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Translocation and metabolism of tricresyl phosphate in rice and microbiome system: Isomer-specific processes and overlooked metabolites

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ABSTRACT

Tricresyl phosphate (TCP) is extensively used organophosphorus flame retardants and plasticizers that posed risks to organisms and human beings. In this study, the translocation and biotransformation behavior of isomers tri-p-cresyl phosphate (TpCP), tri-m-cresyl phosphate (TmCP), and tri-o-cresyl phosphate (ToCP) in rice and rhizosphere microbiome was explored by hydroponic exposure. TpCP and TmCP were found more liable to be translocated acropetally, compared with ToCP, although they have same molecular weight and similar K_{ow}. Rhizosphere microbiome named microbial consortium GY could reduce the uptake of TpCP, TmCP, and ToCP in rice tissues, and promote rice growth. New metabolites were successfully identified in rice and microbiome, including hydrolysis, hydroxylated, methylated, demethylated, methoxylated, and glucuronide- products. The methylation, demethylation, methoxylation, and glycosylation pathways of TCP isomers were observed for the first time in organisms. What is more important is that the demethylation of TCPs could be an important and overlooked source of triphenyl phosphate (TPHP), which broke the traditional understanding of the only man-made source of toxic TPHP in the environment. Active members of the microbial consortium GY during degradation were revealed and metagenomic analysis indicated that most of active populations contained TCP-degrading genes. It is noteworthy that the strains and function genes in microbial consortium GY that responsible for TCP isomers' transformation were different. These results can improve our understanding of the translocation and transformation of organic pollutant isomers in plants and rhizosphere microbiome.

1. Introduction

Organophosphate esters (OPEs) are extensively applied as plasticizers and flame retardants in various kinds of industrial products. As typical nonreactive additives, OPEs were liable to leach out to the environment, including water, sediment, soil, and dust (Lee et al., 2018; Li et al., 2019). For example, Liu et al. (2019) reported that the mean concentration of OPEs in the water sources of the Nanjing section of the Yangtze River was 291.2 ng/L, meanwhile, the mean concentration of OPEs detected in the surface water and sediments from Luoma Lake was 127 and 21.0 ng/L, respectively (Xing et al., 2018). Recent evidences suggested that the OPEs could be accumulated in biological samples through the food webs (Greaves et al., 2016; Ma et al., 2021), resulting in cardiotoxicity, neurotoxicity, endocrine disruption, and immune system toxicity (Alzualde et al., 2018). As such, the prolonged exposure and accumulation of OPEs may elicit high risk to human and living beings.

Rice, the staple food for nearly half of the world population, was widely cultivated in Asia. Various kinds of unintended organic pollutants such as OPEs could be uptaken into plants, when a large amount of irrigation water was introduced into paddy fields during rice growth. Microbial consortium isolated from the rhizosphere of rice was considered as part of the secondary genome of a plant and have major impacts on plant as well as soil health (Kumawat et al., 2022). Beyond that, these

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microorganisms have acquired an evolutionary relationship with their phytobiont, and could degrade organic compounds to benefit the rice by removing the negative effects of contaminants on rice growth (Singha et al., 2018). Currently, no information was available for the removal of OPEs in rhizobacteria-rice based system. Within this context, more comprehensive studies were warranted to reveal the fate of OPEs in rice and microbiome system.

The uptake and transformation of triphenyl phosphate (TPHP), tris (1,3-dichloro-2-propyl) phosphate (TDCPP), tri-n-butyl phosphate (TnBP), triethyl-chloro-phosphate (TCEP), and, tris(1-chloro-2-propyl) phosphate (TECPP) in wheat and white lupin have been reported (Liu et al., 2021; Wan et al., 2017), and the physical and chemical properties, such as molecular weight and log K_{ow} were deemed to play a crucial role in this bioprocess. Still, different from these OPEs, tricresyl phosphate (TCP) have three isomers, ie., tri-p-cresyl phosphate (TpCP), tri-m-cresyl phosphate (TmCP), and tri-o-cresyl phosphate (ToCP), depending on the position of methyl groups on the benzene ring. In recent reports, TCP was detected at concentrations of 0–39.9 ng/L in source water of eastern China, and was the unique OPEs that posed a medium risk to the fish, algae, and crustacea (Zhu et al., 2022), compared with other OPEs' low risk. TpCP, TmCP, and ToCP have same molecular weight and similar logK_{ow} (Calculated by EPI Suite^{TM}), however, it is unclear whether the isomers owned the similar translocation factor (TF) and degradation pathways in rice. In previous study, the isomer-specific transformation and accumulation of nonylphenol has been observed in carrot, which suggested that the structural variations in the length and substitution position on the benzene ring could affect the uptake and transformation of nonylphenol (Sun et al., 2021). In this situation, there is a practical need to study the uptake, translocation, accumulation, and transformation of TpCP, TmCP and ToCP in rice.

Currently, only two literatures reported the transformation of TCP by organisms, and proposed its degradation pathway (Liu et al., 2019; Yu et al., 2022). However, the whole biotransformation process of TCP might be more complicated than previously expected. The methylation, demethylation, and methoxylation reactions of TCP were completely ignored, which limited the understanding of the implications of methylation and demethylation on the environmental behavior and potential risks of OPEs. What is more important is whether TCP could be transformed to other manmade OPEs such as TPHP, which is vital to the understanding of the occurrence and distribution of OPEs in natural environment.

Herein, the main objectives of this study are to I) investigate and compare the uptake and translocation potential of TCP isomers in rice tissues; II) identify the biodegradation intermediates and explore the associated biotransformation pathway of TCP isomers; III) reveal the molecular mechanism and functional genes related to TCP biodegradation by microbial consortium.

2. Experimental section

2.1. Materials and methods

TpCP (purity: >98%), TmCP (purity: >95%), and ToCP (purity: >96%) standards were obtained from Tokyo Chemical Industry (Japan), and d_{21}-TpCP was supplied by Hayashi Pure Chemical Ind. Ltd. (Osaka, Japan). Impurities of standards were determined to ensure the authenticity of results, and no metabolites were found as impurities. Other reagents were purchased from Guangzhou chemical reagent company (Guangzhou, China). The composition of mineral salt medium (MSM) and exposure solutions used were in accordance with the previous study (Yu et al., 2022; Sun et al., 2016). All the media were sterilized at 121℃ for 30 min with an autoclave.

