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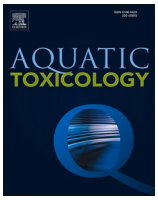

Transcriptomic analysis reveals interactive effects of polyvinyl chloride microplastics and cadmium on *Mytilus galloprovincialis*: Insights into non-coding RNA responses and environmental implications

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ABSTRACT

Despite increasing concerns regarding the interactions of microplastic and heavy metal pollution, there is limited knowledge on the molecular responses of marine organisms to these stressors. In this study, we used whole-transcriptome sequencing to investigate the molecular responses of the ecologically and economically important bivalve *Mytilus galloprovincialis* to individual and combined exposures of environmentally relevant concentrations of PVC microplastics and cadmium (Cd). Our results revealed distinct transcriptional changes in *M. galloprovincialis*, with significant overlap in the differentially expressed genes between the individual and combined exposure groups. Genes involved in cellular senescence, oxidative stress, and galactose metabolism were differentially expressed. Additionally, key signaling pathways related to apoptosis and drug metabolism were significantly modulated. Notably, the interaction of PVC microplastics and Cd resulted in differential expression of genes involved in drug metabolism and longevity regulating compared to single exposures. This suggests that the interaction between these two stressors may have amplified effects on mussel health. Overall, this comprehensive transcriptomic analysis provides valuable insights into the adaptive and detrimental responses of *M. galloprovincialis* to PVC microplastics and Cd in the environment.

1. Introduction

Microplastics and heavy metals pose significant threats to marine ecosystems (Lithner et al., 2011; Santana et al., 2017; Sun et al., 2021, 2022). Microplastics, defined as plastic particles less than five millimeters in diameter, enter the marine environment through various pathways, including wastewater treatment plants, stormwater runoff, and the degradation of larger plastic items (Thompson et al., 2004; Andrady, 2011; Setälä et al., 2014; Yang et al., 2021; Zhang et al., 2016, 2020). Polyvinyl chloride (PVC), one of the most widely used plastics, is particularly concerning due to its ease of fragmentation and documented in vitro toxicity in primary rat and human pulmonary cells (Xu et al., 2020; Andrady, 2011).

Cadmium (Cd), a highly toxic heavy metal even at low doses (Genchi et al., 2020), is a severe marine pollutant due to its ability to accumulate in marine species and induce health problems (Sandbichler and Hockner, 2016). Cd exposure can trigger oxidative stress, immune dysfunction, and detoxification issues in marine animals, particularly mollusks (Cuypers et al., 2010; Pouil et al., 2018). Marine organisms readily ingest both microplastics and heavy metals, leading to their bioaccumulation in tissues and organs (Bakir et al., 2014; Kedzierski et al., 2018; Xu et al., 2020). Ingested microplastics and heavy metals can induce a range of adverse effects, either individually or synergistically, including inflammation, oxidative stress, and cellular apoptosis (Lu et al., 2018; Wen et al., 2018; Zhu et al., 2020; Chen et al., 2022).

The interaction between microplastic and heavy metal pollution is a

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particular concern. Microplastics can act as carriers, facilitating the transport of heavy metals into marine organisms. Additionally, they can impair the gut lining, increasing vulnerability to heavy metal toxicity. Studies have shown that microplastics can promote the accumulation of Cd and other heavy metals, leading to tissue damage, oxidative stress, cell death, and reduced reproductive capacity (Lu et al., 2018; Wen et al., 2018; Zhu et al., 2020). Co-exposure of zebrafish to microplastics and Cd resulted in enhanced oxidative damage and inflammatory responses (Lu et al., 2018). Similarly, combined exposure to polystyrene microplastics and Cd in ironfish caused severe oxidative stress (Wen et al., 2018). Furthermore, microplastics can increase heavy metal accumulation in oysters, ultimately affecting their growth and reproduction (Zhu et al., 2020). Studies on aquatic model species have demonstrated that co-exposure to microplastics and heavy metals can induce cell death, alter swimming behavior through increased acetylcholinesterase (AChE) activity, and ultimately lead to other detrimental effects (Santos et al., 2022).

Despite growing concerns about the combined effects of microplastic and heavy metal pollution, knowledge regarding the molecular responses of marine organisms to these combined stressors remains limited (Santos et al., 2022; Banaee et al., 2023). Non-coding RNAs (ncRNAs) are a class of RNA molecules that play critical roles in regulating gene expression and various cellular processes (Hansen et al., 2013; Kozomara et al., 2013; Rybak-Wolf et al., 2015; Chen et al., 2016). While ncRNAs have been implicated in diverse stress responses in marine organisms (Mercer et al., 2009; Biggar and Storey, 2015; Masaomi et al., 2016; Gajigan and Conaco, 2017; Yu et al., 2020), their role in mediating the combined effects of microplastic and heavy metal pollution is poorly understood.

Mytilus galloprovincialis is an essential, low-cost model organism, it is also prevalent and ecologically significant (Pagano et al., 2020; Zhan et al., 2021; Curpan et al., 2022; Wang et al., 2022; Tresnakova et al., 2023; Impellitteri et al., 2024). In this study, we employed whole-transcriptome sequencing method, to examine the molecular responses of the individual and combined exposures of environmentally relevant concentrations of PVC microplastics and cadmium (Cd) on mussel *M. galloprovincialis*. We hypothesized that the simultaneous exposure to PVC microplastics and Cd would induce differential expression patterns of the ncRNA transcriptome of *Mytilus galloprovincialis*.

2. Methods

2.1. Experimental samples and RNA extraction

We obtained 240 mussels, with shell lengths (4.72 ± 0.14 cm), from the Qingdao fishery market. These mussels were acclimated in aerated seawater (31 psu salinity, 20 °C temperature) for seven days, with a 12 h light/dark cycle and daily feedings of *Chlorella vulgaris* (2 % of dry tissue weight). Post-acclimation, the mussels were randomly assigned to four groups: a control group, a group exposed to 10 μg/L Cd, a group exposed to 20 items/L PVC (Fig. 7), and a group exposed to both 10 μg/L Cd and 20 items/L PVC. Each group, consisting of 20 mussels in 20 L of aerated seawater, was replicated thrice. During the 96 h exposures, all group of mussels were fed with *Chlorella vulgaris* per day. After 96 h of exposure, whole soft tissue of mussels was dissected and stored at −80 °C for subsequent heavy metal and ncRNA analysis. Total RNA extraction was performed using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. To maximize ncRNA identification, equal fractions of total RNAs from all samples were pooled.

2.2. Cadmium content measurement

Total cadmium (Cd) content in mussel tissues was quantified using established methods (Zhang et al., 2012). Tissue samples were dried at 80 °C to a constant weight, treated with 1 mL of concentrated HNO_3 (65 % analytical reagent grade, Fisher Scientific, Shanghai, China), and digested in a water bath at 80 °C until complete. The digested samples were then diluted to 10 mL using double-deionized water. Additional details on Cd content analysis are provided in the Supporting Information.

