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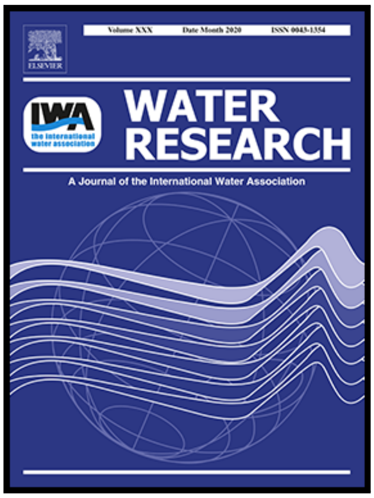

Chlorination enhances the phthalates release and increases the cytotoxicity and bacterial functions related to human disease of drinking water in plastic pipes

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Highlights

- Phthalate acid esters (PAEs) released from PVC pipes were higher than PE pipes.
- Chlorine disinfection enhanced PAEs release, leading to higher water cytotoxicity.
- More PAEs release increased bacterial metabolic pathways linked to human diseases.
- Biofilm bacterial community and chlorine increased the hydrophilicity of pipes.
- Greater PVC pipe surface hydrophilicity increased the release of PAEs.

Chlorination enhances the phthalates release and increases the cytotoxicity and bacterial functions related to human disease of drinking water in plastic pipes

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Abstract

The interaction between water and pipe surfaces can deteriorate drinking water quality, thus threatening public health. However, uncertainties remain in the release mechanism of phthalates acid esters (PAEs) from plastic pipes and their effects on drinking water quality. Our study indicated that PAEs released from polyvinyl chloride (PVC) pipes was higher than polyethylene (PE) pipes. Chlorine disinfection increased the released PAEs concentration in effluents of PE-Cl_2 and PVC-Cl_2 pipes to 6.60~7.87 μg/L and 7.45~8.88 μg/L, respectively. PAEs release varied the CHO and tannins numbers in dissolved organic matter (DOM), increasing the cytotoxicity of water. Although chorine disinfection reduced the abundance of pathogenic bacteria, it upregulated the relative abundance of bacterial metabolic pathways related to human disease, such as drug resistance: antimicrobial and cancer: overview. In addition, various biofilm bacterial community compositions affected the interactions between bacteria and pipe surfaces, and the roughness of pipe surfaces increased after biofilm formation. The hydrophilicity of pipe surfaces also increased due to biofilm formation and chlorine disinfection. After five months of running, higher hydrophilicity of PVC pipe surface was observed than that of PE pipes, especially after chlorine disinfection, consequently enhancing PAEs release. In conclusion, chlorine disinfection accelerated PAEs release from plastic pipes by increasing the hydrophilicity of pipe surfaces, resulting in higher cytotoxicity and microbial risk of drinking water, especially in PVC-Cl_2 pipes. This study revealed the influence of chlorine disinfection on PAEs release and its potential risk to public health, which provided insightful visions for the future drinking water security monitoring.

Keywords: chlorine disinfection, PE and PVC pipes, PAEs release, biofilm, cytotoxicity, human disease

1. Introduction

Drinking water quality is crucial for human health and living standards. The physiochemical and microbial interaction between water and pipe surfaces can deteriorate the drinking water quality when the water transports through the distribution pipes (Ke et al., 2024; Zhang et al., 2020). In recent decades, various types of plastic pipes, especially polyvinyl chloride (PVC) and polyethylene (PE) pipes, have been used in water distribution and plumbing systems worldwide due to their superior characteristics including lightweight, corrosion resistance and great endurance (Xu et al., 2019). Phthalate acid esters (PAEs) are commonly used as plasticizers to maintain the flexibility and stability of plastic pipes (Henkel et al., 2021). The leaching of PAEs from plastic pipes into the water has been recognized as a potential threat to drinking water quality and human health (Kasper-Sonnenberg et al., 2012). However, knowledge remains unknown about the release mechanism of PAEs from plastic pipes and the effects of released PAEs on the drinking water quality.

PAEs are endocrine disruptions and can negatively affect the reproductive system of humans (Adeogun et al., 2015). PAEs have been considered as prior pollutants by the water regulations of many countries (Paluselli et al., 2019). Our previous investigation has found that the total PAEs concentration in rural drinking water of China was 0.1~16.5 μg/L (Yin et al., 2023). During the aging process, the oxygen content, molecular weight, and surface morphology of the plastics would be changed, resulting in the leaching of PAEs from the plastics (Yan et al., 2021, Sun et al., 2021). The UV/H_2O_2 and UV/Cl_2 treatments are two principal processes responsible for accelerated plastic aging (Sun et al., 2021). Our previous work also found that chlorine dioxide (ClO_2) disinfection enhanced the PAEs release from the aged PVC pipes (Wang et al., 2023). Compared to ClO_2, chlorine is a commonly used disinfectant in urban drinking water treatment plants to control the growth of pathogenic bacteria, including *Legionella pneumophila*, *Mycobacterium avium*, and *Pseudomonas aeruginosa* (Oliveira et al., 2023; Ke et al., 2024). However, no study investigates how residual chlorine affects the release of PAEs from plastic pipes and the drinking water quality in water distribution systems using PE or PVC pipes.

Biofilm is prone to form on the surface of plastic pipes (Lehtola et al., 2004), and bacteria have been confirmed to increase the PAEs release from PVC cables in seawater (Paluselli et al., 2019). The mechanism hidden behind this phenomenon may be that biofilm, as a new interface between plastic and the surrounding environment, can influence the transport of PAEs through adsorption and degradation (Zhao et al., 2024). In addition, different pipe materials (e.g., PVC, copper, iron) can shape biofilms with different bacterial community compositions (Buse et al, 2014; Wang et al, 2014; Aggarwal et al, 2018). However, the effects of different biofilm bacterial community compositions on the PAEs release from plastic pipes, especially PVC and PE pipes, remain poorly understood.

Zheng et al. (2024) have indicated that chloramine disinfection increased the toxicity risk of drinking water due to the potential formation of nitrogenous disinfection by-products. However, the correlation between the PAEs released from the plastic pipes and the cytotoxicity of drinking water hasn't been studied. Moreover, there are many micropollutants in drinking water, such as PAEs, perfluoroalkyl substances (PFAS), and antibiotics (Li et al., 2020). It is difficult to figure out the role of each micropollutant in inducing the cytotoxicity of drinking water. Dissolved organic matter (DOM) is another type of complex in drinking water that contains various organic molecules (Leenheer et al., 2003). The assimilable organic carbon (AOC) in DOM is the main carbon source for bacterial growth and biofilm formation (Pick et al., 2021), which might also contribute to the release of PAEs and the cytotoxicity increase of drinking water.

In this paper, we present new information that sheds light on the effects of released PAEs on the drinking water quality in plastic distribution pipes, and importantly, decipher the associated mechanism of PAEs release. Here, various comparison studies were carried out. The released PAEs from PE and PVC pipes with and without chlorination were analyzed with a gas chromatography triple quadrupole tandem mass spectrometer. The DOM at the molecular level was determined by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). The surface functional groups and morphology of PE and PVC pipes were analyzed using a Thermo Fisher Scientific FTIR spectrometry, a scanning electron microscope (SEM), and an atomic force microscopy (AFM). The hydrophilicity of the pipe surface was measured using a contact angle meter. The bacterial community composition was performed on an Illumina HiSeq-PE250 platform and quantified by quantitative polymerase chain reaction (qPCR). The objective of this study was to provide in-depth insight into the interactions between chlorine disinfection, biofilm bacterial community composition, and pipe surface characteristics, and their effects on PAEs release and drinking water quality.