2.2. In vivo hydroponic exposure

Three treatment groups were conducted in this *in vivo* hydroponic exposure experiment. Group 1 was the single rice group, group 2 was the microbial consortium GY group, groups 3 was microbial consortium GY-rice group.

Rice (*Oryza sativa* L.) used in this study was provided by the Chinese Academy of Agricultural Sciences, Beijing, China. Rice seeds with similar size (approximately 10 cm in height) were chosen and sterilized by 3% (V/V) H_2O_2. The sterile glass reactor was used to conduct the exposure experiments, containing five rice seedlings and 50 mL exposure solutions with the selected TCP concentration of 1 mg/L. This concentration exceeded their environmentally relevant concentration (Zhu et al., 2022), but was necessary for the determination of their transformation products in plant tissues and exposed solutions. The reactor was fully wrapped with aluminum foil and parafilm to prevent photolysis and volatilization of the chemicals. The reactors were placed in the controlled growth chamber and the rice seedlings exposed to TpCP, TmCP, and ToCP separately were harvested at intervals of 8, 12, 24, 48, 72, 120, 168, and 240 h. The roots were rinsed with deionized water thoroughly and dried on tissue paper, and rice seedlings were separated into roots and shoots for the subsequent analysis. All the sampled plants were freeze-dried at −50 ℃ for 48 h in a lyophilizer, homogenized, weighed, and then stored at −20 ℃ before analysis. TpCP, TmCP, and ToCP in plant tissues and solution were extracted and cleaned according to previous studies with some modification (Liu et al., 2021; Wan et al., 2017). Blank controls (without TCP) and unplanted controls (without seedlings) were set up. All the groups were prepared in triplicate.

Microbial consortium GY group: We firstly planted the rice in e-waste contaminated sites in Guiyu, South China, where the concentration of TpCP, TmCP, and ToCP was 4.31, 6.43, and 4.62 ng/g respectively in soil, detected in our laboratory. Microbial consortium GY with high potential for degradation of TpCP, TmCP, and ToCP was isolated from the rice root and the detailed procedure for isolation was described in text S1, and the biodegradation experiments of TCP by microbial consortium GY was described in text S2.

Microbial consortium GY-rice group: The setup process of microbial consortium GY-rice group was similar to the method described for single rice group, with the addition of microbial consortium GY (10% v/v) (Fig. S1). The selected concentration of microbial consortium GY was based on the optimum degradation effect of TCP, and then, the rhizosphere microbiome could strongly interact with the rice root, and form a complex ecological interaction web.

2.3. Analytical methods of OPEs and metabolites

The Agilent 1100 series liquid chromatograph (Agilent Technologies, Santa Clara, USA) equipped with an Applied Biosystems/Sciex API 4000 triple-quadrupole mass spectrometer (LC − MS/MS, Applied Biosystems, Foster City, USA) was applied to determine the concentration of TCP, and the details were provided in text S3. The liquid chromatogram (LC20D, Shimadzu Nexera Prominence) coupled with high resolution hybrid quadrupole time-of-flight mass spectrometer (AB SCIEX X500R QTOF) was used to identify the potential metabolites of TCP having no corresponding commercial standards. The MS resolution of the instrument was 30,000 and MS^2 resolution was 25000. The analysis mode was IDA, parameters of mass spectrometer were ESI positive, full scan mode from m/z 50 to 1000, curtain gas (30 psi), ion source gas 1 (50 psi), ion source gas 2 (60 psi), ionspray voltage (5500 V), temperature (400 ℃). The chromatographic separation was performed by a ZORBAX SB-C18 column (4.6 × 150 mm, 5 μm). The mobile phase was water containing 0.1% formic acid (A) and acetonitrile (B). A gradient run was used as follows: mobile phase B was set from 50.0% to 90.0% in 30 min and maintained for 5 min, then decreased from 90% to 10% in 0.1 min and maintained for 25 min. The flow rate was 0.3 mL/min and the injection volume was 5 μL for each sample, all data were acquired and processed using SCIEX OS Software 1.3.1.

2.4. Structure and composition of bacterial community

The DNA was extracted using E.Z.N.A^{TM} Mag-Bind Soil DNA Kit from the rhizosphere microbiome during TCP biodegradation. PCR was started immediately after the DNA was extracted. The 2 × Hieff® Robust PCR Master Mix (Yeasen, 10105ES03, China) was used to amplify the 16S rRNA V3-V4 amplicon. Two universal bacterial 16S rRNA gene amplicon PCR primers (PAGE purified) were used: the amplicon PCR forward primer (CCTACGGGNGGCWGCAG) and amplicon PCR reverse primer (GACTACHVGGGTATCTAATCC). The details were provided in text S4.

2.5. Metagenome analysis

The E.Z.N.A.Soil DNA Kit (Omega, M5635-02, USA) was applied to extract the total community genomic DNA from the experiment group and control, after 12 h during the biodegradation of TCP, respectively. The concentration of the DNA was measured by the Qubit 4.0 (Thermo, USA). Sequencing libraries were generated using Hieff NGS® MaxUp II DNA Library Prep Kit for Illumina® (12200ES96, YEASEN, China) following manufacturer's instructions and index codes were added to attribute sequences to each sample. The details were provided in text S5.

2.6. Toxicity assessment

The toxicity alteration during TCP degradation by microbial consortium GY was investigated. The experimental procedures were based on our previous study (Yu et al., 2019). E-4, E-5, and E-6 were extracted from 1 mg/L TpCP, TmCP, and ToCP samples respectively after the treatment by microbial consortium GY, while E-1, E-2, and E-3 were extracted from the control group without the addition of microbial consortium GY. Both E-1, E-2, E-3, E-4, E-5, and E-6 were further employed to analyze the change in the toxicity before and after TpCP, TmCP, and ToCP degradation. *Escherichia coli*, regarded as the model microorganism, was incubated in 25 mL Erlenmeyer flasks with 10 mL MSM and the corresponding extracts for 12 h. Subsequently, the *Escherichia coli* cells were harvested using centrifugation at 6000 g for 10 min. The toxicity of the extract was evaluated using the cell apoptosis and flow cytometry (Gallios, USA) was applied to analyze the apoptosis rate.

