

The influence of pH, HA content and divalent cations concentrations on the concentrations of the PbPNPs and free Pb-ions were investigated as a function of time (Fig. 2). Parameters of the nonlinear fit of dissolution kinetics for PbPNPs and the ion release profile are given in Table S2. Fig. 2A shows the effect of pH (5.0–8.5) on the dissolution of PbPNPs and the concentration of released Pb-ion. At pH 5.0 after 5-h incubation, the total PbPNPs concentration was 0.507 mg/L with 0.369 mg/L of Pb-ions released from PbPNPs. The ion release rate and the total concentration of PbPNPs decreased with increasing pH value. When the pH value increased to 8.5, the concentrations of total PbPNPs and free Pb-ion in the water column were reduced to 0.411 and 0.318 mg/L, respectively.

The total PbPNPs concentration increased in a manner that was dependent on the HA concentration (Fig. 2B). After 5-h incubation, an increase of the HA concentration from 0 to 50 mg C/L induced an increase of the total PbPNPs suspension concentration from 0.445
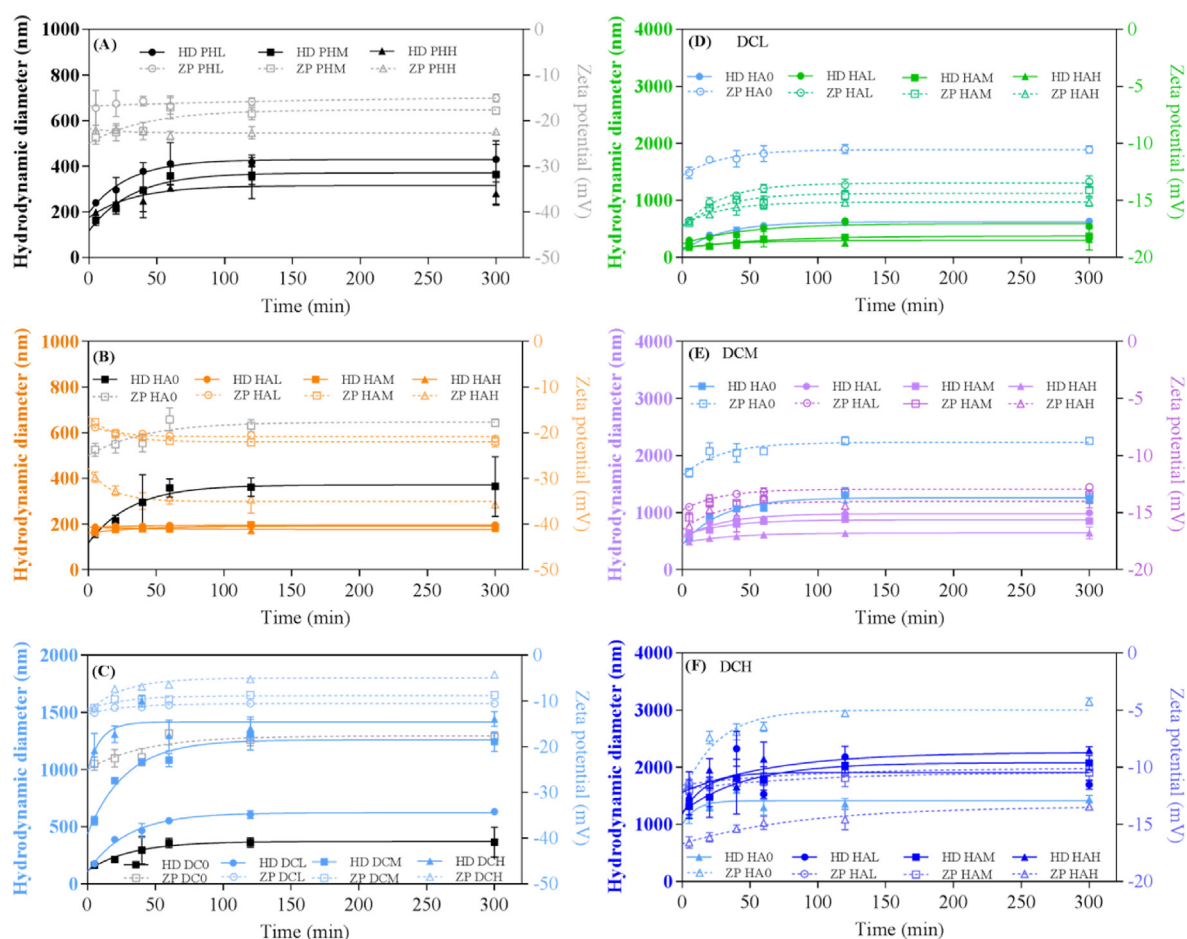
Fig. 1. Zeta potential and size distribution of PbPNPs over time under different environmental conditions of (A) pH, (B) HA and (C) divalent cations; HA combined with divalent cations at (D) 1 mM, (E) 2.5 mM and (F) 10 mM. ZP: zeta potential; HD: hydrodynamic diameter; DCL: divalent cations 1 mM, DCM: divalent cations 2.5 mM, DCH: divalent cations 10 mM; HAL: HA 10 mg/L, HAM: HA 25 mg/L, HAH: HA 50 mg/L.