2. Materials and methods

2.1. Simulated drinking water distribution pipes

Four PE and four PVC pipes, with a diameter of 100 mm and a height of 100 mm for each pipe, were utilized to simulate the plastic drinking water distribution pipes (Text S1). All the pipes were purchased from Wai Fung Plastic Products Co. Ltd (China). In each pipe, 10 coupons (20*20*4 mm) were fixed at the bottom for biofilm growth and morphology analysis. There were four groups of simulated distribution pipes. Each group was run in duplicate. For groups 1 and 2, the water, which was collected from the effluents of active carbon filtration in a drinking water treatment plant in North China, was used as the influents (feed water) of PE and PVC pipes. These two groups were named PE and PVC groups, respectively. For groups 3 and 4, the sampled water was treated with sodium hypochlorite (NaClO) and then used as the influents (chlorinated feed water) of PE and PVC pipes. These two groups were named PE-Cl_2 and PVC-Cl_2, respectively. The chlorine residual in the influents of these two groups was controlled at about 1.0 mg/L. According to previous studies (Al-Jasser 2007; Yan et al., 2022), the influents were stirred by a motor with 100 rpm to simulate the flow rate in the real distribution pipes. The influents of each pipe were displaced with fresh water at 48 h intervals, mimicking dead zones or the worst case in actual distribution pipes (Wang et al., 2014). These four groups of pipes were run for five months. The actual water quality parameters of each experiment are shown in Table S1.

2.2. PAEs release detection and DOM analysis using FT- ICR- MS

During the whole experiment, the released PAEs in different pipes after 48 h running were analyzed for eleven times. The sampling date for the released PAEs is shown in Fig. 1a. The detection of released PAEs from the PE and PVC pipes was the same as our previous studies (Yin et al 2022; Wang et al 2023). The reagents used in this study were as follows: standard PAEs mixture containing 15 PAEs: dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DIBP), dibutyl phthalate (DBP), dimethoxyethyl phthalate (DMEP), diisohexyl phthalate (BMPP), bis(2-ethoxyethyl) benzene-1,2-dicarboxylate (DEEP), dipentyl phthalate (DPP), dihexyl Phthalate (DnHP), benzyl butyl phthalate (BBP), dibutoxyethyl phthalate (DBEP), dicyclohexyl phthalate (DCHP), di-n-octyl phthalate (DNOP), di-2-ethylhexyl phthalate (DEHP), and dinonyl phthalate (DNP). The high-performance liquid chromatography (HPLC) grade PAEs mixture was obtained from Aladdin

(Shanghai, China).

During the entire sampling and pretreatment process, contact with plastics was avoided to minimize background contamination. PAEs were analyzed with an Agilent 7890B\\7000D gas chromatography triple quadrupole tandem mass spectrometer (Agilent Technology, USA). The quantification of all PAEs was carried out using external quantification curves with different concentrations. The correlation coefficients (R^2) for the calibration curves for all target compounds were > 0.99. The details of PAEs detection, quality control (QC), and quality assurance (QA), are shown in Text S2. The limit of detection (LOD) was defined as the lowest concentration when the signal-to-noise ratio (S/N) was >3, and the limit of quantification (LOQ) was defined as the lowest concentration when the S/N was >10. LOD and LOQ were determined after three tests for each analysis with a standard deviation of less than 20%. The LOQ, LOD, and PAEs concentrations in blank samples are shown in Table S2 and Table S3, respectively.

The standard comparison evaluates the degree of PAEs in water by calculating the risk quotient (RQ). RQ was defined as follows (equation 1) (Mak et al., 2009):

$$RQ = \frac{Cs}{Cg} \qquad (1)$$

Where RQ is the relative risk of each pollutant, C_S (μg/L) is the measured concentration of each pollutant in the sample, and C_g (μg/L) is the recommended, or standard value of relevant pollutants. RQ > 1, 0.1 < RQ < 1, and RQ < 0.1 are considered as high risk, medium risk, and low risk, respectively.

The FT-ICR-MS has the unique ability to determine the exact masses of different components present in DOM (Antony et al., 2017). Therefore, DOM characterization at the molecular level was analyzed using the FT-ICR MS. The electrospray ionization (ESI) was in negative ion mode. The main parameters were as follows: continuous injection, injection rate of 120 μL/h, capillary inlet voltage of -4.0 kV, ion accumulation time of 0.06 s, a mass range of 100-1600 Da, 4 M 32-bit samples, and 300 time-domain superimpositions to improve the signal-to-noise ratio. The details of the ESI detection method are shown in Text S3. The mass spectrometry data were calibrated using the known CHO analogs in DOM.

2.3. Water quality analysis

A multiparameter water quality analyzer (Hach HQ40d, Mettler Toledo, Switzerland) was used to measure the pH, oxidation-reduction potential (ORP), dissolved oxygen (DO), and total dissolved solids (TDS) of water. The residual chlorine and the total chlorine concentration were analyzed according to the N, N-diethyl-1, 4-phenylenediamine (DPD) method. The adenosine triphosphate (ATP) was measured to characterize the microbial activity. The abundance of total bacteria and *Pseudomonas aeruginosa* were counted by the heterotrophic plate count (HPC) method and ISO 16266:2006 method, respectively (Casanovas-Massana et al., 2010). The cytotoxicity of the drinking water was evaluated by colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Text S4).

2.4. DNA extraction, qPCR, and bacterial community analysis

DNA of bacteria in water and biofilm was extracted by the FastDNA SPIN Kit (MP Biomedicals, Solon, OH, USA). The number of 16S rRNA genes for total bacteria and different opportunistic pathogens, including *Legionella spp.* and *Pseudomonas aeruginosa*, were quantified by quantitative polymerase chain reaction (qPCR) using a 7300 qPCR system (ABI 7300, Applied Biosystems, Singapore) in a 25 μL reaction system. All primers, probes, amplification efficiencies, and the LOQ are given in Table S4 and Table S5. The 25 μL mixture consisted of: 0.5 μL ROX (50 ×, TaKaRa, China), 12.5 μL SYBR Premix Ex Taq (TaKaRa, China), 1 μL extracted DNA, 1 μL of 10 μM forward and reverse primer, 1 μL of 3 μM probe (if necessary) and 9 or 10 μL double-distilled H_2O. The negative control (double-distilled H_2O) and ten-fold gradient dilutions of the positive standard were included in each qPCR run. A melt curve analysis was conducted to verify the specificity of the primers in each run.

Moreover, the bacterial community analysis was performed on an Illumina HiSeq-PE250 platform. QIIME software package was utilized to analyze the sequences. RDP classifier was used to assign taxonomic data to each representative sequence. Sequences were assigned to operational taxonomic units (OTUs) with 97% similarity. The metabolic pathways of the bacterial community were predicted through the PICRUSt software. The raw sequence data has been uploaded to NCBI with accession number PRJNA1132754.

2.5. PE and PVC pipes surface characterization

The plastic coupons were removed from the bottom of PE and PVC pipes and dried in a dark environment for 24 hours before measurement. The surface functional groups of PE and PVC pipes were analyzed using a Thermo Fisher Scientific FTIR spectrometry (Nicolet iN 10MX). The hydrophilicity was measured by the sessile droplet method using an OCA15EC contact angle meter (Dataphysics, Germany). Each sample was measured three times. The morphology of the PE and PVC pipes surface was analyzed using a S4800 scanning electron microscope (SEM, Hitachi, Japan). In addition, the morphology and surface roughness of PE and PVC pipes were characterized by an atomic force microscopy (AFM, FASTSCANBIO, Bruker, Germany) that held a conventional silicon nitride (Si_3N_4) tip (NP-10, Bruker) with a nominal spring constant of 0.06 N/m.