2.3. mRNA identification and analysis

We used Stringtie to reconstruct transcripts and identify protein-coding genes (Pertea et al., 2015). The expression level of mRNAs was estimated using fragments per kilobase per million reads (FPKM) values, corrected for sequencing depth and transcript length. Differentially expressed mRNAs (DEmRNAs) were identified using a cutoff of $\log_2FC > 1$ and FDR < 0.05. A heat map was generated based on Z-score for the DEmRNAs. Gene ontology (GO) annotation and functional enrichment analyses of DEmRNAs were performed using an online database.

2.4. Long non-coding RNA identification and analysis

Reconstructed transcripts were filtered to retain only those ≥200 bp in length and with ≥1 exon. Transcripts with no coding potential, as predicted by both CPC2 and CNCI, were selected for further analysis by categorizing into five types based on positions to protein-coding genes in the genome etc. (Sun et al., 2013; Kang et al., 2017). The genomic distributions of these lncRNAs were represented by Circos plots. The FPKM values and DESeq2 was used for differential expression analysis. Pathway enrichment analysis was performed based on the database of the Kyoto Encyclopedia of Genes and Genomes (KEGG, Release 104.0, October 1, 2022) (Kanehisa et al., 2008; Love et al., 2014). Additional details on long non-coding RNA analysis are provided in the Supporting Information.

2.5. Circular RNA identification and analysis

To identify circRNAs, bowtie2 software was applied to map the reference genome for circRNA analysis and identification (Langmead and Salzberg, 2012; Memczak et al., 2013). The differential expression levels of circRNAs were determined by calculating the back-spliced reads per million mapped reads (RPM). Pathway enrichment analysis was performed based on the database of the KEGG.

2.6. MicroRNA identification and analysis

Small RNAs (18–30 nucleotides) were extracted from the total RNA using agarose gel electrophoresis. The small RNAs were then ligated with 3′ and 5′ adapters, reverse transcribed, and PCR amplified. The resulting PCR products (140–160 bp) were extracted and purified to construct a cDNA library for miRNA. Sequencing was performed using the Illumina HiSeq™ 4000 platform. MiRBase and edgeR were applied to identify miRNAs and perform differentially expressed miRNAs (DEmiRNAs) analysis were identified using edgeR (dispersion set to 0.01). Additional details on microRNA analysis are provided in the Supporting Information.

2.7. Competing endogenous RNAs regulatory network (ceRNA) construction and analysis

Based on the ceRNA theory, we used Patmatch_v1.2.3 to predict interaction pairs involving DEmiRNAs, DElncRNAs, and DEcircRNAs. The Spearman Rank correlation coefficient (SCC) was calculated to assess pairwise correlations. Interaction networks were constructed considering gene pairs with an SCC value of less than −0.5.

2.8. Statistical analysis

Statistical analysis was analyzed by SPSS 19.0 (SPSS Inc., Chicago,

Table 1
Cd concentrations in soft tissues of mussels *Mytilus galloprovincialis* after exposure to Cd and PVC for 96 h.

Treatments	Cd accumulation (μg $^{-1}$ g dw)
Control	6.56 ± 2.19
10 μg/L Cd	**14.30 ± 1.85
20 items/L PVC	6.60 ± 1.33
10 μg/L Cd + 20 items/L PVC	*13.97 ± 2.75

Data are expressed as mean ± standard deviation. Significant differences among groups were identified by one-way analysis of variance with Tukey's test.
** means $p < 0.01$.
* means $p < 0.05$.

USA). The differentially expressed mRNAs and ncRNAs were selected using a *t*-test with significance set at $p < 0.05$. We used the R statistical software package (Auckland, NZ) for analyses of RNA-seq data. The heat maps were drawn with R package pheatmap, The GO and KEGG results were drawn with Graphpad 5.0 (San Diego, CA, USA). The Cd accumulation data were expressed as the mean ± standard error, with $p < 0.05$ indicating a significant difference, significant differences among groups were identified by one-way analysis of variance (ANOVA) with Tukey's test.