to 0.537 mg/L. However, the concentration of free Pb-ion decreased from 0.340 to 0.241 mg/L. This indicated that the presence of HA promoted the dispersion of PbPNPs while inhibiting the amount of free Pb-ion in the water column. Fig. 3C shows the dissolution of PbPNPs and the release of Pb-ion in response to various divalent cations concentrations. The increase of the concentration of divalent cations from 0 to 10 mM decreased the PbPNPs concentration and the concentration of free Pb-ion from 0.445 mg/L and 0.340 mg/L to 0.248 and 0.241 mg/L, respectively.

The combined effects of HA content and divalent cations concentrations on the dissolution and Pb-ion release of PbPNPs were further analyzed (Fig. 2D–F and Table S3). The concentration of divalent cations was the most influential factor, which explained 90.4% and 59.4% of the variation of the concentration of the PbPNPs suspension and the free Pb-ion, respectively. The co-presence of HA and divalent cations resulted in a decrease in the amount of free Pb-ion and dissolved PbPNPs. Increasing concentrations of HA (from 10 to 50 mg C/L) and Ca^{2+}/Mg^{2+} (from 1 to 10 mM) reduced the suspension concentration and ion release of PbPNPs from 0.334 to 0.139 mg/L to 0.100 and 0.031 mg/L, respectively.

3.2. Influence of pH, HA content and divalent cations concentrations on the toxicity of PbPNPs

The effects of pH, HA content and divalent cations concentrations on the toxicity of PbPNPs on the bacterial community were

investigated based on the CLPP. The diversity indices as well as the metabolic capacity across a range of environmental conditions are provided in Table 1. Although there was no significant change in CLPP of the bacterial community at different pH levels, the metabolic capacity was slightly lower at pH 5.0 than at pH 7.0 and 8.5. The presence of HA significantly increased the metabolic capacity in a manner that was dependent on the HA concentration, and 50 mg C/L HA significantly increased the richness of the bacterial community. The presence of divalent cations at low concentration decreased the richness, diversity and metabolic capacity of the bacterial community, while the toxicity was reduced as the divalent cations concentration increased. The combination of HA and divalent cations reduced the toxicity of PbPNPs to the bacterial community, where community richness, diversity and metabolic capacity significantly increased along with increasing concentrations of HA and divalent cations.