3. Results and discussion

3.1. Release of PAEs from PE and PVC pipes

A total of six PAEs were detected in the effluents of four groups of simulated drinking water distribution pipes (Table S6), including DMP, DEP, DBP, DIBP, DMEP, and DEHP. The total released PAEs content ($\sum_6$PAEs) varied from 5.07 to 8.88 μg/L (Fig. 1a). Without disinfection, the total concentration of released PAEs in the effluents of PE and PVC pipes ranged from 5.07 to 6.44 μg/L and 5.85 to7.27 μg/L, respectively. With NaClO disinfection, the total concentration of released PAEs in the effluents of PE-Cl_2 and PVC-Cl_2 pipes were 6.60~7.87 μg/L and 7.45~8.88 μg/L, respectively. PAEs released from PVC pipes were higher than PE pipes. The different plasticizer content in PE and PVC pipes may be one reason for the difference in PAEs release of these two groups (Yan et al., 2021). However, it is still reasonable to conclude that NaClO disinfection accelerated the PAEs release from PE and PVC pipes. This result is consistent with our previous findings that the release of PAEs increased after the addition of disinfectants to plastic pipes (Yin et al., 2023; Wang et al., 2023).

Due to the low concentration of PAEs, PAEs released from different groups of pipes didn't change the DOC concentration in effluents ($p > 0.05$) (Fig. 1 b). However, the dominant PAEs were different in the PE and PVC groups. In effluents of PE pipes, the concentration of released DMP, DEP, DIBP, DBP, DMEP, and DEHP, was 0.21~0.50 μg/L, 0.13~0.36 μg/L, 2.29~3.59 μg/L, 1.71~2.14 μg/L, 0.09~0.20 μg/L, and 0.11~0.17 μg/L, respectively (Table S6). In effluents of PVC pipes, the concentration of these released PAEs was 1.89~2.52 μg/L, 1.75~2.27 μg/L, 0.15~1.19 μg/L, 1.00~1.48 μg/L, 0.10~0.20 μg/L, and 0.32~0.43 μg/L, respectively. DBP and DIBP were the main PAEs components in the effluents of PE and PE-Cl_2 groups, while DMP, DEP, and DEHP were the main components of PVC and PVC- Cl_2 groups. Paluselli et al. (2019) claimed that PE bags easily release DIBP and di-n-butyl phthalate (DnBP), while PVC cables are prone to release DMP and DEP. The RQ results of this study indicated that DBP exposure risk was the highest, followed by DEHP and DEP (Fig. S1). According to the Chinese drinking water standard (Standardization Administration of China, 2022), the RQ values of DBP in all samples were at a medium risk level. According to the USEPA drinking water standard (2009), the RQ values of DEHP in 72.7% of the samples were at a medium risk level after adding chlorine to PVC pipes. Hence, chlorine disinfection indeed enhanced the PAEs release in PE and PVC pipes and increased chemical risk of the drinking water in plastic distribution pipes. Moreover, our previous research also indicated that PAEs released from aged PVC pipes facilitated the growth of ClO_2-resistant bacteria, which elevated the microbial risk of drinking water (Wang et al., 2023). Therefore, it is clear that the release and the composition of PAEs could affect the drinking water quality.

3.2. Effects of PAEs release on the molecular characterization of DOM

DOM molecular characteristics were analyzed using FT-ICR-MS (Table S7). No significant difference was observed in the FT-ICR-MS average elemental ratios and the molecular parameters of DOM between the feed water with and without chlorination. However, the DOM molecular characteristics, such as double bond equivalents (DBE_{wa}), $DBE\text{-}O_{wa}$, and nominal oxidation state of carbon ($NOSC_{wa}$), changed greatly after running the unchlorinated feed water/chlorinated feed water in the simulated distribution pipes. For PE pipes, chlorine disinfection resulted in an increase in DBE_{wa} and $DBE\text{-}O_{wa}$ of DOM in effluents, indicating the presence of more compounds with C=C formation (Chen et al., 2021). However, for PVC pipes, chlorine disinfection significantly decreased $DBE\text{-}O_{wa}$ but not DBE_{wa}, which means that the chlorine disinfection induced more compounds with C=O formation (Gonsior et al., 2009). In addition, the value of H/C_{wa} (1.27/1.27) was higher in PE and PE-Cl_2 pipes than

(1.23/1.23) in PVC and PVC-Cl_2 pipes. The value of $NOSC_{wa}$ (-0.27/-0.27) was lower in PE and PE-Cl_2 pipes than (-0.21/-0.21) in PVC and PVC-Cl_2 pipes. Compounds with higher H/C_{wa} and lower $NOSC_{wa}$ indices are hard to react with chlorine and are not easy to be used by microorganisms (Chen et al., 2021a; LaRowe et al., 2011). Therefore, the compounds in PE and PE-Cl_2 pipes are not conducive to the growth of bacteria in distribution pipes.

The MS intensity of molecular formulas related to $C_{10}H_{10}O_4$, $C_{12}H_{14}O_4$, and $C_{16}H_{22}O_4$ in the effluents of PE and PVC pipes with chlorine disinfection was higher than that in pipes without disinfection (Fig. S2). This tendency was consistent with the variation of DMP, DEP, and DBP (or DIBP) concentration in the effluents of different pipes. In addition, the number of CHO was 2348, 2506, 2478, and 2536 in the effluents DOM of PE, PE-Cl_2, PVC, and PVC-Cl_2 pipes, respectively (Fig. 2). The number of CHO in the DOM of effluents from different plastic pipes was higher than that in the influents (feed water/chlorinated feed water, Fig. S3), showing similar changes to the change in the total released PAE concentration in the effluents of various distribution pipes. Therefore, PAEs released from pipes may potentially affect the DOM composition. The DOM can be divided into lipids, proteins, carbohydrates, unsaturated hydrocarbons, lignins, tannins, and aromatic structures (Xu et al., 2020). The number of proteins, lignins, and tannins in the DOM of effluents from different plastic pipes were higher than that in influents (feed water/chlorinated feed water, Fig. S4). Moreover, chlorine disinfection increased the number of proteins, lignins, and tannins in DOM (Fig. S5). The number of tannins showed the same changes with the total PAEs concentration in the effluents of different distribution pipes. Therefore, it can be inferred that the release of PAEs altered the DOM composition, hence affecting the drinking water quality.

3.3. Cytotoxicity of water and quantification of opportunistic pathogens

The cytotoxicity evaluation results indicated that the relative viability of LO2 cells incubated with the unchlorinated feed water and chlorinated feed water was 75.2% and 70.8%, respectively (Fig. S6). In addition, the relative viability of LO2 cells incubated with water samples from PE, PE-Cl_2, PVC, and PVC-Cl_2 pipes was 69.3%, 52.6%, 61.8%, and 45.8%, respectively (Fig. 3a). Compared to feed water (with and without chlorination), the cytotoxicity increased after the water went through the different plastic pipes. Water from plastic pipes with higher PAEs release has higher cytotoxicity. The DBP, DIBP, DEHP, DEP, and DMP detected in our studies are also common PAEs components found in bottled water and tap water (Yin et al., 2023; Luo et al., 2018). DBP and DIBP have been identified as dominant contributors to androgen receptor antagonistic potencies (Hu et al., 2013; Li et al., 2010). In addition, PAEs, including DBP, DIBP, and DEHP, are considered to be the major contributors to cytotoxicity (Chang et al., 2023). Thus, PAEs release leads to an increase in the DOM with CHO, which increases the cytotoxicity of drinking water, especially in PVC pipes with disinfection.