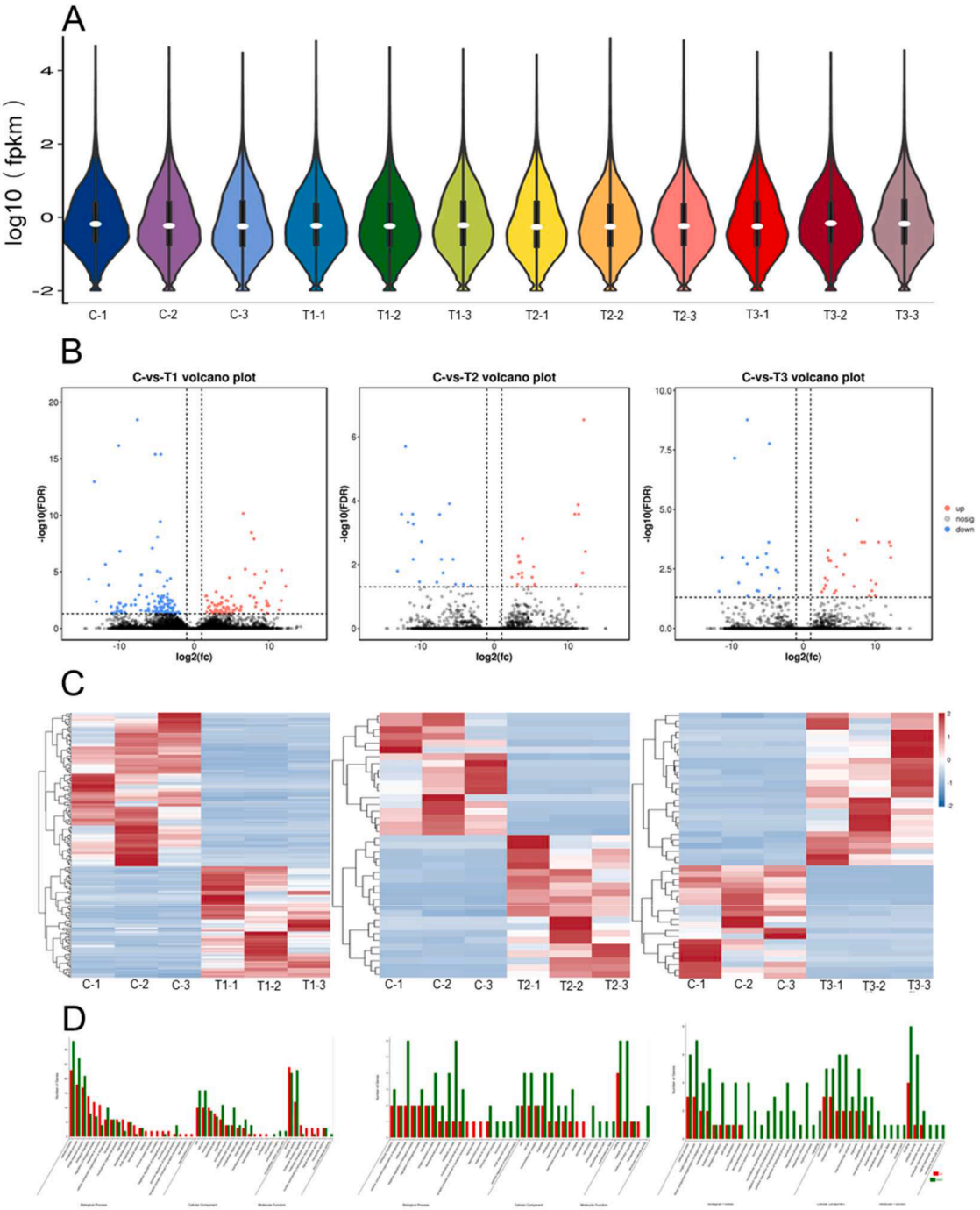

Fig. 1. Identification and analysis of mRNAs and DEmRNAs in *Mytilus galloprovincialis* under PVC and Cd stress. (A) FPKM distribution of mRNAs in 12 samples. (B) DEmRNAs statistics after PVC and Cd treatment. (C) Heat map of all DEmRNAs. (D) GO enrichment of all DEmRNAs.

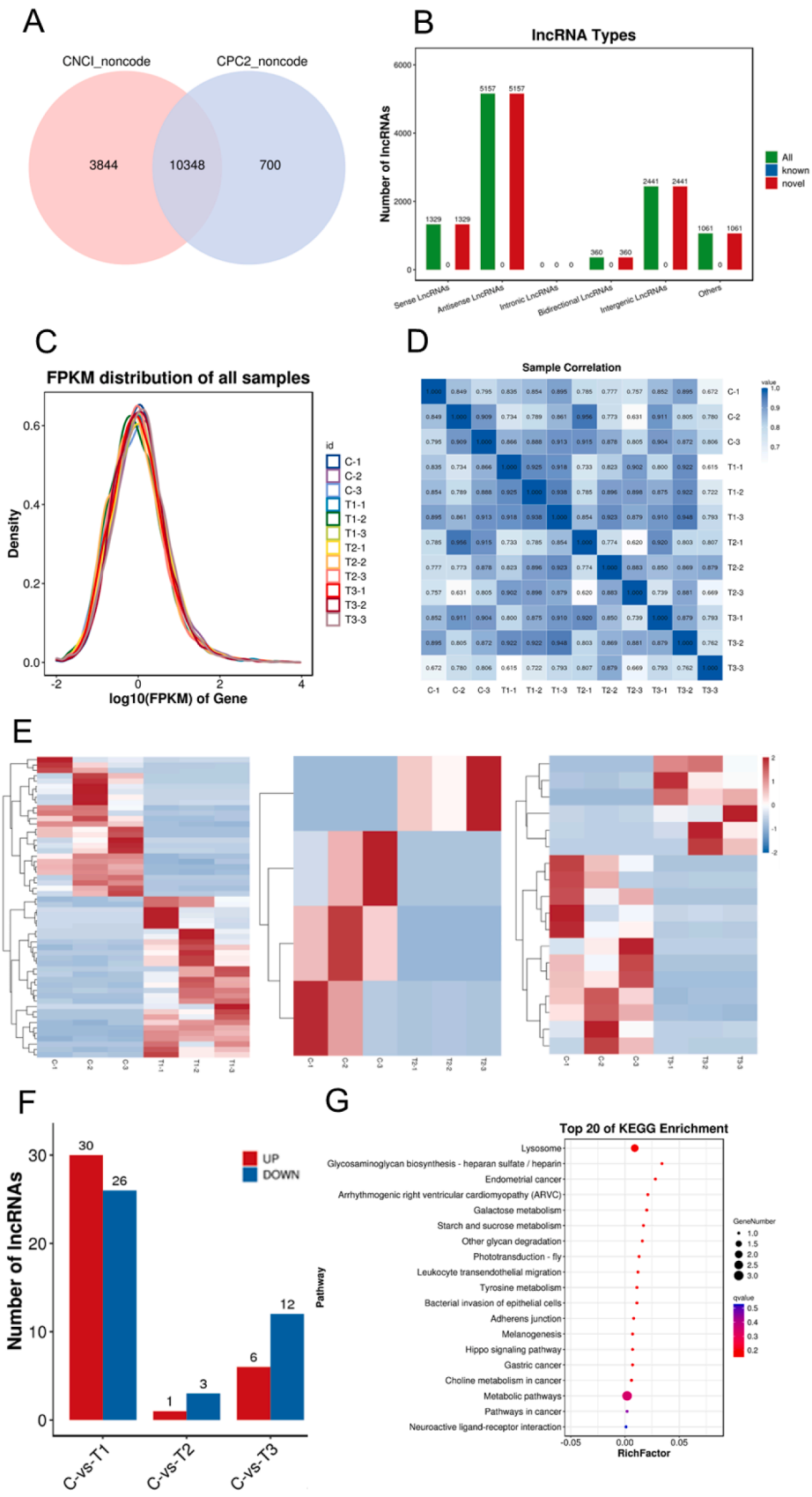

Fig. 2. Identification and distribution of lncRNAs and DElncRNAs in *Mytilus galloprovincialis* under PVC and Cd stress. (A) The number of lncRNAs identified by the CNCI and CPC2 methods. (B) lncRNA type distribution in *M. galloprovincialis*. (C) FPKM distribution of lncRNAs in 12 samples. (D) Sample correlation of lncRNAs in 12 samples. (E) Heat map of all DElncRNAs. (F) The expression levels of lncRNA in the Cd, PVC and the Cd and PVC groups. (G) Top 20 of KEGG enrichment of lncRNA.