The metabolic profiles of the bacterial communities in response to different exposure conditions were further compared based on the substrates utilization (Fig. 3). The bacterial communities clustered together when exposed to PbPNPs at different pH levels, indicating that pH did not significantly affect the toxicity of PbPNPs on the community metabolic profiles (Fig. 3A). The presence of only HA or divalent cations in the PbPNPs suspensions changed the community metabolic profiles (Fig. 3B and C). The impact of the joint presence of HA and divalent cations in the exposure system on the toxicity of PbPNPs was further analyzed using canonical
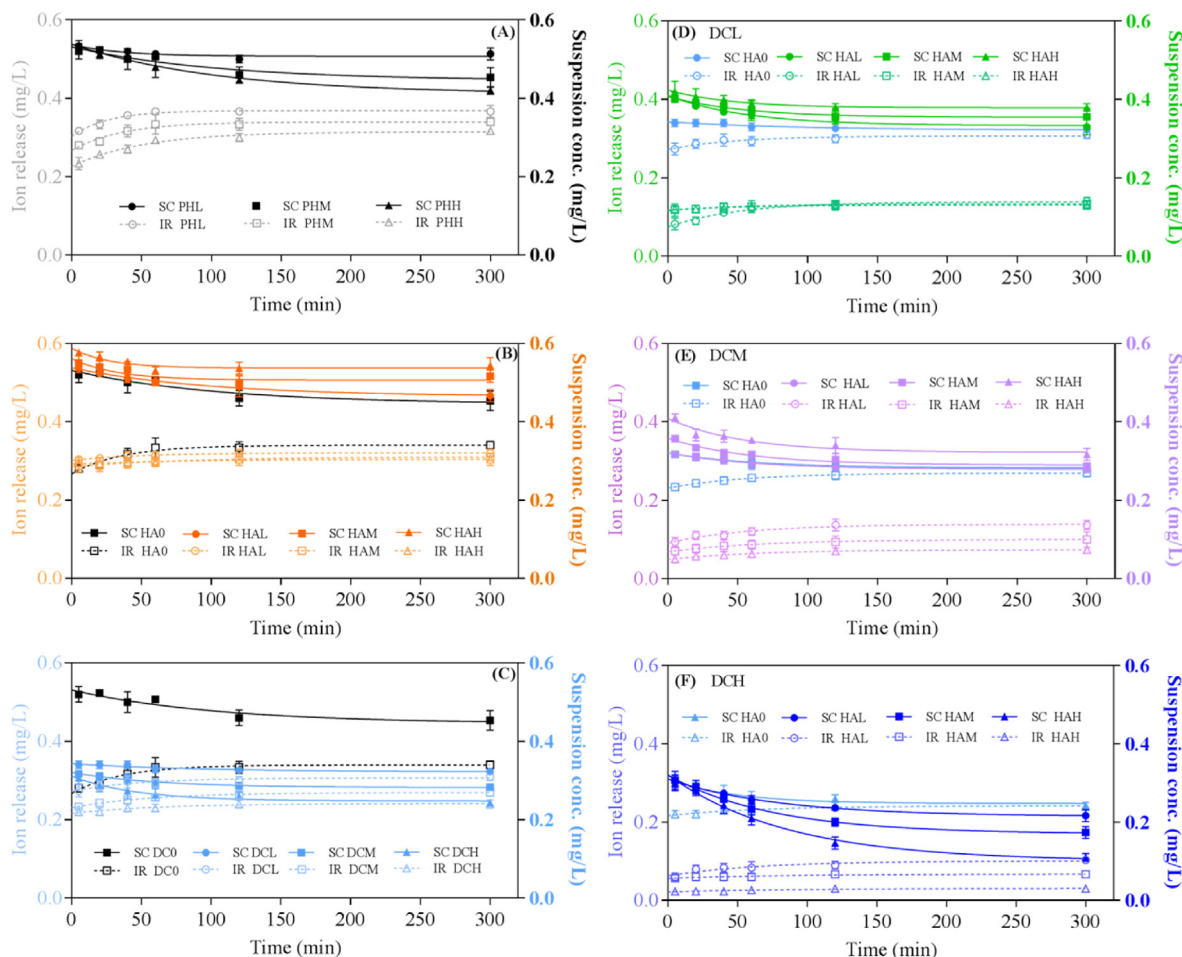
Fig. 2. Dissolution kinetics of PbPNPs under different environmental conditions of (A) pH, (B) HA and (C) divalent cations; HA combined with divalent cations at (D) 1 mM, (E) 2.5 mM and (F) 10 mM. SC: total PPNPs suspension concentration; IR: Pb ion release. DCL: divalent cations 1 mM, DCM: divalent cations 2.5 mM, DCH: divalent cations 10 mM; HAL: HA 10 mg/L, HAM: HA 25 mg/L, HAH: HA 50 mg/L.

correspondence analysis based on the bacterial substrate utilization. The concentration of divalent cations was found to be the main factor in determining the community shift (Fig. S1). When the concentration of divalent cations in the suspension was low (1 mM), the bacterial communities were separated into four groups based on the HA concentration (Fig. 3D). As the concentration of divalent cations increased in the suspension, the bacterial communities in the presence of HA (ranging from low to high concentration) clustered together and were separated from the community present in the absence of HA (Fig. 3E and F). This indicated that when the divalent cations concentration in the PbPNPs suspension increased, the impact of divalent cations on the bacterial community was higher than the impact of increasing HA concentration.

Fig. 4 (A-F) presents an illustration of the fate of PbPNPs in response to the variations of pH, concentrations of HA and divalent cations. The combination of electrostatic repulsive forces and Van der Waals forces allowed the PbPNPs-particles to keep their initial stability in the suspension (Fig. 4A). The increase of the concentration of divalent cations enhanced the aggregation of PbPNPs (Fig. 4B and C), whereas the presence of HA prevented the aggregation of PbPNPs (Fig. 4D). The co-existence of HA and divalent cations promoted the PbPNPs particle aggregation to a larger extend (Fig. 4E and F). The toxicity patterns of PbPNPs in response to the variations of pH, HA content and concentrations of divalent

cations are schematically given in Fig. 4 (G-J). The toxicity of PbPNPs to bacteria was mainly driven by the interactions between PbPNPs-particles and the cell membranes, as well as by the ionic Pb-induced toxicity (Fig. 4G). The presence of divalent cations reduced the toxicity of PbPNPs to gram-negative bacteria due to the repulsive force between the cell membrane, Pb-ions and PbPNPs-particles (Fig. 4H). For gram-positive bacteria, the presence of divalent cations could promote the interaction between PbPNPs and the bacteria, while the competition between cations and free Pb-ions reduced the toxicity of PbPNPs (Fig. 4H). The addition of HA resulted in the formation of coated PbPNPs-particles and reduced the bioavailability of PbPNPs to gram-negative bacteria (Fig. 4I). Moreover, HA present in suspension also binds with free Pb-ion, which reduces the amount of free Pb-ion and subsequently the toxicity to gram-positive bacteria (Fig. 4I). The co-existence of HA and divalent cations promoted the PbPNPs particle aggregation and reduced the toxicity of PbPNPs (Fig. 4J).