Except for cytotoxicity, the microbial risk of the water in different pipes was also analyzed (Fig. 3b-d). The average HPC of effluents from PE, PE-Cl_2, PVC, and PVC-Cl_2 pipes was 613 CFU/mL, 152 CFU/mL, 1113 CFU/mL, and 400 CFU/mL, respectively (Fig. 3b). The difference between PE and PVC groups is because the DOM with a higher NOSCwa index in PVC and PVC-Cl_2 pipes is conducive to bacteria growth in distribution pipes. Correspondingly, the mean ATP, which can be used to reflect bacterial activity (Liu et al., 2013), was 0.097 nmol/mL, 0.082 nmol/mL, 0.191 nmol/mL, and 0.099 nmol/mL, respectively, in effluents of these four pipes (Fig. 3c). Moreover, the average plate counts of *P. aeruginosa* were 424 CFU/mL, 221 CFU/mL, 577 CFU/mL, and 337 CFU/mL, respectively, in effluents of PE, PE-Cl_2, PVC, and PVC-Cl_2 pipes (Fig. 3d). The qPCR results for 16S rRNA and *P. aeruginosa* were consistent with the plate counts of HPC and *P. aeruginosa* (Fig. S7). Although *Legionella* spp. was not detected using plate methods, the qPCR results also showed higher concentrations in effluents of PVC and PVC-Cl_2 pipes (Fig. S7b). In brief, the release of PAEs from plastic pipes changes the DOM composition, promotes biofilm formation and the growth of opportunistic pathogens, and increases the microbial risk of drinking water. Besides, the PVC and PVC-Cl_2 pipes have higher microbial risk than PE and PE-Cl_2 pipes.

3.4. Bacterial community composition in biofilm and effluents of different pipes

The alpha diversity of bacterial communities in the influents, effluents, and biofilms of different drinking water distribution pipes is shown in Table S8. For the influents, the alpha diversity index was lower in chlorinated feed water than in feed water without chlorination, indicating that chlorine disinfection effectively reduced the alpha diversity of the bacterial community in influents. For effluents and biofilm, the alpha diversity index was similar in PE and PVC pipes and was higher than that in PE-Cl_2 and PVC-Cl_2 pipes, revealing that chlorine disinfection can decrease the alpha diversity of the bacterial community in both PE and PVC pipes.

In terms of dominant bacteria, for undisinfected feed water and chlorinated feed water, the dominant bacteria were Proteobacteria (50.4%~76.5%) and Cyanobacteria (13.0%~28.6%) at the phylum level. At the genus level, the dominant bacteria were *Achromobacter* (30.7%~37.5%), *unclassified_o_Chloroplast* (9.33%~26.9%), and *Sphingomonas* (1.48%~17.7%) (Fig. S8). For biofilm, at the phylum level, the main bacteria were Proteobacteria (60.2%~89.8%), followed by Planctomycetota (0.03%~19.0%), Actinobacteriota (1.27%~10.7%), and Firmicutes (0.013%~13.3%)

(Fig. 4a). In both kinds of pipes, chlorine disinfection increased the relative abundance of Proteobacteria but decreased the relative abundance of Planctomycetota in biofilm. Proteobacteria has high adaptability and wide prevalence in the biofilm of plastic pipes (Goraj et al., 2021). One previous study reported that the abundance of Proteobacteria increased in the water after chlorination treatment (Hou et al., 2018). At the genus level, the main bacteria in the biofilms were *unclassified_f__Isosphaeraceae* (13.1%~17.6%), *Elstera* (14.6%~14.7%), and *Methyloversatilis* (8.60%~11.5%) in PE pipes, while *Brevundimonas* (18.2%~20.3%) and *Methylobacterium-Methylorubrum* (17.1%~18.9%) were the main bacteria in PVC pipes (Fig. 4b). Chlorine disinfection reduced the abundance of these bacterial genera, making *Hyphomicrobium* (22.2%~31.4%), *Sphingomonas* (17.9%~22.9%), *norank_f__Hyphomonadaceae* (10.0%~13.5%), and *Phreatobacter* (8.50%~22.8%) became the predominant bacteria in PE-Cl_2 and PVC-Cl_2 pipes. *Hyphomicrobium* has been identified as the predominant bacteria in the biofilm of PVC taps (Liu et al., 2014). *Sphingomonas* has a stronger disinfectant resistance (Yin et al., 2023) and may be a potential hazard to immunocompromised patients (Johnson et al., 2018). The Stamp analysis indicated that *Mycobacterium* and *Rhodobacter* were enriched in the biofilm of PVC-Cl_2 pipes, while *Blastomonas* was enriched in the biofilm of PE-Cl_2 pipes (Fig. S9). The different bacterial community composition in biofilm can affect the pipe surfaces characteristics, which would influence the PAEs release in different pipes.

For effluents, at the phylum level, the main bacteria were also Proteobacteria (71.7%~96.9%) and Planctomycetota (0.05%~14.9%) in PE and PVC pipes (Fig. 4c). Chlorine disinfection increased the abundance of Proteobacteria but decreased the abundance of Planctomycetota, which was similar to the situation in biofilm. At the genus level, *Brevundimonas* (2.57%~54.8%), *Sphingomonas* (2.97%~23.5%), *Methylobacterium-Methylorubrum* (6.13%~7.33%), *Sphingobium* (1.21%~13.5%), and *Blastomonas* (4.32%~7.07%) were the main bacteria in both kinds of pipes (Fig. 4d). Chlorine disinfection resulted in the predominance of *Sphingomonas* (29.8%~80.9%), DSSF69 (5.17%~21.9%), and uncultured_f_*Hypomonadaceae* (1.43%~14.7%) in both types of pipes. The Stamp analysis showed that *Methyloversatilis* and *Aquabacterium* were enriched in PE pipes, while *Brevundimonas* was enriched in PVC pipes (Fig. S10). Moreover, *unclassified_f__Isosphaeraceae* was enriched in PE-Cl_2 pipes, while *Amphiplicatus*, *Novosphingobium*, and *Blastomonas* were enriched in PVC-Cl_2 pipes. The different bacterial community composition in effluents would result in different microbial risk of the drinking water from PE and PVC pipes with or without chlorine disinfection.

3.5. Bacterial community metabolic pathways in different pipes

For influents, the mean metabolic pathway of the bacterial community was metabolism (76.0%~76.5%) at level 1 (Fig. S11). At level 2, the main metabolic pathways related to human disease were drug resistance: antimicrobial (16.3%~22.2%), infectious disease: bacterial (15.7%~18.1%), and Cancer: overview (15.0%~16.4%). For biofilm, the mean metabolic pathways of bacterial community in different pipes were metabolism (74.9%~76.3%), environmental information processing (5.50%~6.45%), genetic information processing (5.44%~6.13%), cellular processes (4.97%~5.84%), human diseases (5.21%~5.64%), and organismal systems (1.81%~1.92%) at level 1 (Fig. 5a). After chlorine disinfection, the relative abundance of pathways related to metabolism, genetic information processing, and human diseases increased, while the relative abundance of other pathways decreased. At level 2, the mean pathways included global and overview maps (38.7%~39.0%), carbohydrate metabolism (8.29%~8.53%), amino acid metabolism (7.54%~7.84%), energy metabolism (4.29%~4.63%), and metabolism of cofactors and vitamins (4.02%~4.42%) in different pipes (Fig. 5b). The amino acid metabolism is related to the extracellular polymeric substances (EPS) secretion, which plays a significant role in the attachment of bacteria onto the surface of pipes (Wu et al., 2023). Carbon metabolism can determine the bioaugmentation efficiency under different carbon source conditions (Wu et al., 2024). Micropollutants can induce toxicity by influencing energy metabolism (Zheng et al., 2022). Moreover, the metabolic pathways of the bacterial community in the biofilm of PE and PVC with or without chlorine disinfection showed differences (Fig. S12). For example, the relative abundance of cellular community-prokaryotes and membrane transport pathways was higher in PE-Cl_2 pipes, while the abundance of biosynthesis of other secondary metabolites pathways was higher in PVC-Cl_2 pipes. The above results reflected that chlorine disinfection could affect the metabolism pathway of bacteria, resulting in different interactions between bacteria and pipe surfaces.