2.7. Quality assurance and quality control

Quality controls were conducted through the regular analysis of procedural blanks. No mutual interference was observed in the instrumental analysis of target pollutants used in this study. Blank samples were analyzed to monitor possible contamination, showing an absence of background interference. Average recoveries of TpCP, TmCP, and ToCP in the solutions and plant tissues were 87.5%-92.4%, 86.3–90.1%, and 90.2%-92.8%, respectively, where the relative standard deviation (RSD) for the spiked samples was lower than 15% (n = 3). Recoveries of the d_{15}-TpCP was 80.1%-88.5%. The method limits of detection (MLODs) were estimated on the basis of a signal-to-noise ratio of 3. MLODs for TCP in water was 1.53–5.04 μg/L, and in plants was 0.0008–0.0013 μg/g, respectively.

2.8. Data analysis

All statistical analyses were completed by IBM SPSS 20.0 software. The logK_{ow} of the TpCP, TmCP, and ToCP was calculated by estimation program interface v4.11 (EPI Suite^{TM} Version 4.11). The mean and standard deviations (SD) of all the data were calculated from the results in triplicate. One-way analysis of variance (ANOVA), followed by least significant difference (LSD), was used to examine significant differences between values. A linear regression was used to derive the relationships among variables. The statement of significant difference is based on $p <$ 0.05.

3. Results and discussion

3.1. Uptake, translocation and toxicity of TCP in rice

TpCP, TmCP, and ToCP were detected in the roots and shoots of rice (Fig. 1A, B and C). Concentrations of TCP in the roots increased first, reaching the peak levels at 95.2, 133.2, and 127.9 μg/g dry weight for TpCP, TmCP, and ToCP in single rice group and 60.3, 99.0, and 92.6 μg/g dry weight for TpCP, TmCP, and ToCP in microbial consortium GY-rice group respectively, at different exposure times, and slightly decreased afterward. The reason behind this phenomenon was that the TpCP, TmCP, and ToCP could be degraded by the microbial consortium GY, reducing the root uptake potential. Translocation factor (TF) of TpCP, TmCP, and ToCP were calculated for each exposure time point. Collectively, The TF followed the order of TpCP ≈ TmCP > ToCP (Fig. 1D), which indicated that TpCP and TmCP were translocated from root to shoot more easily compared with ToCP, even though the three isomers have the same molecular weight and similar log K_{ow} (6.34) (Calculated by EPI Suite^{TM}). Previous studies confirmed that the TF of OPEs was opposite to the trend of their log K_{ow} values (Liu et al., 2021), but some researches still pointed out that this regularity was not always effective (Olisah et al., 2021; Fan et al., 2022).

The biomass of rice was calculated for each exposure time point in the single rice group, and microbial consortium GY-rice group. As shown in Fig. 2, TpCP, TmCP, and ToCP could evidently restrain the growth of rice ($p < 0.05$), and microbial consortium GY could alleviate the negative impact. After 240 h, compared with the control group, the biomass of rice decreased by 29.3%–32.4%, in the single rice exposure group, and 15.7%-19.0% in the microbial consortium GY-rice exposure group. The reasons behind this phenomenon were that (1) GY1 could reduce the uptake potential of rice for the TCP directly, which avoided the excessive accumulation of TCP in rice, and then alleviate the toxicity of the TCP to the rice; (2) the biodegradation of TpCP, TmCP, and ToCP by GY1 was proved to be a detoxification process based on the cell apoptosis rate of *Escherichia coli* (Fig. S2), which could be conducive to eliminate negative effects of TCP on the growth of the rice. Furthermore, although previous study suggested that ToCP showed stronger biotoxicity than TmCP and TpCP (Yu et al., 2022), no obvious difference in biomass was observed among TpCP, TmCP, and ToCP exposure in the single rice group.

3.2. Metabolic pathways of TCP by microbial consortium GY and rice

The possible metabolic pathways of TCP were speculated based on the characteristic fragmentation and molecular weight. A total of 15 metabolites were successfully identified during TCP biodegradation by microbial consortium GY, including 4 metabolites of TpCP, 6 metabolites of TmCP, and 5 metabolites of ToCP (Fig. 3). None of them was found in the blank controls. Hydroxylated, methylated and demethylated products were identified during TpCP biodegradation. Product P1 (CH_3-TpCP) (*m/z* 383.1405) was identified as the methylated product. Biotic methylation is a phase II metabolism mediated by methyltransferases, and this process happens commonly during the transformation of various organic pollutants (Bartikova et al., 2015), and CH_3-TpCP identified in this study was reported for the first time. Hydroxylation has been extensively perceived as a crucial step for the oxidative conversion of aromatic compounds (Chen et al., 2019). In this research, P2 (OH-TpCP) (*m/z* 385.1199) was detected, which was also reported by other study, suggesting that Brevibacillus brevis could hydroxylate TCP (Liu et al., 2019). Biotic demethylation is a phase I metabolism process facilitated mainly by cytochrome P450 enzymes that are ubiquitous in organisms (Chuang et al., 2018; Hagel and Facchini, 2010). In this study, the identification of product P3 (CH_3-TPHP (triphenyl phosphate)) (*m/z* 341.0939) and product P4 (TPHP) (*m/z*