4. Discussion

4.1. Influence of pH, HA content and divalent cations concentrations on the fate of PbPNPs

pH. The aggregation and dissolution of PbPNPs were found to be pH-dependent, as reflected by the decreased hydrodynamic
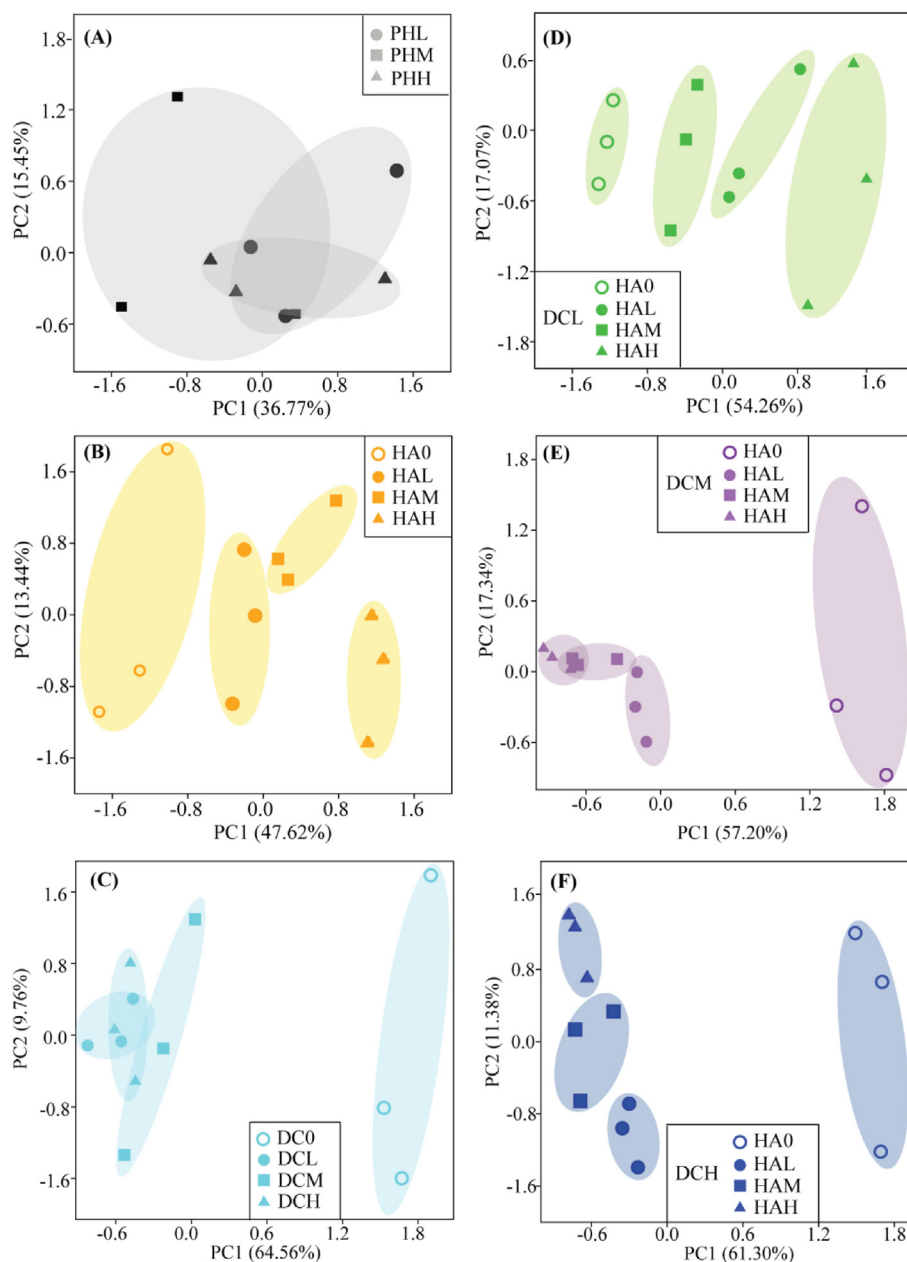
Fig. 3. Principle component analysis of the substrates utilization profiles of the soil bacterial community in response to PbPNPs exposure under different environmental conditions of (A) pH, (B) HA, and (C) divalent cations; HA combined with divalent cations at (D) 1 mM, (E) 2.5 mM and (F) 10 mM. DCL: divalent cations 1 mM, DCM: divalent cations 2.5 mM, DCH: divalent cations 10 mM; HAL: HA 10 mg/L, HAM: HA 25 mg/L, HAH: HA 50 mg/L.