In addition, the mean metabolic pathway of the bacterial community in effluents of different pipes was also the metabolism (75.4%~76.1%) (Fig. 5c). The pathway related to human disease is essential for water quality evaluation. The mean pathways related to human disease in effluents included infectious disease: bacterial (13.3%~18.1%), cancer: overview (13.7%~15.4%), drug resistance: antimicrobial (13.4%~16.7%), neurodegenerative disease (12.4%~12.8%), and infectious disease: viral (9.84%~11.9%) (Fig. 5d). Although chlorine disinfection can inhibit the pathogenic bacteria growth in drinking water, only the relative abundance of infectious disease: bacterial, infectious disease: viral, and drug resistance: antineoplastic pathways decreased after disinfection. The relative abundance of all other pathways related to human disease increased after chlorine disinfection, such as drug resistance:

antimicrobial, cancer: overview, and cardiovascular disease. The relative abundance of these human disease pathways in PVC-Cl_2 pipes was higher than that in PE-Cl_2 pipes. In short, although chlorine disinfection can effectively reduce the activity of several infectious disease-related pathways in bacterial community metabolic, higher risks of other diseases were induced, especially for PVC pipes.

3.6. PAEs release mechanism in different pipes

SEM results showed the surface morphology of PE and PVC pipes with different treatments (Fig. S13). There were more cracks and cavities on the surface of PVC pipes than PE pipes, which contributed to the enhanced PAEs release from PVC pipes. After five months of running, the biofilm was formed on the surface of PE and PVC pipes. Although chlorine disinfection inhibited biofilm formation, it still caused more cracks and cavities, especially for PVC pipes. The formation of cavities on plastic surfaces suggested an increase in surface roughness (Yan et al., 2021). AFM results showed the surface morphology and roughness of different pipes (Fig. 6). The roughness of virgin PE and PVC pipes was 1.71 nm and 2.70 nm, respectively. Since the biofilm formed on the pipe surface can enhance the pipe surface roughness (Wang et al., 2022), the surface roughness of PE and PVC pipes fed with undisinfected water increased to 42.6 nm and 45.5 nm, respectively. In contrast, chlorine disinfection reduced the biomass of biofilms, resulting in the lower roughness of the pipe surface treated with chlorinated water. The roughness was 19.0 nm and 43.8 nm for PE-Cl_2 and PVC-Cl_2 pipes, respectively. The surface morphology and roughness revealed by the AFM images were consistent with the SEM results.

According to FTIR spectra results, the peaks of virgin PE pipes were observed at 2990 cm^{-1}, 2840 cm^{-1}, 1460 cm^{-1}, and 720 cm^{-1}, while the peaks of virgin PVC pipes were found at 2925 cm^{-1}, 2850 cm^{-1}, 1740 cm^{-1}, 1430 cm^{-1}, 1430 cm^{-1}, and 615 cm^{-1}.

After five months of running, new peaks at 1740 cm^{-1}, 1260 cm^{-1}, 1010 cm^{-1}, and 860 cm^{-1} were observed on the surface of PE and PE-Cl_2 pipes (Fig. 7a). These peaks might be attributed to the tensile vibration of carbonyl groups (C=O), the double bond stretching of P=O of general phosphoryl groups and phosphodiester of nucleic acids, C-O-C of polysaccharides, and C-C stretching vibrations (Sun et al., 2021; Ojeda et al., 2008; Chen et al., 2013; Dittrich et al., 2005). Moreover, the intensity of peaks at 1740 cm^{-1} and 1260 cm^{-1} was enhanced on the surface of PVC and PVC-Cl_2 pipes (Fig. 7b). The new peaks at 1010 cm^{-1} and 860 cm^{-1} also occurred on the surface of PVC and PVC-Cl_2 pipes. Chlorine disinfection enhanced the peak intensity of C=O but decreased the peak intensity of P=O, C-O-C, and C-C on the surface of both PE and PVC pipes. The P=O, C-O-C, and C-C formation on the surface of pipes are key indicators of biofilm formation. These results suggested that chlorine disinfection accelerated the oxidation process of the plastic pipes and inhibited bacteria growth on the pipe surface. The peak intensity of C=O groups on the pipe surface reflected the oxidation potential of the pipes by oxygen or chlorine in the water (Yan et al., 2021; Sun et al., 2021). The increase in hydrophilicity was probably due to the incorporation of oxygen-containing functional groups on plastic surfaces (Sun et al., 2021). In addition, the static water contact angle was detected to characterize the hydrophilicity of various plastic pipe surfaces (Fig. 7c). The contact angle of virgin PE and PVC pipes was 101.9º and 98.4º, respectively. After five months of running, biofilm formed on the surface of different pipes, and the contact angle of PE and PVC pipes (fed with undisinfected water) decreased to 89.0º and 82.0º, respectively. Chlorine disinfection was applied to prevent biofilm formation on the PE-Cl_2 and PVC-Cl_2 pipe surfaces, while it also induced lower contact angles of these pipe surfaces. The increase in hydrophilicity changed the interactions between plastic pipes and plasticizers, such as PAEs, which promoted the release of PAEs from the plastic pipes (Yan et al., 2021; Sun et al., 2021). PVC material has been reported to be conducive to the assimilable organic carbon (AOC) concentration increase in water, therefore promoting bacterial growth (Chen et al., 2021). In this study, the PAEs released from PVC pipes were more than those released from PE pipes, and the DOM composition contributed to the bacteria growth and biofilm formation on pipe surfaces. The release mechanism of PAEs could be generally concluded as the chlorine disinfection used for inhibiting biofilm formation increased the hydrophilicity of pipe surfaces. The accelerated PAEs release increased the cytotoxicity and bacterial metabolic pathways related to human disease of the drinking water.

4. Conclusion

In this study, PAEs released from PVC pipes were higher than from PE pipes. Chlorine disinfection accelerated the PAEs release, and the total concentration of released PAEs in the effluents of PE-Cl_2 and PVC-Cl_2 pipes were 6.60~7.87 μg/L and 7.45~8.88 μg/L, respectively. FT-ICR-MS results indicated that CHO number and tannins composition of DOM varied in a similar trend to the total PAEs concentration in effluents of different distribution pipes. The cytotoxicity test suggested that a higher water cytotoxicity was observed in the plastic pipes with a higher concentration of released PAEs. Moreover, the average plate counts of *P. aeruginosa* were 424 CFU/mL, 221 CFU/mL, 577 CFU/mL, and 337 CFU/mL, respectively, in effluents of PE, PE-Cl_2, PVC, and PVC-Cl_2 pipes. Chorine disinfection reduced the abundance of pathogenic bacteria but increased the abundance of metabolic pathways related to human diseases, such as drug resistance: antimicrobial, cancer: overview, and cardiovascular disease. In addition, chlorine disinfection could affect the metabolism process of bacteria, resulting in different interactions between bacteria and pipe surfaces. Chlorine disinfection reduced the biofilm biomass and further decreased the roughness and contact angle of the pipe surface. The increased hydrophilicity changed the binding interactions between plastic pipes and the PAEs, which accelerated the release of PAEs from plastic pipes. Thus, it can be briefly concluded that chlorine disinfection enhanced the PAE release from plastic pipes to water, leading to higher cytotoxicity and microbial risk of drinking water, especially in PVC-Cl_2 pipes. Considering all the above findings, a series of factors, including the optimal pipe types, disinfection method, and disinfectant dose, should be comprehensively optimized to monitor and maintain drinking water quality in distribution pipes.

Declaration of competing interest

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.

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Supplementary materials

Supplementary material associated with this article can be found in the online version.