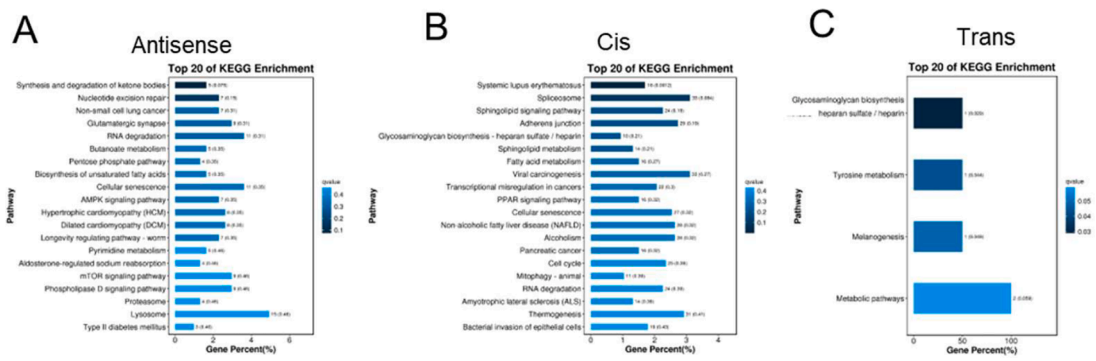

Fig. 3. The KEGG pathway analysis of the target genes of DElncRNAs in *Mytilus galloprovincialis* under PVC and Cd stress. (A) The KEGG pathway analysis of the antisense-targeted genes of DElncRNAs. (B) The KEGG pathway analysis of cis-targeted genes of DElncRNAs. (C) The KEGG pathway analysis of trans-targeted genes of DElncRNAs.

3. Results and discussion

The regulation of ncRNAs is widespread in invertebrates, but there has been limited research on their role in environmental adaptation or specific physiological processes under stress from PVC and Cd, especially in non-model organisms like molluscs (Kozomara et al., 2013). Marine species face various environmental pollution stressors, and researchers have explored how ncRNAs respond to these stresses (Biggar and Storey, 2015). Marine mussels are capable of accumulating large amounts of heavy metals and can be used to monitor metal contaminants (Jernelov, 1996; Bebianno and Serafim, 1998; Burgos-Aceves et al., 2018a, 2018b). Previous studies have identified multiple miRNAs in *M. galloprovincialis* (mg-miRNAs) and discussed their potential functions and responses to Cd exposure (Yu et al., 2020, 2021). However, no integrated study of ncRNAs has been conducted on *M. galloprovincialis* under PVC microplastics and Cd stress. Our whole-transcriptome sequencing results reveal the complex molecular responses of *M. galloprovincialis* to environmentally relevant concentrations of PVC microplastic and Cd. In this study, we investigated the differential expression of various RNA pathways in response to co-exposure to Cd and PVC compared to exposure to Cd and PVC individually. The results revealed different patterns of differential expression in circRNA, lncRNA, miRNA, and mRNA.

3.1. Bioaccumulation of cadmium (Cd) and polyvinyl chloride (PVC) in mussels

Marine organisms can ingest both microplastics and heavy metals, leading to their bioaccumulation in tissues and organs (Bakir et al., 2014; Kedzierski et al., 2018; Xu et al., 2020). Marine mussels *M. galloprovincialis* exposed to solutions containing 10 μg/L Cd exhibited a significant increase in Cd accumulation within their soft tissues compared to the control group ($p < 0.05$) (Table 1). This effect was not further amplified by the co-exposure to 10 μg/L Cd and 20 items/L PVC, as Cd concentrations in these groups were statistically similar. Similarly, the control group and the group exposed solely to 20 items/L PVC displayed no significant difference in Cd content. PVC particles were identified in the soft tissues of both the PVC-exposed group and the co-exposure group (Fig. 8). The number of particles observed varied between 0 and 3 pieces, with no statistically significant difference between the groups.

3. 2 mRNA expression profiles under PVC and Cd stress

Whole-transcriptome RNA sequencing was employed to obtain FPKM (Fragments per kilobase million) values for gene expression. Based on the distribution of transcript abundance across the 12 samples, overall gene expression levels were categorized into four groups: control (seawater-treated; C-1, C-2, and C-3), Cd-treated (T1–1, T1–2, and T1–3), PVC-treated (T2–1, T2–2, and T2–3), and co-exposure (Cd and PVC; T3–1, T3–2, and T3–3) (Fig. 1A). Differential expression (DE) analysis identified a total of 281 mRNAs (130 up-regulated and 151 down-regulated; $p < 0.05$) (Fig. 1B, Supplementary Table 1). Heatmap visualization revealed distinct clustering of the control group from the treatment groups (Fig. 1C).

To understand the functional roles of DEmRNAs, Gene Ontology (GO) enrichment analysis was performed. In all treatment groups (Cd, PVC, and co-exposure), the majority of DEmRNAs were associated with biological processes like metabolism and cellular activities (Fig. 1D). Transcriptome analysis of genes expressed in the earthworm *Eisenia fetida* in response to cadmium exposure reveals that differential expressional genes (DEGs) mainly enriched in metabolism and oxidative stress (Chai et al., 2020), which are consistent with our study. Similarly, the molecular function category consistently showed enrichment in binding and catalytic activities across all groups. Additionally, terms related to cellular components, including “cell” and “cell part”, were consistently enriched. Notably, the co-exposure group displayed additional enriched terms in the biological process category, such as protein processing in the endoplasmic reticulum, viral infections (Hepatitis C, Epstein-Barr virus, Measles, Herpes simplex, Influenza A) (Fig. 1D). These findings suggest a core response involving cellular processes, binding, and catalysis across all treatments. The co-exposure group, however, exhibited additional enrichment in stress-related pathways like viral infections and protein processing.

3.3. Characterization of long non-coding RNAs (lncRNAs) under PVC and Cd stress

Whole-transcriptome sequencing identified a total of 10,348 lncRNAs (Fig. 2A). These were further categorized based on their genomic location relative to protein-coding genes: 1329 sense, 5157 antisense, 360 bidirectional, 2441 intergenic, and 1060 other lncRNAs (Fig. 2B). Differential expression analysis revealed significant differences ($p < 0.05$) in lncRNA expression compared to mRNA expression in Cd, PVC, and co-exposure groups (Fig. 2F). A total of 37 lncRNAs were up-regulated, and 41 were down-regulated (Figs. 2F and Supplementary Table 2). Heatmap visualization demonstrated distinct clustering of the control group from the treatment groups (Fig. 2E).

Antisense lncRNA prediction based on complementary base pairing identified 6327 antisense pairs between 5157 lncRNAs and 5039 mRNAs. However, only 17 pairs were found between differentially expressed lncRNAs (DElncRNAs) and differentially expressed mRNAs (DEmRNAs) ($p < 0.05$) (Fig. 2B). KEGG pathway enrichment analysis revealed the lysosome, cellular senescence, and RNA degradation

Table 2
Summary of detected associations (antisense, *cis* and trans) of ncRNAs for the mussel *Mytilus galloprovincialis* exposed to Cd and PVC for 96 h.

Association	list	lncRNA	mRNA	pair
Antisense	all	5157	5039	6327
	diff	16	17	17
Cis	all	8980	20,167	28,885
	diff	4	2	6
Trans	all	7839	45,238	2,282,581
	diff	53	91	107

pathways as the most significantly enriched (Fig. 2G).