diameter as well as the zeta potential with increasing pH value (Fig. 2). It is reported that the zeta potential of PbPNPs decreased along with increasing pH with the point of zero charge being approximately around pH 4.0 (Wang et al., 2017). This well explains the negative charge found in our study at pH values ranging from 5.0 to 8.5. The repulsive forces between the negatively charged particles prevented the PbPNPs-particles to aggregate and thus increased the stability of PbPNPs suspensions (Derjaguin and Landau, 1941). Moreover, the concentrations of dissolved PbPNPs as well as the amount of released Pb-ions were found to decrease with increasing pH values. It is suggested that PbPNPs could be degraded to PbI_2 and carbonated moieties that ultimately are converted into hydroiodic acid (HI) and methylamine (Conings et al., 2015). The further decomposition pathways and the

speciation of Pb at different pH values are presented in Fig. S2. The predominant species of released Pb in acidic suspension is Pb^{2+}. When the pH value increases, the formation of $Pb(OH)_2$ decreased the amount of ionic Pb (Jiang et al., 2016). Therefore, adjusting the suspension pH changes the fate of both ionic and particulate PbPNPs.

Humic acid (HA). The negative charge of the hydrophobic fraction of HA is associated mainly with the carboxylic (pKa values in between 2.5 and 5) and phenolic groups (pKa values around 9 or 10) (Edwards et al., 1996). In aqueous environments, natural organic matter can adsorb on the surface of metallic NPs due to the van der Waals interactions (Omar et al., 2014). The decreased zeta potential of the PbPNPs indicated that HA was adsorbed on the surface of PbPNPs. Meanwhile, the hydrodynamic diameter was

Table 1
Effects of PbPNPs on the community level physiological profiles of the soil bacterial community under different environmental conditions.

Environ. factors	Expt.[a]	pH	HA (mg/L)	DC (mM)	Diversity indices[b]			Metabolic capacity[c]
					S	H	E	AWCD
pH	PHL	5.0	0	0	13[a]	2.56[a]	0.99[a]	0.51[a]
	PHM	7.0	0	0	14[a]	2.63[a]	0.99[a]	0.58[a]
	PHH	8.5	0	0	14[a]	2.64[a]	0.98[a]	0.62[a]
HA	HA0	7.0	0	0	14[a]	2.63[a]	0.99[a]	0.58[a]
	HAL	7.0	10	0	21[b]	2.97[b]	0.98[a]	0.77[b]
	HAM	7.0	25	0	22[b]	3.02[b]	0.98[a]	0.88[c]
	HAH	7.0	50	0	22[b]	3.03[b]	0.99[a]	0.99[d]
Divalent cations	DC0	7.0	0	0	14[a]	2.63[a]	0.99[a]	0.58[a]
	DCL	7.0	0	1	13[a]	2.61[a]	1.00[a]	0.59[a]
	DCM	7.0	0	2.5	14[a]	2.63[a]	0.99[a]	0.63[ab]
	DCH	7.0	0	10	20[b]	2.93[b]	1.00[a]	0.67[b]
Divalent cations	HA0	7.0	0	1	13[a]	2.61[a]	1.00[a]	0.59[a]
Low	HAL	7.0	10	1	14[a]	2.63[a]	0.98[a]	0.62[ab]
	HAM	7.0	25	1	19[b]	2.91[b]	0.99[a]	0.65[ab]
	HAH	7.0	50	1	20[b]	2.94[b]	1.00[a]	0.68[b]
Divalent cations	HA0	7.0	0	2.5	14[a]	2.63[a]	0.99[a]	0.63[a]
Medium	HAL	7.0	10	2.5	24[b]	3.13[b]	0.99[a]	0.86[b]
	HAM	7.0	25	2.5	23[b]	3.11[b]	0.99[a]	0.89[b]
	HAH	7.0	50	2.5	24[b]	3.12[b]	0.99[a]	0.89[b]
Divalent cations	HA0	7.0	0	10	20[a]	2.93[a]	1.00[a]	0.67[a]
High	HAL	7.0	10	10	25[ab]	3.16[b]	0.98[a]	1.09[b]
	HAM	7.0	25	10	26[ab]	3.17[b]	0.97[a]	1.19[c]
	HAH	7.0	50	10	27[b]	3.17[b]	0.97[a]	1.27[d]

[a] Expt: P: pH; HA: humic acid; DC: divalent cations; 0: control; L: low concentration; M: middle concentration; H: high concentration.
[b] Diversity indices: S: richness; H: diversity; E: evenness.
[c] Metabolic capacity: AWCD: average well color development.