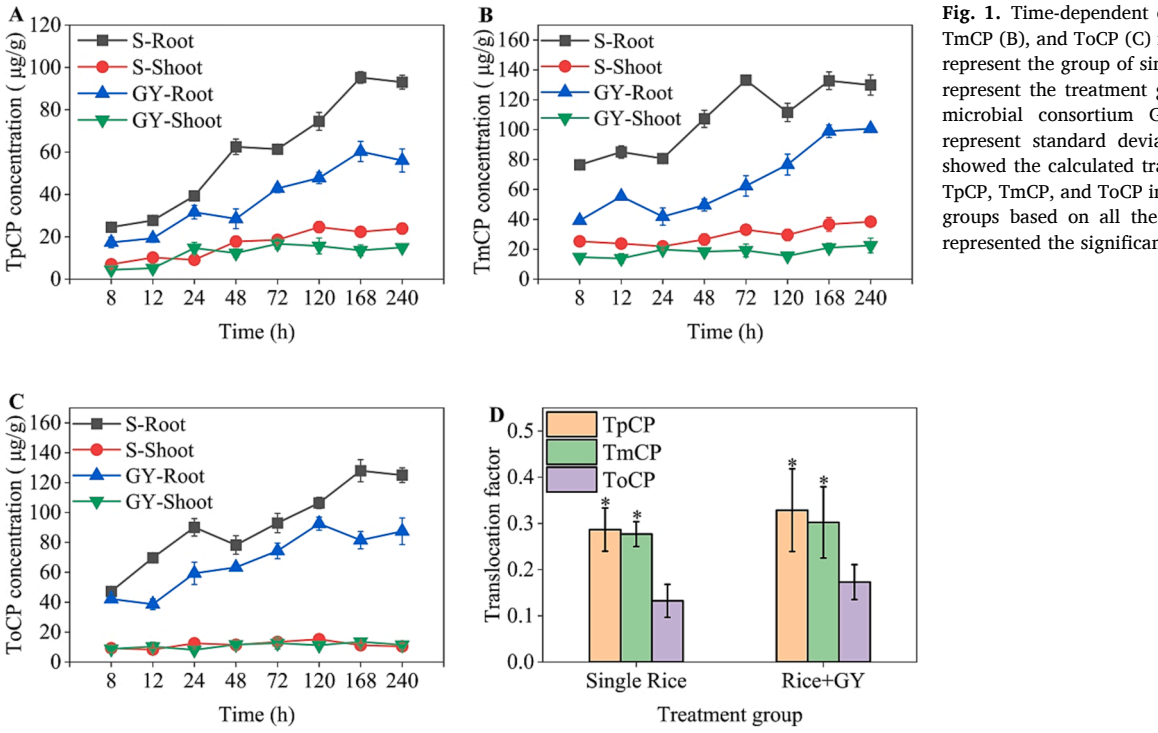

Fig. 1. Time-dependent concentration of TpCP (A), TmCP (B), and ToCP (C) in rice roots and shoots. (S- represent the group of single rice treatment and GY- represent the treatment group with combination of microbial consortium GY and rice). Error bars represent standard deviation values (n = 3). (D) showed the calculated translocation factors (TFs) of TpCP, TmCP, and ToCP in rice of different treatment groups based on all the harvest times (n = 8). * represented the significant difference $p < 0.05$.

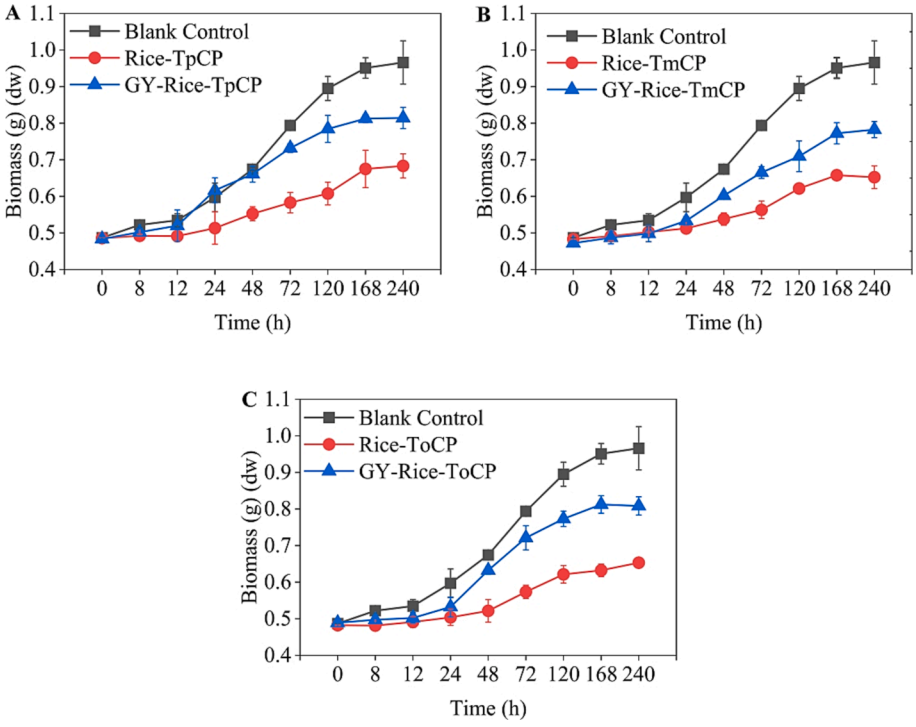

Fig. 2. The biomass of rice under different treatment groups.

327.0781) verified the occurrence of demethylation of TCP by microorganism. This was a novel degradation pathway of OPEs. The similar demethylation bioprocess of other organic pollutants was observed in previous study, such as tetrabromobisphenol A (TBBPA) (Peng et al., 2022), lincomycin (Lei et al., 2022), and chlortetracycline (Zhang and Wang, 2022). TPHP is a synthetic compound and a kind of manmade OPE. This phenomenon suggested the degradation from TCP to TPHP is a new natural "secondary" source of TPHP that has been overlooked before. This founding broke the traditional understanding and provided new insights into the source of widespread and toxic TPHP in the environment.