Six DElncRNA-DEmRNA pairs were identified through cis-interaction analysis (Table 1). The most enriched cis-targeted DEmRNAs belonged to thermogenesis pathways (Fig. 3A). Correlation analysis identified 107 trans-interactions between DElncRNAs and DEmRNAs (Table 2). KEGG pathway analysis of these trans-interacting RNAs indicated the metabolic pathway as the most enriched (Fig. 3B). These findings suggest that the combined exposure to Cd and PVC microplastics modulates lncRNA expression, potentially impacting stress adaptation and detoxification pathways in mussels. This aligns with the observation by Qu et al. (2019) who identified lncRNAs involved in nano-polystyrene (nano-PS) stress in *Caenorhabditis elegans*. This implies a potential role for programmed cell death as a protective response to the toxic effects of PVC microplastics and Cd in mussels.

3.4. Characterization of circular RNAs (circRNAs) under PVC and Cd stress

Whole-transcriptome sequencing identified 5343 circRNAs classified into four types (Fig. 4A and Supplementary Table 5). Their chromosomal distribution is shown in Fig. 4D. Most circRNAs exhibited lengths exceeding 3000 bp (Fig. 4B). GO enrichment analysis revealed distinct patterns in gene annotations of differentially expressed circRNAs (DEcircRNAs) across treatment groups ($p < 0.05$). In all groups (Cd, PVC, and co-exposure of Cd and PVC), the majority of DEcircRNAs were

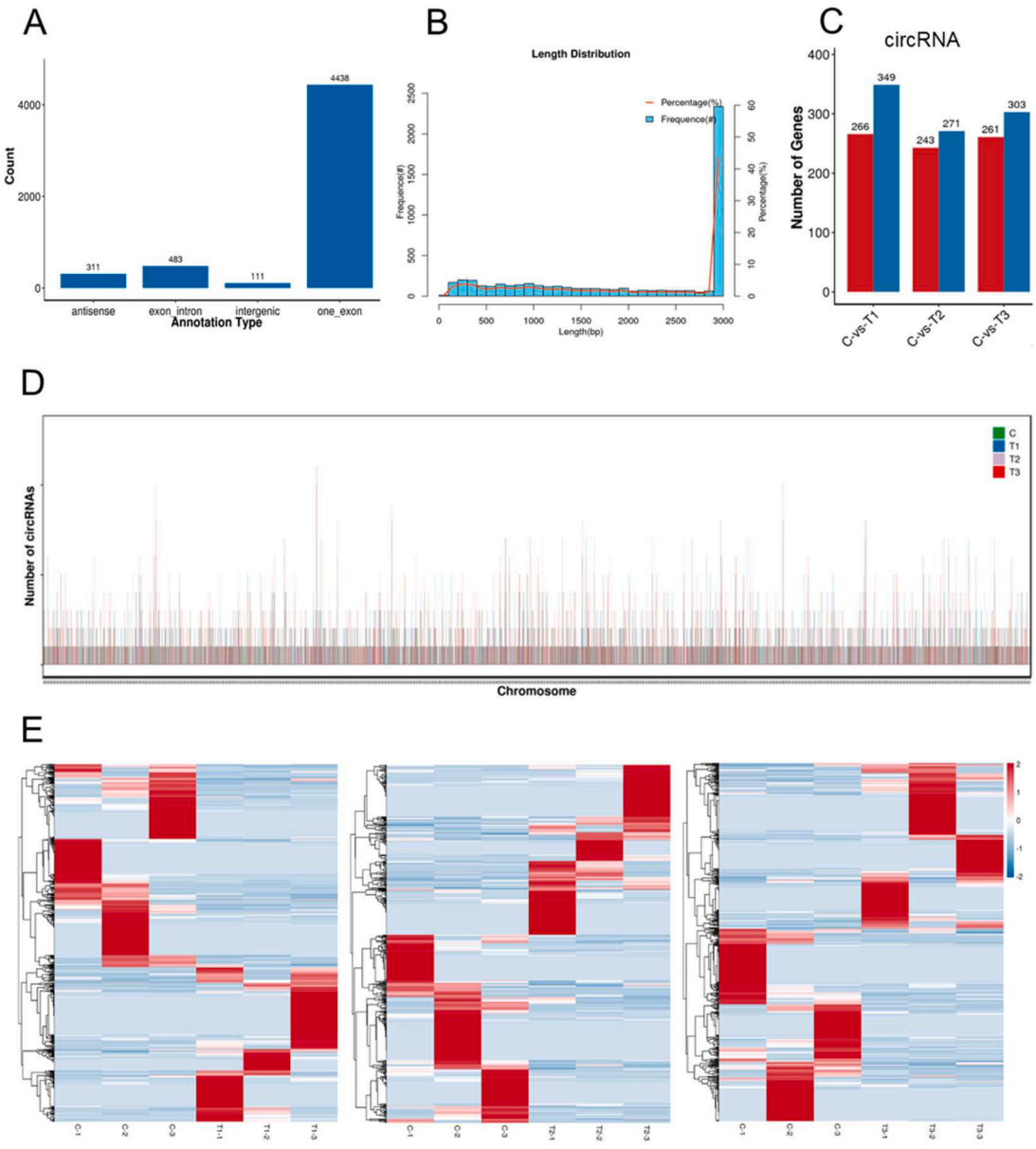

Fig. 4. Distribution and analysis of source genes of circRNAs in *Mytilus galloprovincialis* under PVC and Cd stress. (A) Numbers of annotation types of circRNAs. (B) Number statistics of different circRNAs in length. (C) Chromosome distribution of circRNAs. (D) Heat map of all DEcircRNAs.