found to decrease upon increasing HA concentration. According to the Derjaguin-Landau-Verwey-Overbeek theory (Derjaguin and Landau, 1941), natural organic matter can increase the electrical double layer compression of particles, and the van der Waals attraction thus was relatively small with the net energy between the particles being repulsive (Zhang et al., 2009). This enhanced the disaggregation as well as the dispersion of PbPNPs in suspension (Fig. 4A and D). However, the surface adsorption of HA blocked the reactive sites of PbPNPs, and the HA present in suspension can also combine with the released metal ions, forming HA-metal complexes (Giannakopoulos et al., 2005), which reduced the amount of free Pb-ions in the PbPNPs suspension (Shang et al., 2017). Overall, the presence of HA can affect the aggregation of PbPNPs particles as well as Pb-ion release.

Cations. Divalent cations can absorb on the negatively charged PbPNPs-particles, which neutralizes the surface charge of the particles and reduces the electrical double layer repulsion between the particles. This in turn causes a decrease in thickness of the electrical double layer, resulting in a decreased absolute value of zeta potential (Fig. 4A−C) and a reduced energy barrier (Baalousha et al., 2013). This leads to rapid aggregation of the particles as a result of prevailing van der Waals attraction (Zhu et al., 2014). This is typically an aspect that shows the particle-specific impact. The enhanced aggregation of PbPNPs reduced the amount of PbPNPs dispersed in the exposure medium, which also reduced the Pb-ion release in the water column.

Cations coexisting with humic acid. When cations coexisted with HA in the exposure medium, the concentration of divalent cations was the most significant factor responsible for aggregation and dissolution of PbPNPs (Table S3). At low divalent cations concentration, the stability of PbPNPs increased upon increasing the amount of HA added in the dispersion medium (Figs. 1D and 2D). It is likely that increased steric interactions due to the adsorption of HA macromolecules on the PbPNPs surfaces hinder the attraction between the particles (Chen and Elimelech, 2007) (Fig. 4E). The increased stability promoted the total amount of PbPNPs dispersed

in the exposure medium, while the concentration of released Pb-ions declined due to the blocking of reactive sites by HA adsorption (Shang et al., 2017). In addition, at high divalent cations concentrations, the increasing amount of HA in the dispersion medium can increasingly adsorb on the surface of particles, and subsequently form long chains between divalent cations and particles through a bridging effect (Delay et al., 2011) (Fig. 4F). The formation of humic acid flocs resulted in enhanced particle aggregation and sedimentation (Fig. 1F). Consequently, less dissolved PbPNPs was observed in the exposure medium (Fig. 2F) (Wang et al., 2016).

4.2. Microbial responses to PbPNPs under different exposure conditions

pH. In our results, although the impact of pH-values ranging from 5.0 to 8.5 on the toxicity of PbPNPs was not statistically significant, the diversity and metabolic capacity increased with increasing pH (Table 1). Our previous study found that mainly ionic Pb caused the toxic effects on a bacterial community (Zhai et al., 2017) (Fig. 4G). It has also been reported that the speciation and toxicity of Pb-containing contaminants could be affected by pH in the exposure medium (Wang et al., 2014). In our test conditions, Pb^{2+} and $PbOH^+$ were predominantly present in the dispersion medium across the tested pH range, while part of the Pb-ions transformed into $Pb(OH)_2$-species, which sedimented when pH was increased to 8.5 (Fig. S2). Sedimentation of Pb-species reduced the concentration of reactive Pb-ions and the decreased toxicity was found to be correlated with the decreasing Pb-ions concentration (Fig. S3A). Considering the increased PbPNPs dispersion as well as decreased toxicity with increasing pH (Fig. 1A and Table 1), the Pb ion release and Pb speciation probably dominated the toxicity at different pH levels.