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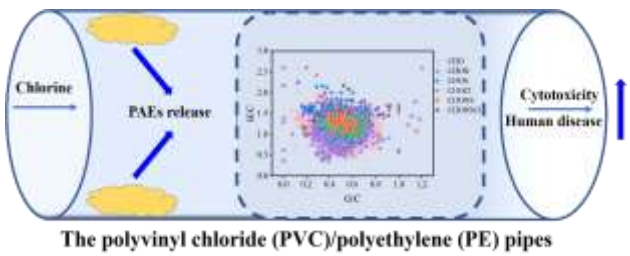
Graphical Abstract



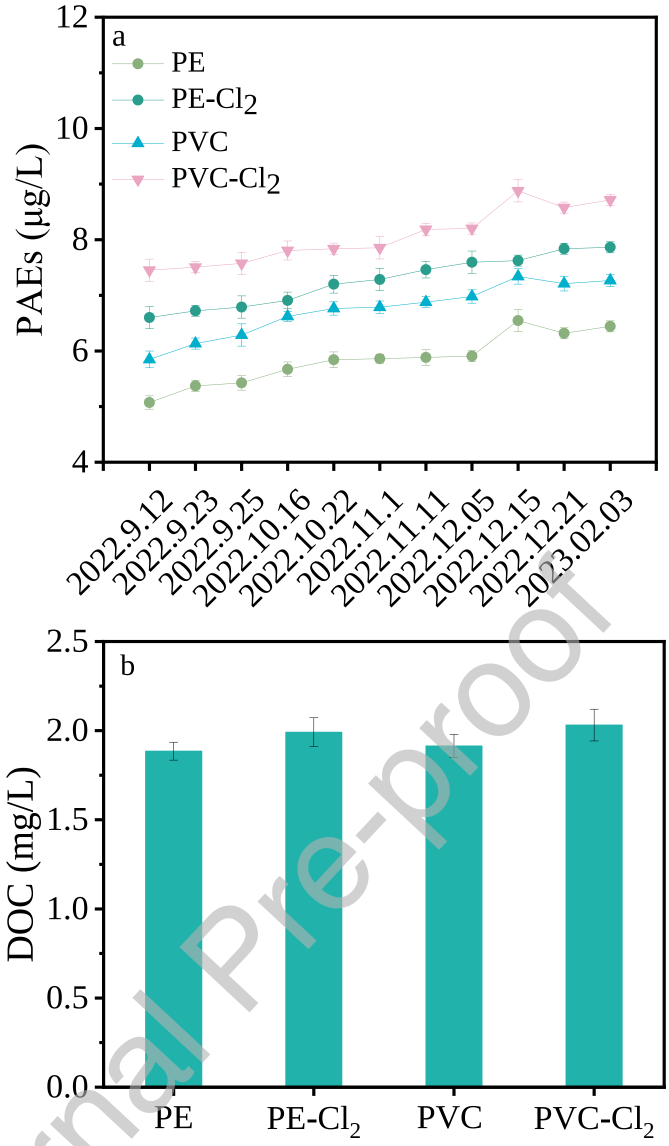
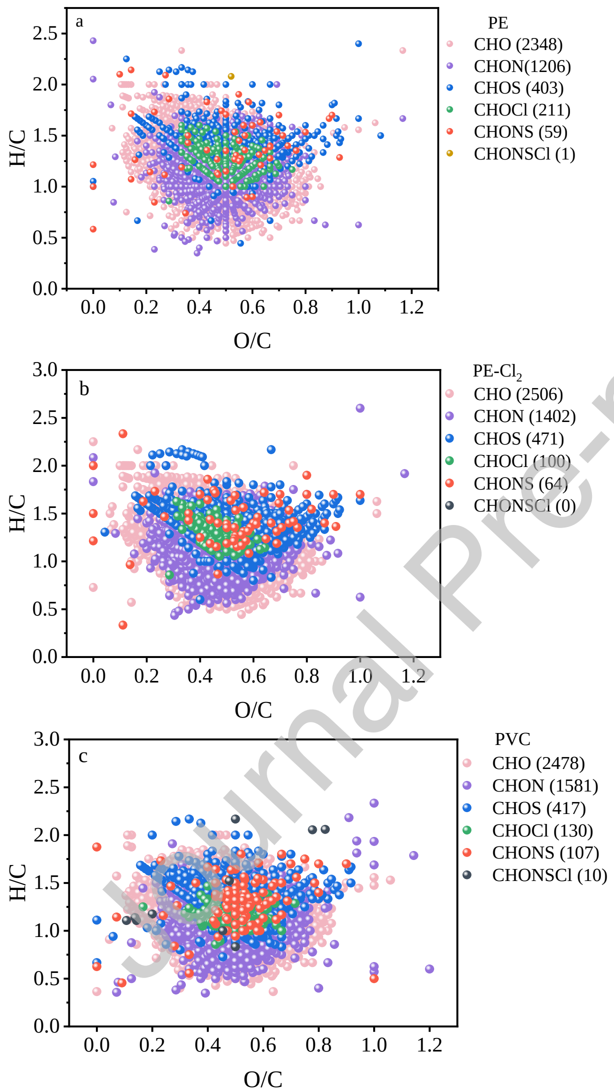


Fig. 1. (a) The concentration of total released phthalate acid esters (PAEs), and (b) the dissolved organic carbon (DOC) concentration in effluents of different groups of drinking water distribution pipes after 48 h running.


a
H/C
O/C
PE
CHO (2348)
CHON(1206)
CHOS (403)
CHOCl (211)
CHONS (59)
CHONSCl (1)
b
PE-Cl2
CHO (2506)
CHON (1402)
CHOS (471)
CHOCl (100)
CHONS (64)
CHONSCl (0)
c
PVC
CHO (2478)
CHON (1581)
CHOS (417)
CHOCl (130)
CHONS (107)
CHONSCl (10)

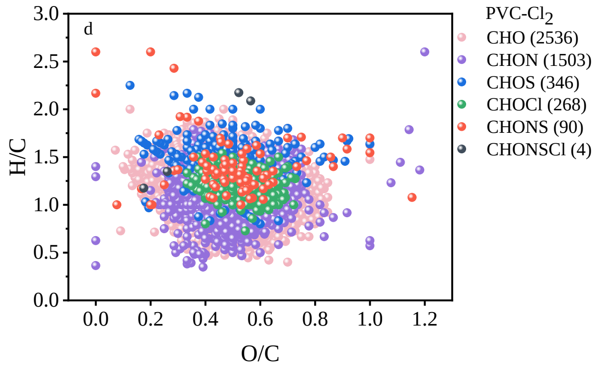


Fig. 2. Van Krevelen diagrams of the molecular formulas of dissolved organic matter (DOM) composition in different groups of water samples for (a) PE, (b) PE-Cl_2, (c) PVC, and (d) PVC- PE-Cl_2 pipes.