M1 (CH_3-TmCP) (*m/z* 383.1405), M2 (CH_3-DmCP (di-m-cresyl phosphate)) (*m/z* 293.0938), M3 (C_7H_8) (*m/z* 93.0698), M4 (OH-TmCP) (*m/z* 385.1202), M5 (CH_3-TPHP) (*m/z* 341.0938), and M6 (TPHP) (*m/z* 327.0780) were identified as the intermediate products of TmCP (Fig. 3). The degradation pathways of TmCP were similar to those of

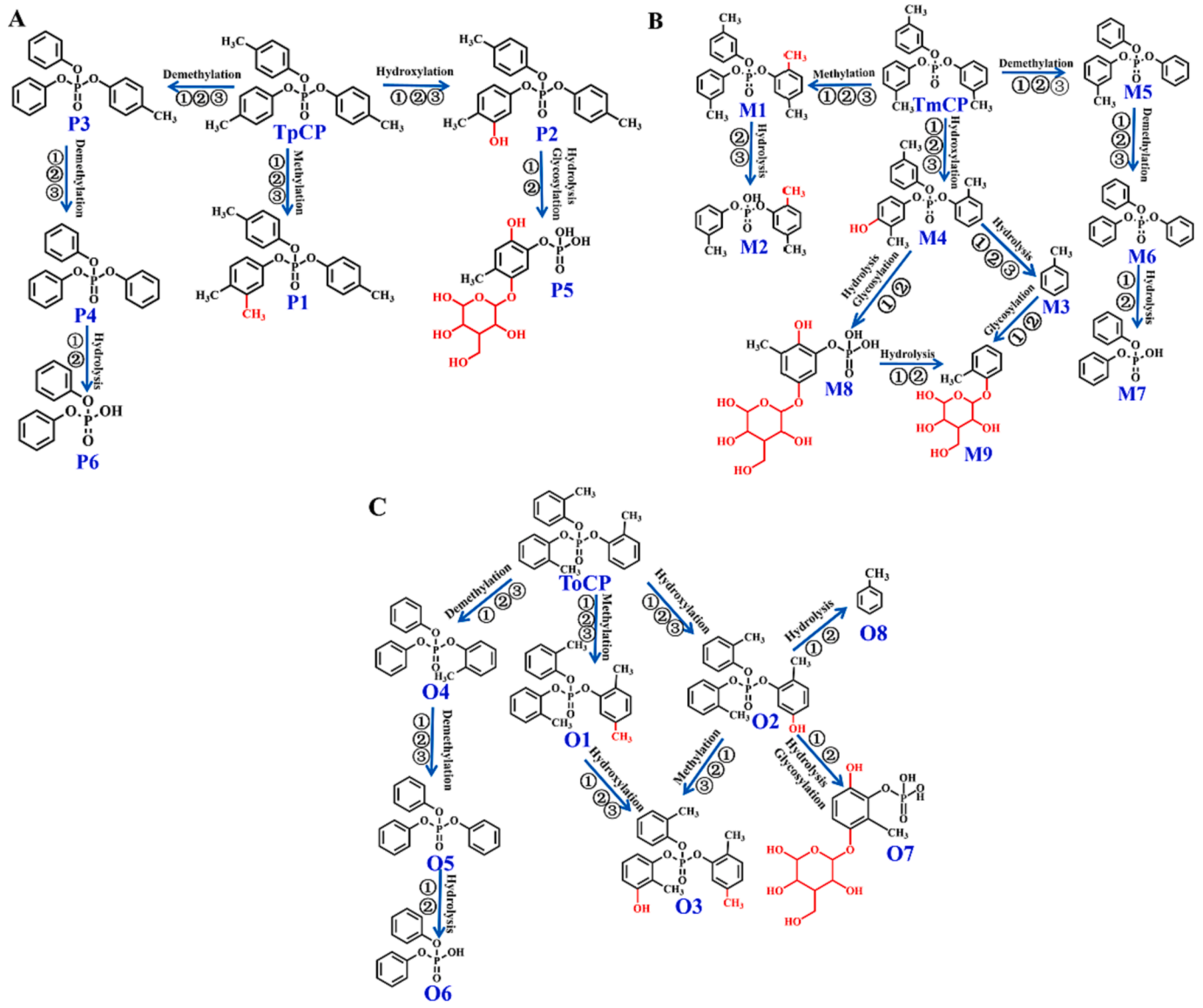

Fig. 3. Possible biotransformation pathways of TpCP, TmCP, and ToCP in rice and microbial consortium GY. (① represent the metabolites identified in rice, ② represent the metabolites identified in the microbial consortium GY-rice group, and ③ represent the metabolites identified in microbial consortium GY).

TpCP. But interestingly, in our study CH_3-DmCP was the unique diester metabolite detected in the degradation of TCP. This product could be formed via the hydrolysis of CH_3-TmCP, and the hydrolysis of phosphate ester bonds was considered as a crucial step for OPEs biotransformation in human body (Van den Eede et al., 2013), zebrafish (Wang et al., 2016), and microorganism (Feng et al., 2021). CH_3-TmCP, CH_3-DmCP, CH_3-TPHP and TPHP were all identified as the intermediate products of TCP for the first time. O1 (CH_3-ToCP) (*m/z* 383.1410), O2 (OH-ToCP) (*m/z* 385.1198), O3 (CH_3-O-ToCP) (*m/z* 399.1359), O4 (CH_3-TPHP) (*m/z* 341.0939), and O5 (TPHP) (*m/z* 327.0779) were identified as the intermediate products of ToCP. Except OH-ToCP, which was reported by previous studies, the other four products were observed for the first time. Different from TpCP and TmCP, the CH_3-O-ToCP detected in our study imply that methoxylation could also be considered as potential mechanism underlying microbial transformation of TCP.

A total of 23 metabolites were successfully identified in rice, including 6 metabolites of TpCP, 9 metabolites of TmCP, and 8 metabolites of ToCP. It should be noted that this was the first report that revealed the transformation process of TpCP, TmCP, and ToCP in plants. In TpCP treatment group, hydroxylated, glucuronic acid-conjugated, hydrolysis, methylated, and demethylated products were observed in both the single rice group and the microbial consortium GY-rice group. The glucuronic acid-conjugated products were never been identified during TpCP transformation before. P5 (OH–(G-O-p-tolyl dihydrogen phosphate)) (*m/z* 383.0747) could be formed via hydrolysis and glycosylation of P2 (OH-TpCP) (*m/z* 385.1198), and this bioprocess was considered as a crucial step for organic compounds detoxication in rice (Li et al., 2022). Similar with the transformation pathways in microbial consortium GY, biotic demethylation and methylation of TpCP occurred simultaneously in rice, and this reaction could be achieved by methyltransferases and cytochrome P450 / NADPH-cytochrome P450 reductase (CYP 450). Currently, The environmental significance of methylation and demethylation of TCP was completely overlooked before, due to the lack of evidences that confirming the existence of these products. This limited the understanding of the implications of methylation and demethylation on the environmental behavior and potential risks of OPEs. Thus, the results obtained in our study provided strong evidence for the existence of methylation and demethylation of TCP. Among the three isomers, TmCP had the largest number of metabolites in rice. It is noteworthy that M9 (G-O-methylbenzene) (*m/z* 271.1174) and M2 (CH_3-DmCP) (*m/z* 293.0937) were only identified in TmCP treatment group. Interestingly, CH_3-DmCP was not observed in

single rice group, implying that the CH_3-DmCP could not be formed via the transformation by rice. CH_3-DmCP was detected as product during the degradation by microbial consortium GY, thus it was speculated that CH_3-DmCP found in the microbial consortium GY-rice group was from the function of microbial consortium GY. O3 (CH_3-O-ToCP) (*m/z* 399.1358) was found in ToCP exposure group, and this product could be formed through the hydroxylation of O1(CH_3-ToCP) (*m/z* 383.1410) or the methylation of O2 (OH-ToCP) (*m/z* 385.1198).

Moreover, it is worth noting that all the intermediate products identified in this study should be considered as an example of potential regioisomers and the accurate positions of introduced groups could not be determined merely using mass spectrometry analysis.

3.3. Degradation of TCP by microbial consortium GY

TpCP, TmCP, and ToCP could be efficiently eliminated by microbial consortium GY (Fig. 4). 1 mg/L of TpCP and TmCP were completely degraded after 24 and 36 h, respectively, meanwhile, 97.7% of 1 mg/L ToCP could be removed during 48 h. These results suggested that TpCP and TmCP were easier to be removed than ToCP, which presented a high substrate specificity exhibited by microbial consortium GY for TCP degradation. Le et al. (2017) revealed that this phenomenon might relate to the affinity between catabolic enzymes and target pollutants. Compared with the results reported by other studies (Liu et al., 2019), microbial consortium GY showed an excellent degradation ability, and exhibited a high substrate tolerance, even though the degradation efficiency of TCP declined with the increase of the initial concentration. This strong degradation ability could protect the soil from OPEs pollution, and contribute to the growth of rice.

3.4. Bacterial community dynamics during degradation of TCP

The microbial community structure and composition associated with TpCP, TmCP, and ToCP degradation was revealed. It can be seen from Fig. 5A, Proteobacteria was the most dominant phylum during the degradation of TpCP (75.1%-75.3%), TmCP (71.6%-75.8%) and ToCP (71.8%-72.7%), followed by Bacteroidetes, Firmicutes, Actinobacteria, Acidobacteria, Chloroflexi, Nitrospirae, and Cyanobacteria. Previous studies confirmed that these phyla were frequently observed during the transformation of OPEs (Liu et al., 2020; Pang et al., 2018). In TpCP degradation group, the relative abundance of Firmicutes (6.25%-6.36%), Actinobacteria (0.48%-0.56%), and Cyanobacteria (0.10%-0.15%) showed a slight uptrend. For TmCP, except Actinobacteria and Bacteroidetes, the relative abundance of the other phyla exhibited an increasing tendency, especially the Proteobacteria (71.6%-75.8%), Acidobacteria (0.036%-0.064%), Chloroflexi (0.018%-0.034%) and Nitrospirae (0.018%-0.034%). Similarly, in ToCP degradation group, except Proteobacteria and Bacteroidetes, the relative abundance of Actinobacteria (0.23%-0.49%), Acidobacteria (0.017%-0.059%), Cyanobacteria (0.05%-0.11%), Firmicutes (5.55%-6.15%), and Nitrospirae (0.001%-0.029%) also gradually increased.

At genus level (Fig. 5B), *Comamonas*, *Pantoea*, and *Sphingopyxis* from Proteobacteria, *Sphingobacterium* from Bacteroidetes, *Bacillus* from Firmicutes, were the predominant genera during the biodegradation of TCP. Among these genera, the relative abundance of *Comamonas*, *Sphingobacterium*, *Pantoea*, and *Bacillus* showed a significant uptrend. In TpCP degradation group, *Comamonas*, *Sphingobacterium, Pantoea,* and *Bacillus* increased from 48.4% to 49.2%, 11.5% to 12.8%, 3.87% to 4.98%, and 2.12% to 3.21%, respectively. In TmCP degradation group, these four genera increased from 47.9% to 50.5%, 13.5% to 14.51%, 3.58% to 5.13%, and 2.12% to 3.20%, separately. In ToCP degradation group, the corresponding genera increased from 55.5% to 62.8%, 11.9% to 14.5%, 3.28% to 5.86%, and 2.12% to 3.22%. Beyond that, different from TpCP and TmCP, *Stenotrophomonas* (0.76%-1.48%) and *Pseudomonas* (0.12%-1.11%) from Proteobacteria also increased in ToCP group. Among these genera, *Bacillus* and *Pseudomonas* were recently reported to be able to degrade OPEs (Wang et al., 2019; Zhou et al., 2020). Particularly, *Comamonas*, the most dominant species in microbial consortium GY, was reported as the typical biodegrader for organic pollutants, such as organophosphorus pesticides (Firdous et al., 2020) and polychlorinated biphenyl (Francova et al., 2004). More recently, *Comamonas* was considered as the host microorganism of alkaline phosphatase and methyltransferases gene (described in metagenomic analysis). Therefore, it was hypothesized that *Comamonas* might have the potential for OPEs biotransformation. *Sphingobacterium* was also the major strain in microbial consortium GY, which could efficiently degrade polycyclic aromatic hydrocarbon (Li et al., 2021), pyridine (Jin et al., 2020), and tetracycline (Tan et al., 2022). Moreover, *Sphingobacterium* and *Stenotrophomonas* were proved to be the core strains for the biodegradation of organophosphorus pesticide, which harbored organophosphorus (OPH) hydrolase catalyzing the cleavage of phosphorous-ester bond (Verma et al., 2020; Deng et al., 2015) This could pave a way for interpreting the intermediate products identified in the present study. Although the degradation ability of *Pantoea* involved in OPEs transformation was not reported, the function of *Pantoea* in TCP biodegradation could not be ignored since microorganisms always cooperate to form complex ecological interaction webs instead of living in isolation.

3.5. Mechanism revealed by microbial metagenomic analysis

Exploration of functional genes is of great importance when analyzing degradation process, since it provides direct evidences of the shifts in microbial community structure upon TCP exposure. Eight representative genes were selected for a comprehensive understanding of the biotransformation process of TpCP, TmCP, and ToCP (Fig. 6A, B, C and D).

The CYP450 was the specific enzyme, well-known for the contribution to the formation of hydroxylated organic compounds, which was described as an important biotransformation pathway for the generation of new active substances (Kim et al., 2007). Moreover, as the typical enzymes during phase I xenobiotics metabolizing, CYP450 was also

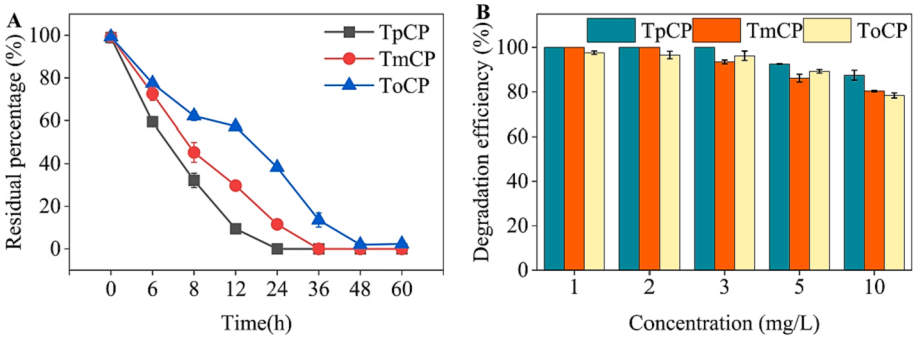

Fig. 4. Effects of contact time (A) and initial concentration (B) on TpCP, TmCP, and ToCP biodegradation by microbial consortium GY.

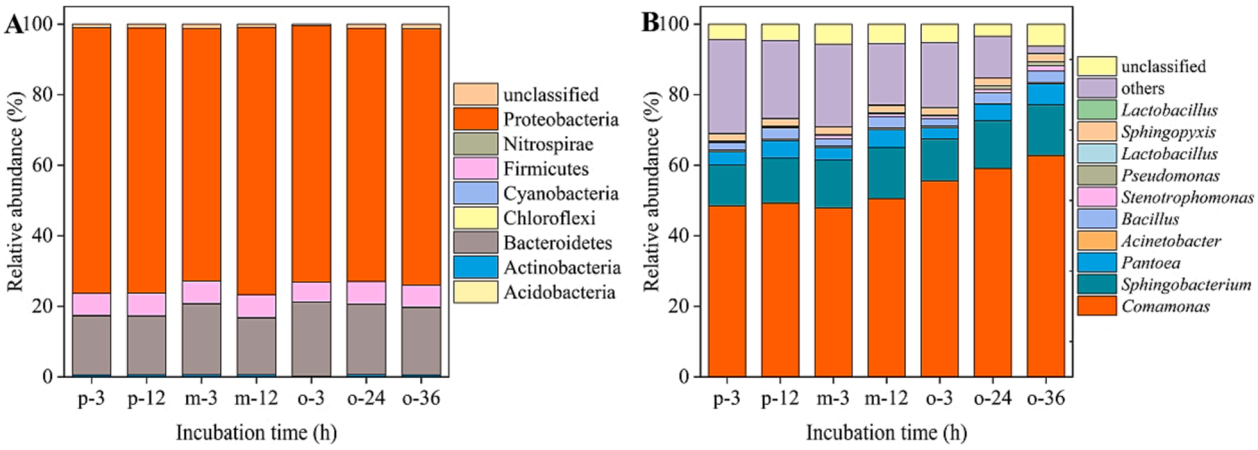

Fig. 5. Shifts in bacterial community at phylum (A) and genus (B) level during TpCP, TmCP, and ToCP degradation process.

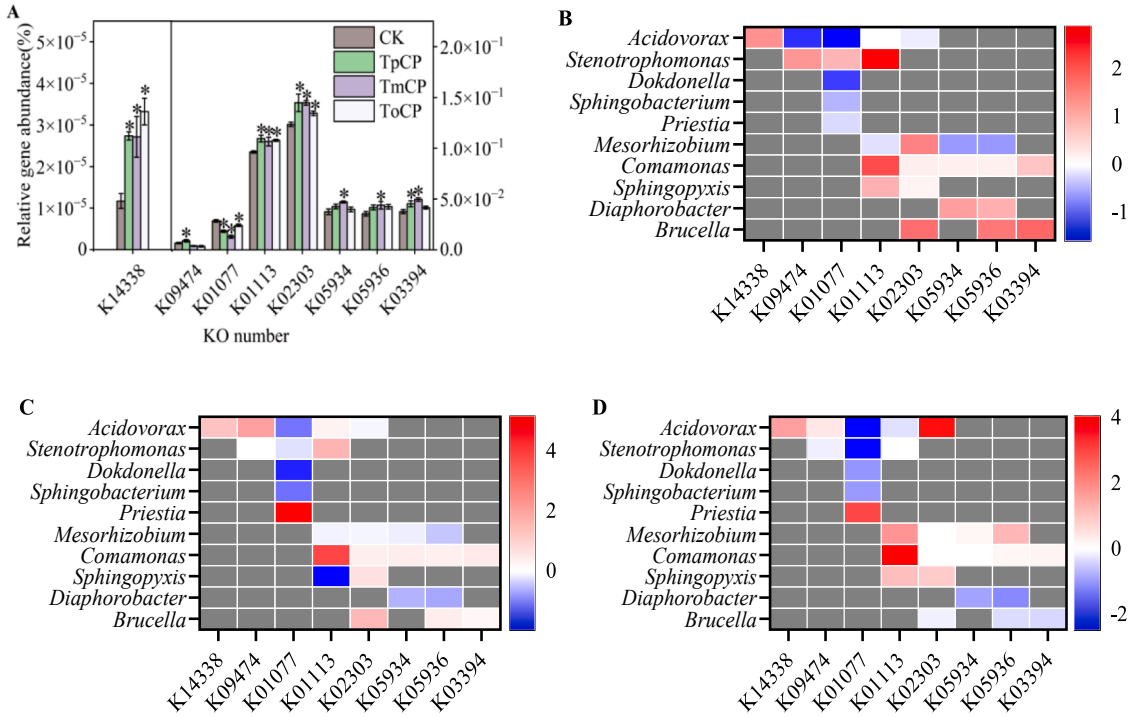

Fig. 6. Relative abundances of genes involved in TpCP, TmCP, and TpCP biodegradation based on metagenomic analysis (A) (The left horizontal coordinates was set for K14388, and the right horizontal coordinates was set for other genes). Heatmap showing the log ratio (log_2 (Fold change)) of relative abundances of TpCP (B), TmCP (C), and ToCP (D) metabolism-related genes assigned to genus.

renowned for its efficiency to mediate the demethylation of organic pollutants (Chuang et al., 2018; Hagel and Facchini, 2010). In this study, the genes (K14338) coding for CYP450 showed a significant up-trend during TpCP, TmCP, and ToCP biodegradation ($p < 0.05$), and this could pave a way for interpreting the identified hydroxylated and demethylated products. In the present study, CYP450 (K14338) was closely associated with *Acidovorax,* and the K14338 assigned to *Acidovorax* were 2.34, 2.32, and 2.84 times higher in the treatment groups compared to the control, suggesting that *Acidovorax* might be the core genus in the formation of hydroxylated and demethylated products, although the relative abundance of *Acidovorax* was not high during the TCP biodegradation according to amplicon sequencing results. In this regard, the ability of *Acidovorax* to organic pollutants hydroxylation and C–C bond cleavage has been well documented (Chu et al., 2017; Zheng et al., 2021).

Gene coding for acid phosphatase (K09474) and alkaline phosphatase (K01077 and K01113) was also found during TCP transformation, which could catalyze the hydrolysis of nearly all phosphate monoesters to produce inorganic phosphoric acid and corresponding sugars, phenols, and alcohols, and was proved to play an important role in the hydrolysis of OPEs (Wang et al., 2022). In this study, the gene coding for K09474 was mainly distributed in *Acidovorax* and *Stenotrophomonas*, and the alkaline phosphatase (K01077 and K01113) genes were closely associated with *Acidovorax, Stenotrophomonas*, *Dokdonella*, *Sphingobacterium*, *Priestia*, *Mesorhizobium*, *Comamonas*, and *Sphingopyxis.* It is worth noting that compared to the control, K01077 assigned to *Priestia* were 34.9 and 7.49 times higher in the TmCP and ToCP treatment groups than control, separately, and K01113 assigned to *Comamonas* were 4.02, 13.31, and 16.09 times higher in the TpCP, TmCP, and ToCP treatment groups, respectively, indicating that *Priestia* and *Comamonas*

might play a critical role in hydrolysis reaction, although the hydrolysis product was only detected during TmCP transformation in this study.

Biotic methylation is a phase II metabolism mediated by methyltransferases (Bartikova et al., 2015). In this study, four representative genes coding for methyltransferases (K02303, K05934, K05936, and K03394) were selected for a comprehensive understanding the methylated-process of TCP. Among these genes, the total abundances of K02303 were obviously higher in all the treatment groups compared to the control, and this gene assigned to *Brucella* were 3.07 and 2.71 times higher in TpCP and TmCP treatment groups, and to *Acidovorax* was 13.94 times higher in ToCP treatment group. Besides, *Brucella*-related methyltransferases gene (K05936 and K03394) was significantly more abundant during TpCP biodegradation (2.86 and 3.07 times higher than control) and *Mesorhizobium*-related methyltransferases gene (K05936) was 2.17 times higher in ToCP than control, indicating that these strains played an important role in methylation of TCP. Beyond that, it should be noted that even though TpCP, TmCP, and ToCP have similar structures, the strains which involved in the methylation of these pollutants were different.

4. Conclusion

Isomer-specific translocation and metabolism processes of tricresyl phosphate in rice and microbiome exposure system were revealed. In conclusion, TpCP and TmCP were prone to be more translocated acropetally in rice, compared with ToCP. Microbial consortium GY could efficiently degrade TpCP, TmCP, and ToCP. GY could reduce the uptake of these pollutants in rice tissues, and promote rice growth. Twelve intermediates were observed for the first time in organisms as the products of TCP. The demethylation pathway of TpCP, TmCP, and ToCP may be an important and overlooked source of TPHP in the environment. *Comamonas*, *Pantoea*, *Sphingopyxis*, *Sphingobacterium*, and *Bacillus* were the predominant genera during the biodegradation of TCP. The metagenomic analysis further indicated that CYP450, acid phosphatase, alkaline phosphatase, and methyltransferases may involve in TCP degradation. However, it should be noted that the strains and function genes in microbial consortium GY that responsible for TpCP, TmCP, and ToCP transformation may be different.

CRediT authorship contribution statement

Yuanyuan Yu: Conceptualization, Methodology, Validation, Data curation, Writing – original draft. **Jiahui Huang:** Conceptualization, Methodology, Validation, Data curation. **Ling Jin:** Writing – review & editing. **Miao Yu:** Writing – review & editing. **Xiaolong Yu:** Data curation. **Xifen Zhu:** Data curation. **Jianteng Sun:** Conceptualization, Writing – review & editing, Funding acquisition. **Lizhong Zhu:** Supervision.

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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.107793.

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