HA. In the presence of HA, the toxicity of PbPNPs was mitigated, as shown by the increased metabolic capacity along with increasing HA concentration (Table 1). This finding is in line with studies where HA bound to the surface of nanoparticles could significantly
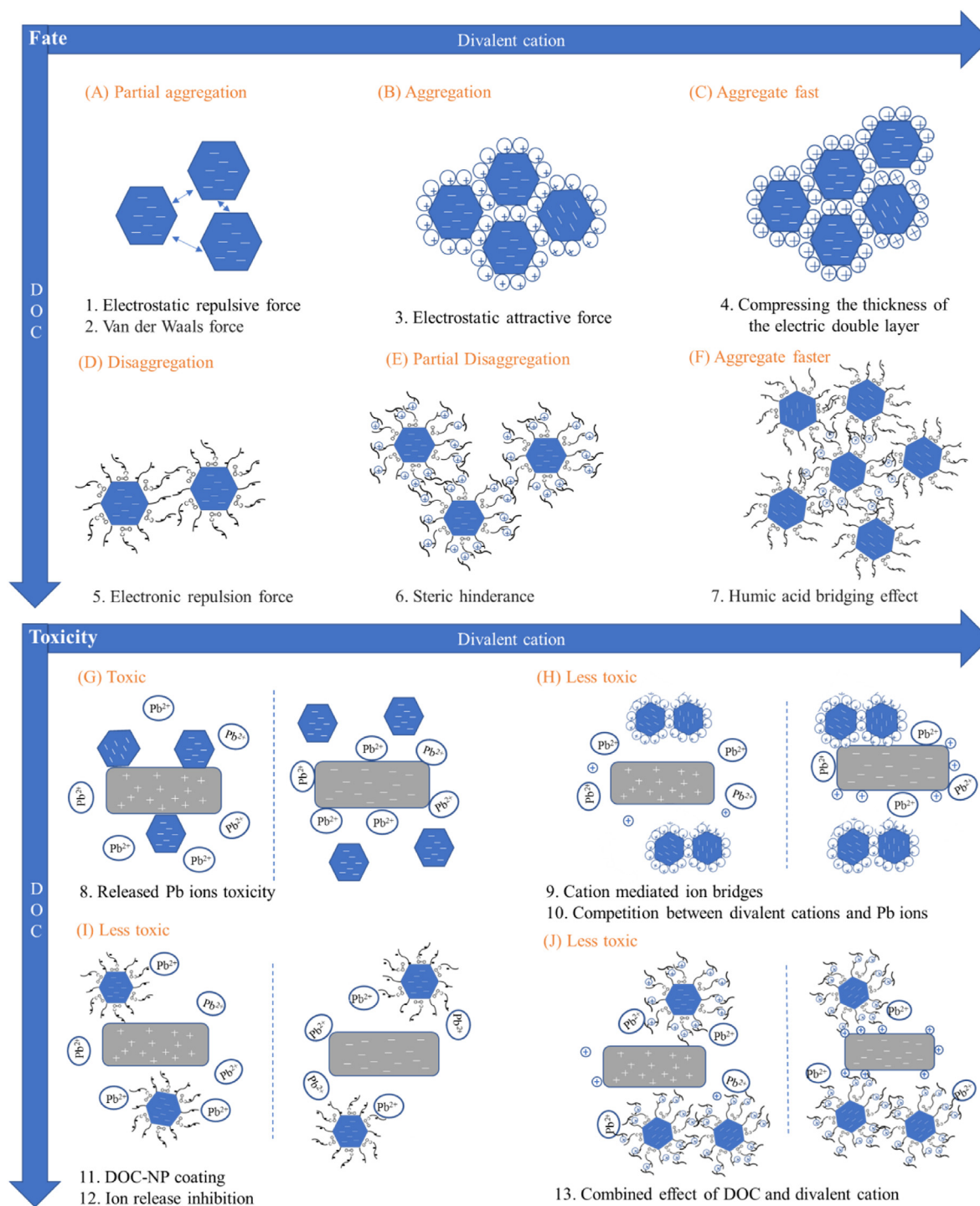
Fig. 4. Schematic diagram of PbPNPs stability and toxicity interacting with HA and electrolyte at pH = 7. A-F representing the impact of HA and divalent cation on PbPNPs fate. G-J representing the impact of HA and divalent cation on PbPNPs toxicity (blue polygons are nanoparticles, and grey rectangles are gram-negative and gram-positive bacteria). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

alleviate the toxicity of nano-TiO₂, nano-ZnO and nanoparticulate zerovalent iron on algal cells and microbes (Li et al., 2010a, 2010b; Lin et al., 2012). We observed a correlation between decreasing Pb-ions concentrations and alleviated toxicity (Fig. S3B). The sorption of HA on the PbPNPs could block the reactive sites of PbPNPs from releasing Pb-ions, and the formation of HA-Pb complexes also reduced the Pb-ion induced toxicity (Shang et al., 2017). In addition, the HA coating increased the negative surface potential of PbPNPs-

particles, thereby reducing the direct interaction by increasing the charge repulsion between PbPNPs and bacterial cells (Wang et al., 2016; Huang et al., 2016). Therefore, both PbPNPs-particles and Pb-ions interacting with bacterial cells need to be accounted for in contributing to the overall toxicity in the presence of HA (Fig. 4I).