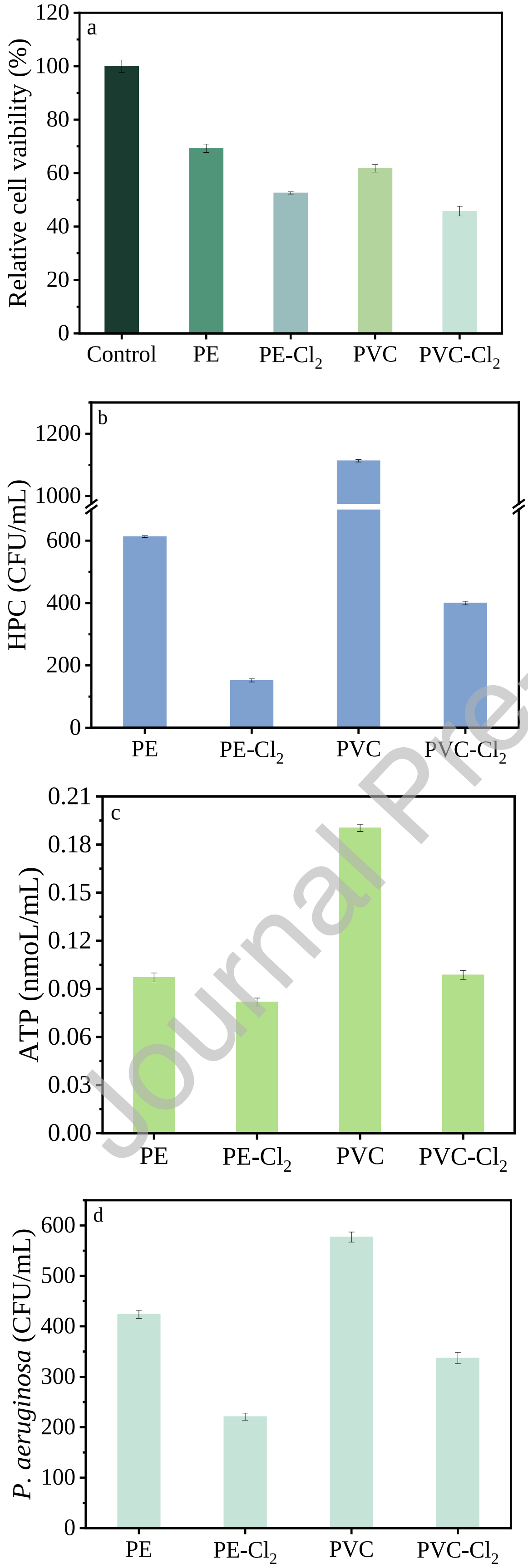

a
Relative cell vaibility (%)
120
100
80
60
40
20
0
Control
PE
PE-Cl2
PVC
PVC-Cl2
b
HPC (CFU/mL)
1200
1000
600
400
200
0
PE
PE-Cl2
PVC
PVC-Cl2
c
ATP (nmoL/mL)
0.21
0.18
0.15
0.12
0.09
0.06
0.03
0.00
PE
PE-Cl2
PVC
PVC-Cl2
d
P. aeruginosa (CFU/mL)
600
500
400
300
200
100
0
PE
PE-Cl2
PVC
PVC-Cl2

Fig. 3. (a) The cytotoxicity, (b) heterotrophic plate counts (HPC) of bacteria, (c) adenosine triphosphate (ATP), and (d) the plate counts of *Pseudomonas aeruginosa* in the effluents of different distribution pipes.

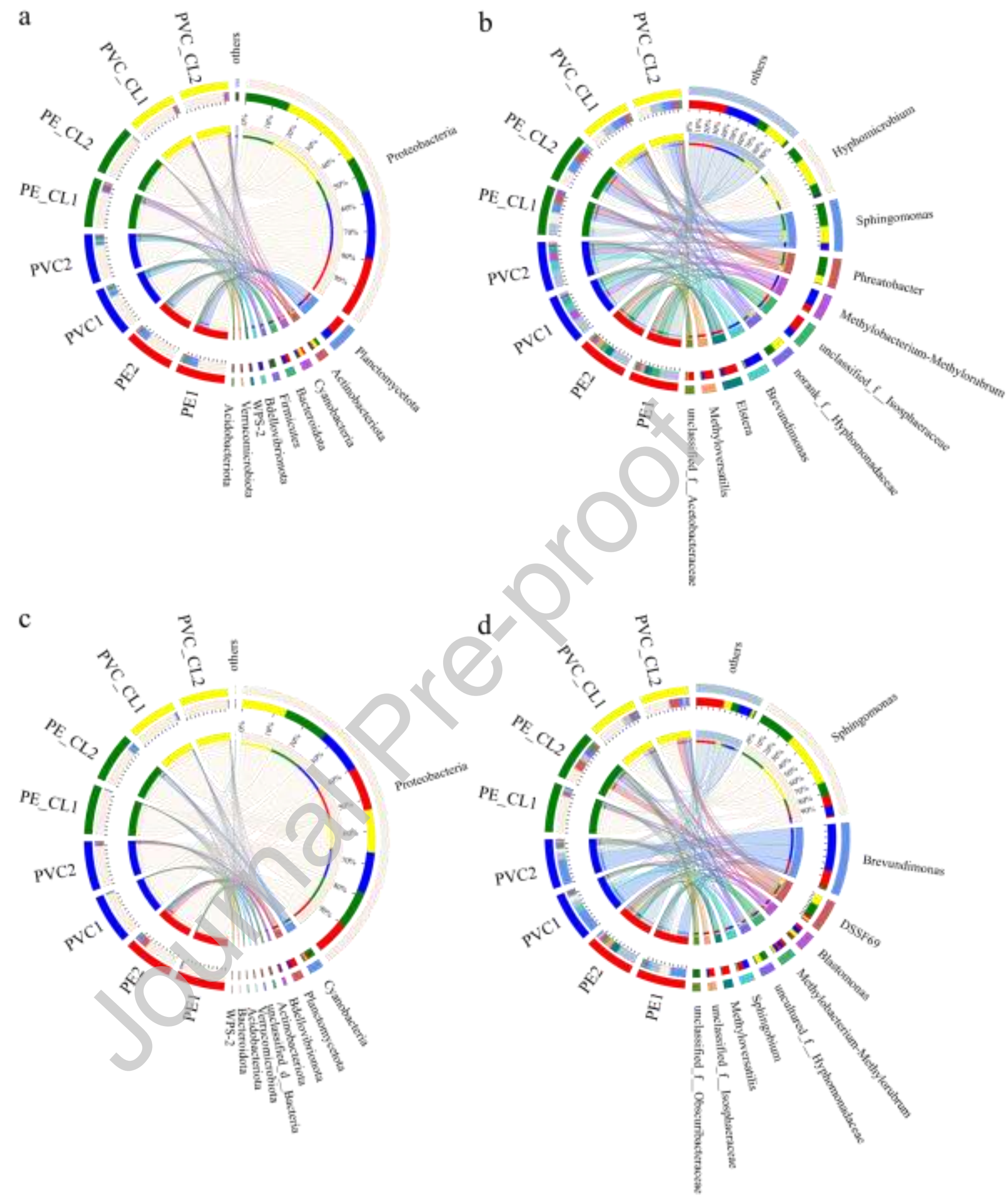


Fig. 4. The relative abundance of bacterial community composition at phylum (a, c) and genus (b, d) level in biofilm (a, b) and effluents (c, d) of different groups of pipes.