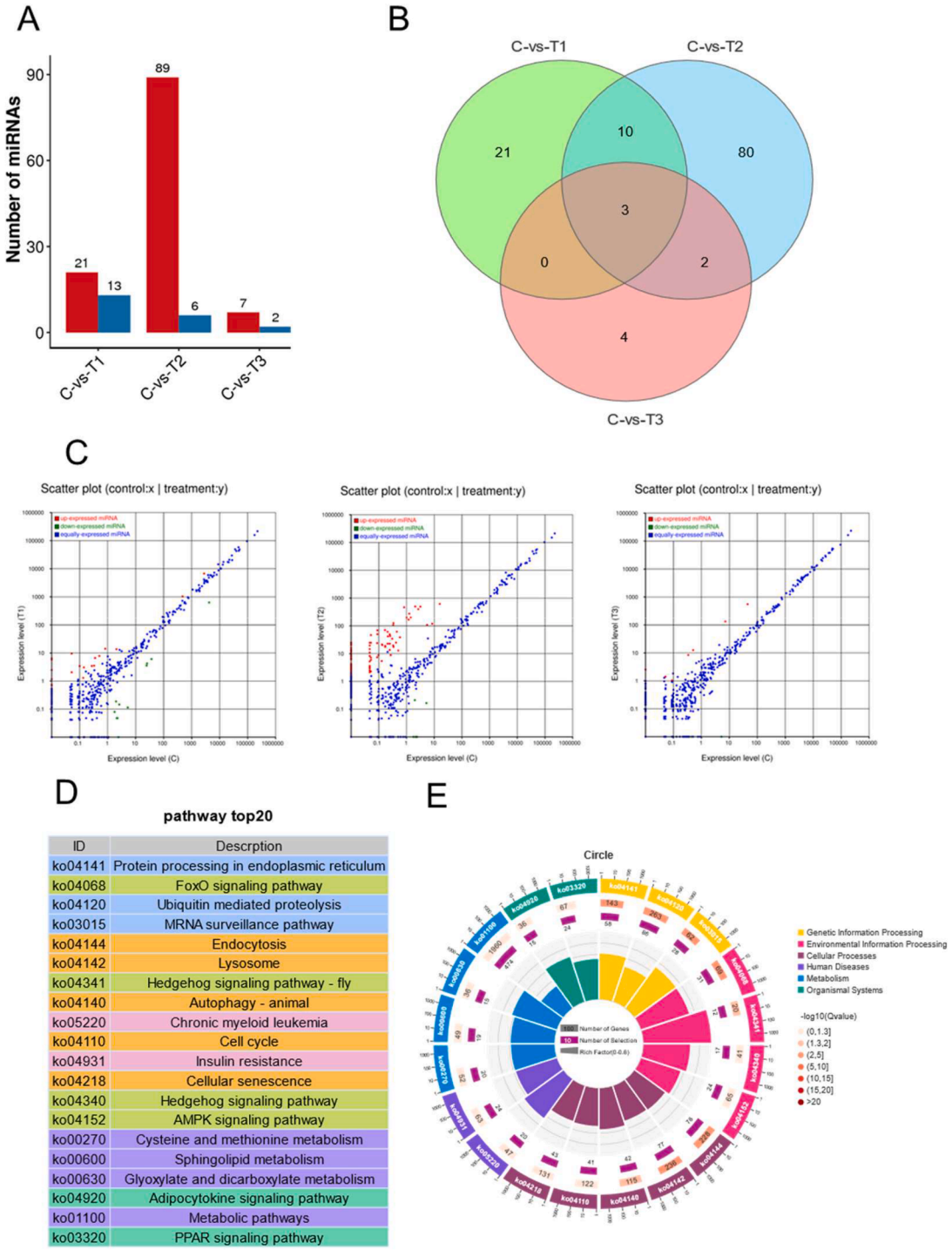

Fig. 5. Expression analysis of miRNA in *Mytilus galloprovincialis* under PVC and Cd stress. (A, B, C) Their expression levels of DEmiRNA in the 12 samples in *M. galloprovincialis*. (D, E) Top 20 of KEGG enrichment of miRNA.

associated with biological processes like cellular and single-organism activities (Supplementary Fig. 1). Similarly, the molecular function category consistently displayed enrichment in binding and catalytic activities. Additionally, terms related to cellular components, such as "cell" and "cell part", were consistently enriched. Notably, KEGG pathway analysis of the co-exposure group identified the Huntington disease pathway as the most enriched (Supplementary Fig. 1).

Circular RNAs (circRNAs) exhibit stability, conservation, abundance, and stage specificity. Additionally, they have the capability to serve as miRNA sponges and modulate gene expression (Lei et al., 2018). Differential expression analysis identified a total of 1693 circRNAs (770 up-regulated and 923 down-regulated; $p < 0.05$) (Figs. 4B and E). Heatmap visualization confirmed distinct clustering patterns between the control and treatment groups (Fig. 4E). More detailed information is provided in Supplementary Table 4. Apoptosis pathway was affected in the three treatment groups. Cd induces apoptosis by regulating circ08409 is reported in bovine mammary epithelial cells (BMECs) and mouse mammary gland (Chen et al., 2021), which are consistent with

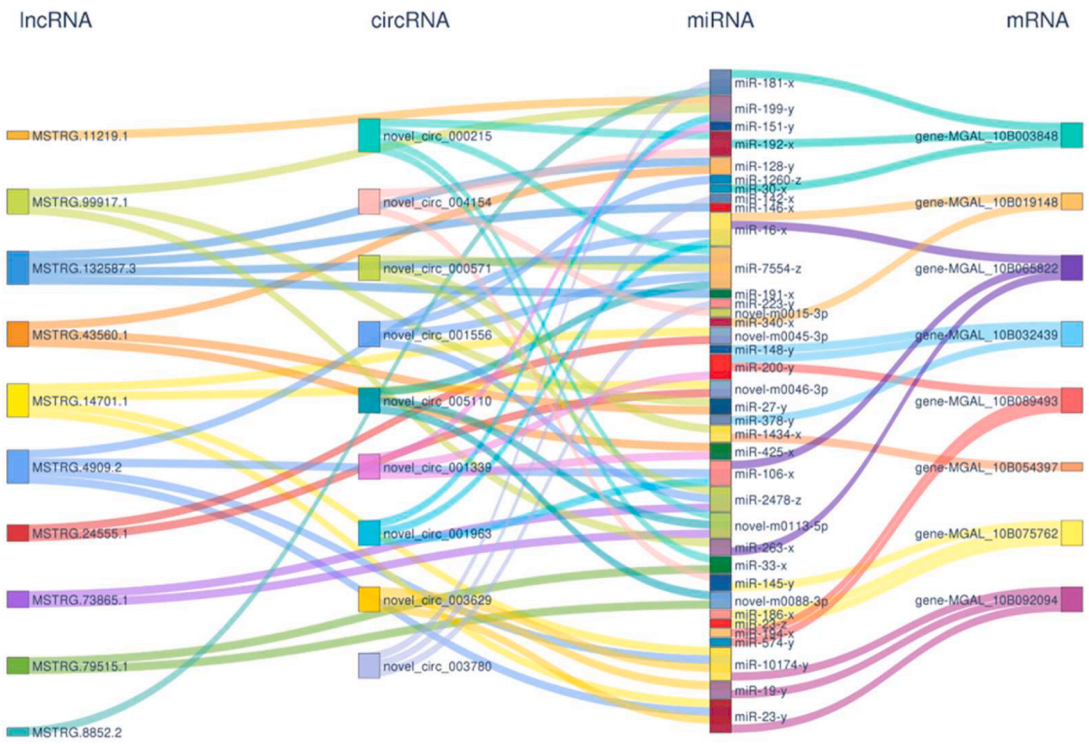

Fig. 6. The ceRNA networks in *Mytilus galloprovincialis* under PVC and Cd stress. The ceRNA networks were constructed with DEmiRNAs, DElncRNAs, DEcircRNAs and DEmRNAs.

our studies.

3.5. Characterization of MicroRNAs (miRNAs) under PVC and Cd stress

Whole-transcriptome RNA sequencing identified both known and novel miRNAs (Supplementary Table 6). Differential expression analysis revealed a total of 138 miRNAs (117 up-regulated and 21 down-regulated; $p < 0.05$) (Fig. 5A, 5B). The expression profiles of these miRNAs across the 12 samples are shown in Fig. 5C. KEGG pathway analysis indicated that protein processing in the endoplasmic reticulum was the most enriched pathway among differentially expressed miRNAs (DEmiRNAs) (Fig. 5D, 5E).

Cadmium (Cd) exposure specifically affected the expression of miRNAs associated with various metabolic pathways, including glyoxylate and dicarboxylate metabolism, steroid biosynthesis, and others involved in glycan degradation, pyruvate metabolism, cytokine-cytokine receptor interaction, retinol metabolism, oxidative stress, and circadian rhythm. Previous pathway analysis revealed miRNAs might increase Cd tolerance by suppressing cellular growth and switch cellular energy allocation to detoxification processes (Chen et al., 2016), these pathway findings are consistent with our study. Conversely, polyvinyl chloride (PVC) stress specifically altered miRNAs related to glycolysis-gluconeogenesis, apoptosis, DNA repair (nucleotide excision repair), drug metabolism (cytochrome P450), splicing (spliceosome), cellular signaling (MAPK and p53 pathways), sphingolipid signaling, inositol phosphate metabolism, Notch signaling, propanoate metabolism, the citric acid cycle, synaptic vesicle cycling, amino acid biosynthesis, and processes involving oxidative stress and chemical carcinogenesis. Notably, the combined exposure to Cd and PVC microplastics resulted in differential expression of miRNAs associated with diverse pathways, including those regulating longevity, phospholipase D signaling, TGF-beta signaling, chemokine signaling, apelin signaling, folate metabolism, purine metabolism, oocyte meiosis, regulation of the actin cytoskeleton, axon guidance, thermogenesis, endocrine resistance, and again, oxidative stress and longevity regulation. These findings align with the observations by Shi et al. (2022) who reported altered miRNA expression and exacerbated oxidative damage in rats treated with PS-MPs (polystyrene microplastics).

3. 6 ceRNA regulatory network and key pathways under PVC and Cd stress

The ceRNA regulatory network plays vital roles in regulating the expression of genetic information, it is constructed in many marine species (Ma et al., 2020; Ning et al., 2021; Wang et al., 2021; Cai et al., 2023; Ibrahim et al., 2023). However, we found no report in marine mussel, *M. galloprovincialis*. To explore the interactions between protein-coding RNAs and ncRNAs in response to PVC and Cd stress, ceRNA regulatory networks were constructed based on the ceRNA hypothesis. Our analysis identified 8576 interactions between differentially expressed miRNAs (DEmiRNAs) and mRNAs, 32,495 interactions between DEmiRNAs and differentially expressed circRNAs (DEcircRNAs), and 13,114 interactions between DEmiRNAs and lncRNAs (Fig. 6).

Among these interactions, 1756 DEmRNAs were predicted as targets for 135 DEmiRNAs (Fig. 6). For example, mg-miR7554 targeted two mRNAs (MGAL_10B020614 and MGAL_10B089122) and six DEcircRNAs (including novel_circ_000215, novel_circ_000263, novel_circ_000337, novel_circ_001556, novel_circ_003590, and novel_circ_005333). These DEmiRNAs, DEcircRNAs, and DEmRNAs may play crucial regulatory roles in *M. galloprovincialis* under PVC and Cd stress. Furthermore, we investigated key genes and pathways affected by

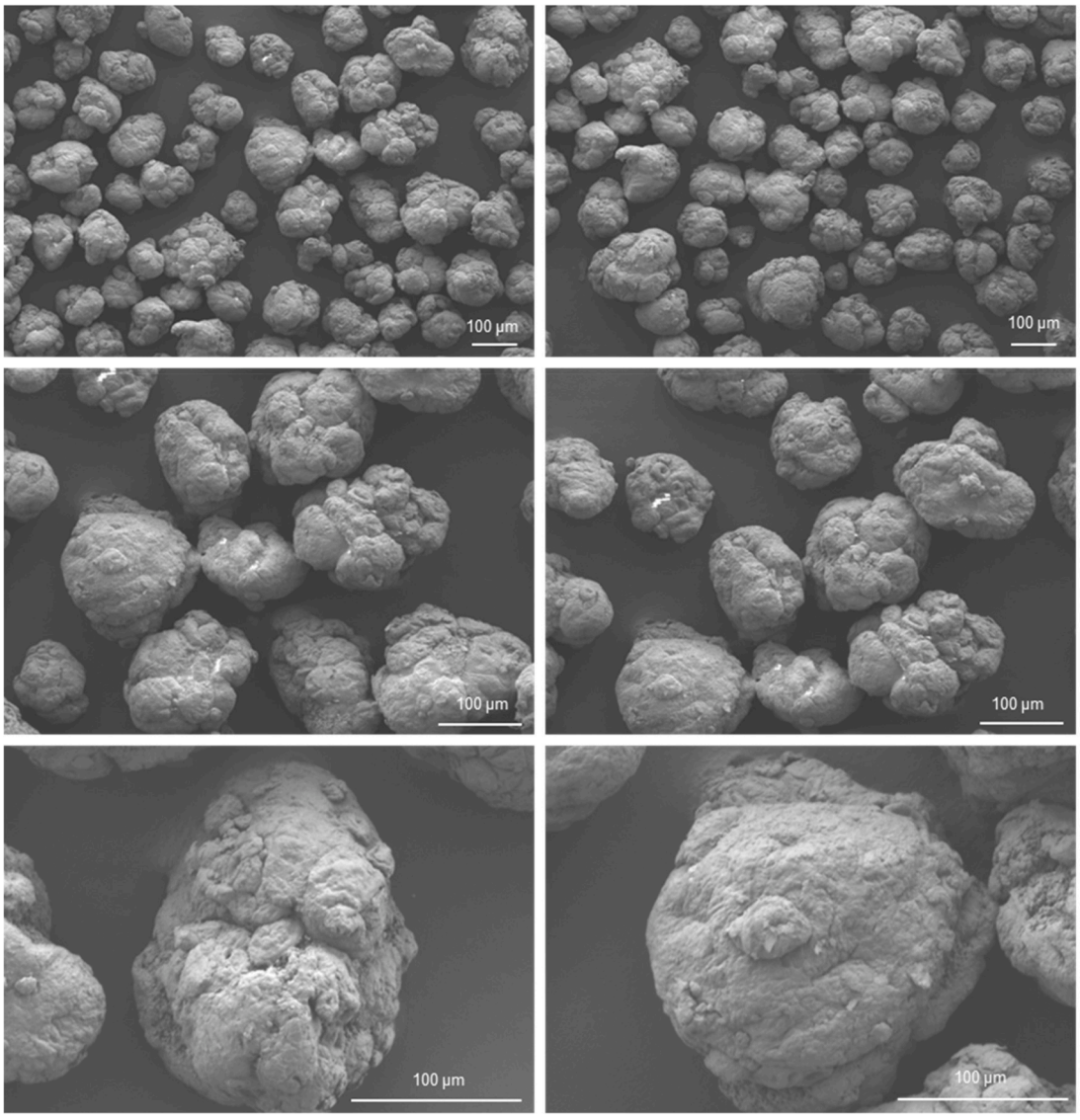

Fig. 7. SEM of experimental PVC particles used in this study.

PVC and Cd exposure in *M. galloprovincialis*. Transcription factors (TFs) are critical regulators of gene expression in the nucleus. Our study revealed downregulation ($p < 0.05$) of TFs, including zf-C2H2 (gene-MGAL_10B094598), following PVC and Cd treatment.

We also identified 11 genes involved in metabolic pathways that displayed significant changes in expression ($p < 0.05$). Among these, MGAL_10B032439 was co-regulated by mg-mir-148, mg-mir-200, and mg-mir-378. Additionally, mRNA pathway enrichment analysis identified the top seven enriched pathways as drug metabolism, steroid hormone biosynthesis, retinol metabolism, drug metabolism-cytochrome P450, Parkinson's disease, chemical carcinogenesis, and metabolic pathways.

4. Conclusion

This study sheds light on the regulatory roles of non-coding RNAs (ncRNAs) in the stress response of the marine mussel *Mytilus galloprovincialis* to PVC microplastics and cadmium (Cd). Exposure to Cd, PVC, or their combined stress revealed complex molecular responses in *M. galloprovincialis*, characterized by differential expression patterns in circRNAs, lncRNAs, miRNAs, and mRNAs. These findings highlight the diverse involvement of ncRNAs in stress adaptation. We observed differential expression of specific pathways associated with circRNAs, miRNAs, and lncRNAs under each stress condition (Cd, PVC, and co-exposure). This suggests the participation of ncRNAs in cellular signaling, metabolism, and the stress response. Notably, co-exposure triggered differential expression in additional pathways, implying potential interactions between PVC microplastics and Cd that influence stress adaptation and detoxification. These results emphasize the intricate interplay between various RNA pathways under multiple stress conditions. Furthermore, the identification of pathways related to cellular senescence, oxidative stress, and galactose metabolism underscores the critical role of ncRNAs in regulating these processes. In conclusion, this study highlights the importance of ncRNAs in *M. galloprovincialis*' response to PVC microplastics and Cd stress, providing valuable insights into the complex molecular mechanisms governing stress adaptation and detoxification.

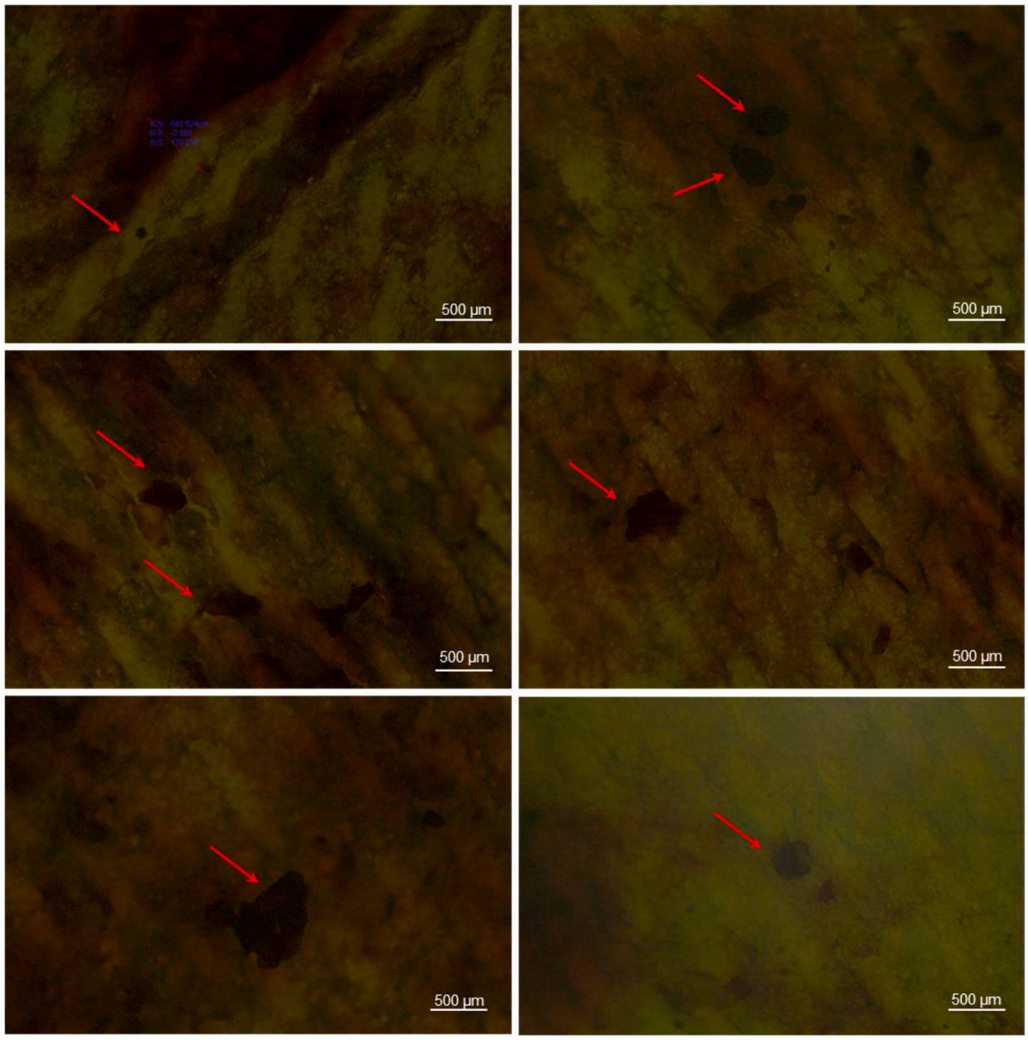

Fig. 8. PVC particles in soft tissues of mussel *Mytilus galloprovincialis*.

CRediT authorship contribution statement

Deliang Yu: Writing – original draft, Project administration, Funding acquisition, Formal analysis. **Shaochong Liu:** Writing – review & editing. **Yaqi Yu:** Writing – review & editing. **Yanhao Wang:** Writing – review & editing. **Lianzhen Li:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Willie J.G.M. Peijnenburg:** Writing – review & editing, Supervision. **Yufeng Yuan:** Writing – review & editing. **Xiao Peng:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

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

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2024.107062.

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