Cations. At a low concentration of divalent cations, toxicity increased (Table 1 and Figure S3 C) while Pb ion release was found to be decreased (Fig. 2C and Table S2). These findings indicated that

Pb^{2+} was not the only factor driving the toxicity, and the toxicity of PbPNPs in the presence of divalent cations was ascribed to enhanced interaction between the aggregated particles and the cell wall of the bacteria within the diverse community (Fig. 4H) (Jin et al., 2009). The divalent cations can neutralize the surface charge of PbPNPs-particles as the negative surface potential of PbPNPs was observed to be decreased (Fig. 1C and Table S1). The cation neutralization weakened the electrostatic repulsion between PbPNPs-particles and bacterial cells and promoted their interaction. Furthermore, cations could form ion bridges which promote the binding of negatively charged PbPNPs to the bacteria, and which consequently facilitated the adsorption/adhesion of PbPNPs-particles to the bacteria (Kikuchi et al., 1997). As the concentration of divalent cations increased, the cations compressed the thickness of the electric double layers of the PbPNPs-particles, forming larger aggregates that reduced the toxicity (Table 1 andFigure S3 C). Overall, the toxicity of PbPNPs in the presence of divalent cations was driven by both PbPNPs-particles and Pb-ions.

Cations coexisting with humic acid. When the divalent cations coexisted with HA, the concentration of divalent cations was found to be the main factor in determining the community shift (Fig. S1). At a lower concentration of divalent cations, the Ca^{2+}/Mg^{2+} ions neutralized the charge of PbPNPs-particles to increase the direct contact of the particles with bacterial cells, but the coexisted HA in the exposure medium could compromise the cations neutralization by forming a HA-PbPNPs coating and HA-Pb complexes that prevented direct interactions between PbPNPs and bacterial cells (Huang et al., 2016). As the concentration of divalent cations increased, the bridging effect of humic acid accelerated the formation of large HA-PbPNPs aggregates and HA-Pb complexes that reduced the toxicity (Fig. 4J).

4.3. Implications for environmental risk analysis and management

In brief, the contribution of PbPNPs-particles and Pb-ions to the overall toxicity needs to be quantitatively accounted for in assessing microbial effects. By knowing the PbPNPs-particle aggregation, Pb-ion release and microbial community responses in different exposure conditions, the toxic effect caused by the released Pb-ions can be assessed by quantifying the fraction of Pb-ions and the Pb-activity at the biotic ligands in different exposure conditions using the terrestrial biotic ligand models (Thakali et al., 2006). The contribution of the PbPNPs-particles therefore can be predicted by exposure levels (concentration addition model) or by the responses (independent action model) based on the mode of action of PbPNPs (Vijver et al., 2018). In future research, both experimentally investigating and mechanistically modelling is needed for the safety assessment of Pb-based nanomaterials assessment towards more realistic exposure conditions. The various toxicity of Pb-based perovskite nanoparticles under natural environmental conditions highlighted the importance for the safe-by-design in both fabrication and risk management of novel perovskite solar cells.

5. Conclusions

In this study, the impacts of pH, humic acid (HA) and divalent cations on the fate and toxicity of PbPNPs were investigated. Increasing the medium pH increased the dispersion of PbPNPs-particles whilst decreasing the Pb-ions release. Coating of the surface of the PbPNPs-particles by HA increased the stability of, while blocking the reactive sites for further Pb-ion release. The divalent cations accelerated the aggregation of PbPNPs-particles and reduced the release of Pb-ions. When the divalent cations coexisted with HA, the steric hindrance of HA-PbPNP coating prevented particles aggregation. As the cations concentration increased, HA

was capable of bridging the PbPNPs-particles and divalent cations together, which enhanced particle aggregation and reduced the release of Pb ions. The metabolic potential of the soil microbial community was investigated to assess PbPNPs toxicity in different exposure conditions. Increasing the pH of the exposure medium reduced PbPNPs toxicity, which was mainly ascribed to Pb-ion release and Pb speciation at different pH levels. The HA-PbPNPs-particle coating weakened the interactions of the particles with bacterial cells. Moreover, HA also bound free Pb-ions, thus reducing ion toxicity. The presence of divalent cations increased the toxicity by providing cation bridges for the PbPNPs-particles interacting with bacteria. When the divalent cations coexisted with HA, reduced toxicity was observed at lower cations concentrations, due to the formation of a HA coating on the PbPNP-particles and the formation of HA-Pb complexes. At higher cations concentrations, enhanced aggregation of PbPNPs and reduced Pb-ion release reduced the toxicity. These findings indicated that both Pb-ions and the interaction between the PbPNPs-particles and bacterial cells contributed to the toxicity in the presence of HA and divalent cations. This study systematically described the impact of environmental factors on the fate and microbial responses of the class of emerging nanomaterials of PbPNPs, as for instance increasingly used within new generations of solar panels.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Yujia Zhai: Conceptualization, Investigation, Methodology, Writing - original draft. **Zhuang Wang:** Data curation, Software, Writing - review & editing. **Guiyin Wang:** Methodology, Visualization, Supervision. **Willie J.G.M. Peijnenburg:** Supervision, Writing - review & editing. **Martina G. Vijver:** Project administration, Funding acquisition, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.126564.

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