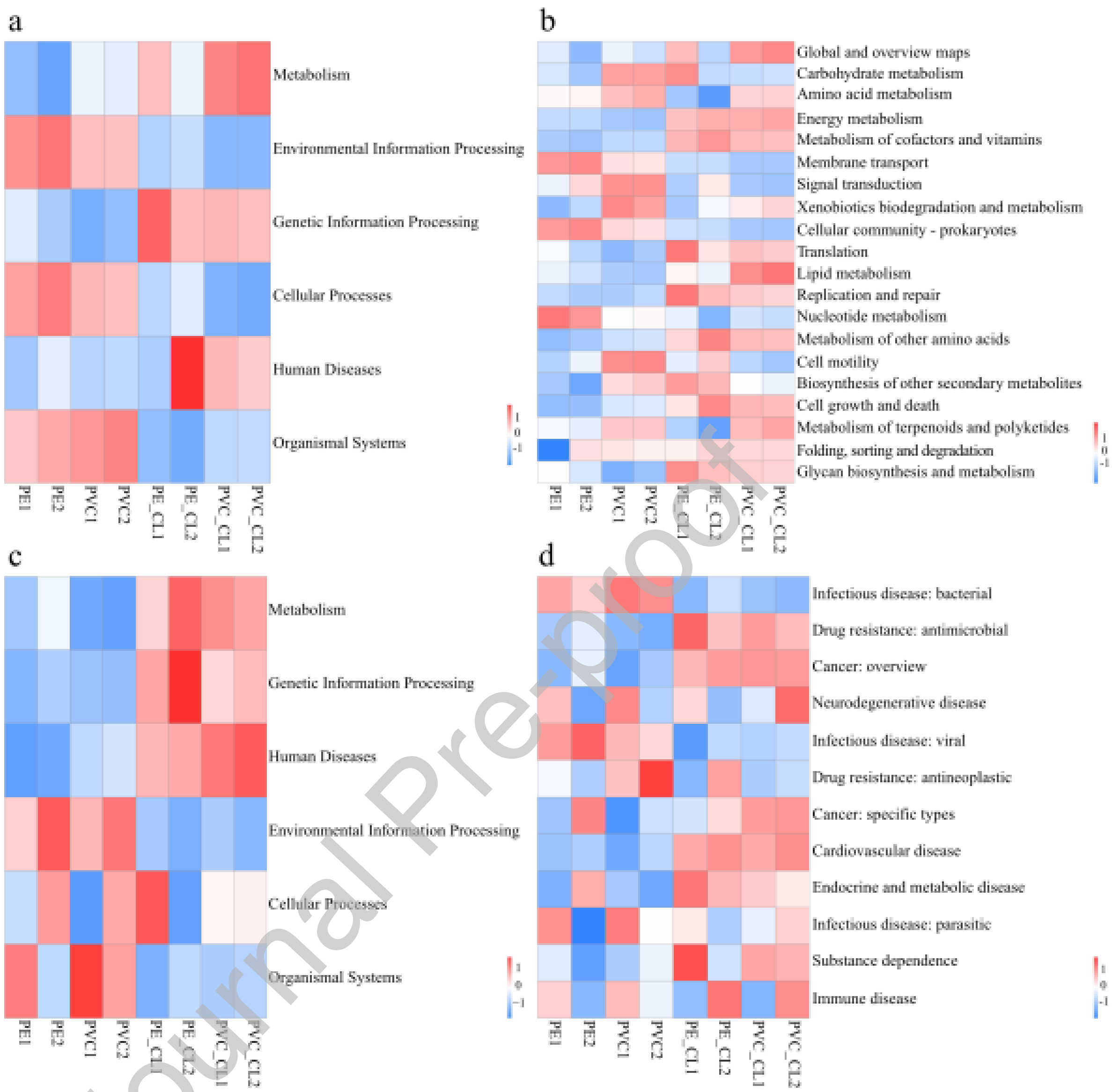


Fig. 5. The heatmap of the relative abundance of different metabolic pathways of the bacterial community at level 1 (a, c), and level 2 (b, d) in the biofilm (a, b) and effluents (c, d) of different groups of pipes.

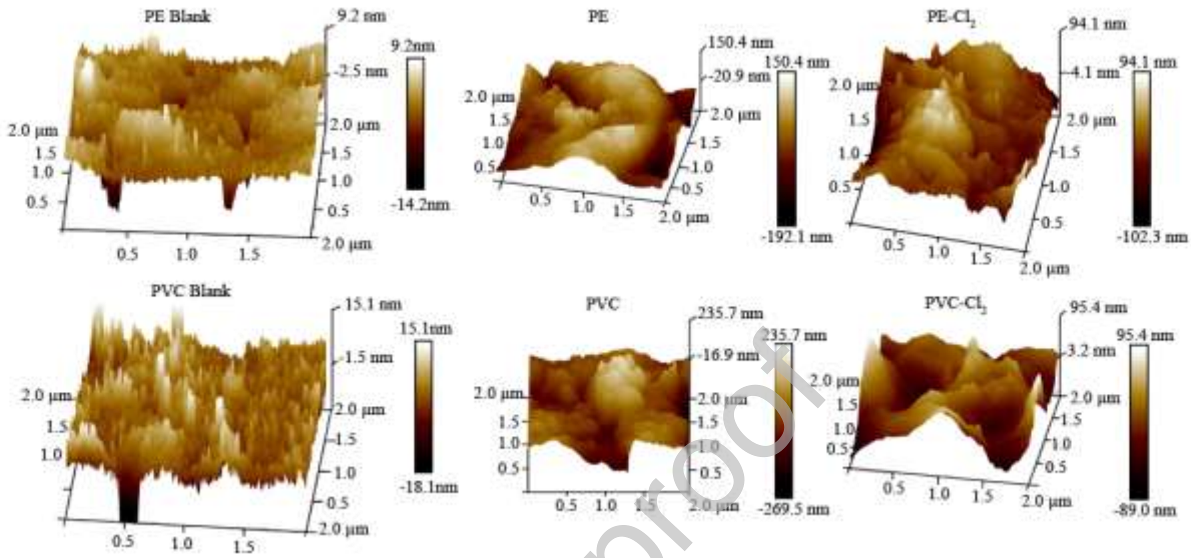


Fig. 6. The surface morphology and roughness of PE and PVC pipes before treatment and after five months of running with and without chlorine disinfection characterized by atomic force microscopy (AFM).

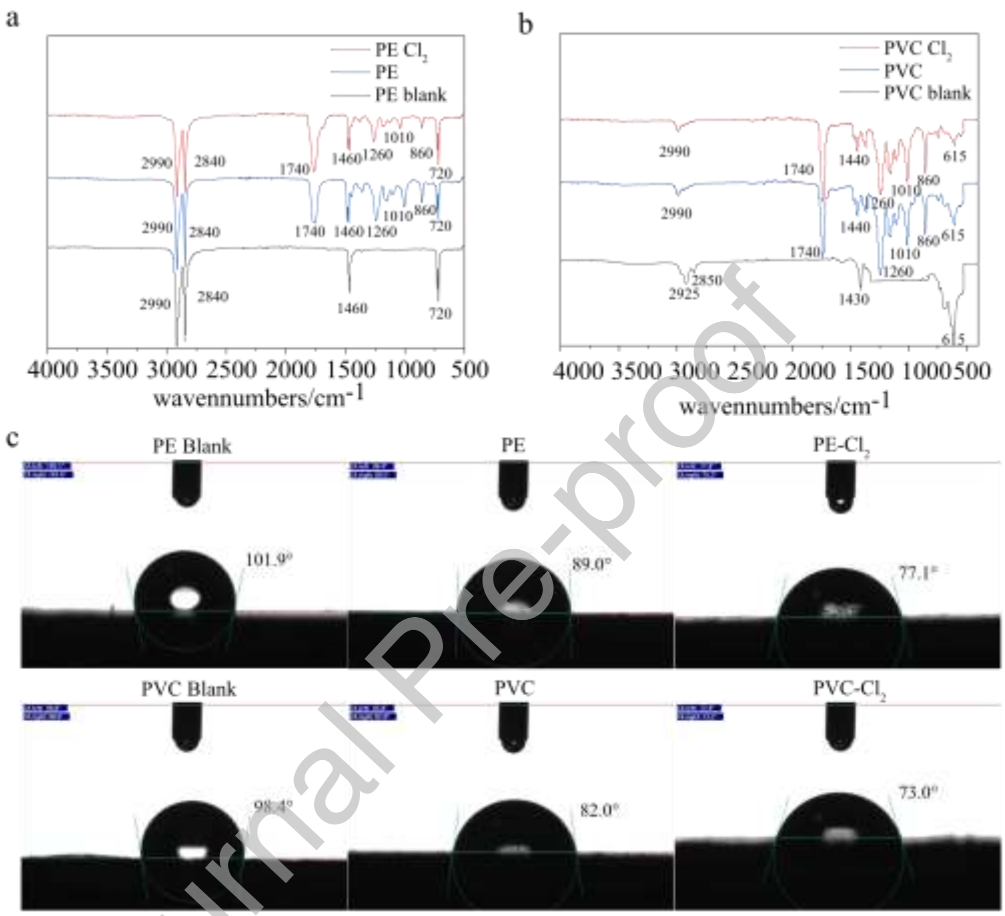


Fig. 7. The FTIR spectra (a, b) and the contact angle images (c) of PE and PVC pipes before treatment and after five months of running with and without chlorine disinfection.

Declaration of Interest Statement

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: