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The Role of Microplastics as Carriers of Heavy Metal Cadmium (Cd) in *Paphia undulata* under different Salinities

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ABSTRACT

The combination of microplastics (MPs) and heavy metals has been documented to result in increased accumulation levels and more severe impacts on organisms. Paphia undulata serves as a bioindicator with a high sensitivity to alterations in salinity. This research examines the involvement of MP particles as carriers of heavy metal cadmium (Cd) pollution in hard clams across various treatment conditions, MP types, and salinity levels. The type of MP particles significantly influences the extent of Cd contamination in hard clams with polypropylene (PP) particles leading to a higher Cd concentration increase compared to other particles. Furthermore, the concentration of heavy metal Cd rises in correlation with increasing water salinity. Clams placed in water with a salinity of 30‰ exhibited a greater accumulation of MP particles compared to those in lower salinity environments. Our findings underscore the critical role of salinity in conjunction with the interaction and type of MP particles in the transport of heavy metals within aquatic ecosystems.

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INTRODUCTION

Microplastics are a ubiquitous environmental concern, representing tiny plastic particles and fibers less than 5 mm in size. They originate from various sources, including the breakdown of larger plastic items and the release of microplastic-containing products. These particles have been found in oceans, rivers, and even in the soil, impacting various aquatic organisms (Peda *et al*., 2016). A growing body of research has focused on the potential of microplastics to act as vectors for toxic substances, such as heavy metals, which can adsorb to their surfaces (Brennecke *et al*., 2016; Barus *et al*., 2021). In recent years, the pervasive issue of microplastic pollution has garnered significant attention due to its detrimental impact on aquatic ecosystems and the organisms inhabiting them (Prokic *et al*. 2019). Microplastics have been found in various aquatic environments worldwide, raising concerns about their potential to transport and introduce contaminants, such as heavy metals, into aquatic organisms (Vieira *et al*., 2021; Barus

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et al., 2023). Negative consequences include oxidative stress, DNA damage, organ malfunction, metabolic problems, immunological response, neurotoxicity, and toxicity to reproduction and development might result from prolonged exposure to microplastics (Prokic *et al*. 2019).

Cadmium (Cd), in conjunction with lead, arsenic, chromium, and mercury is classified as a heavy metal without any discernible physiological purpose and is commonly regarded as a toxic substance (Friberg *et al*., 2018). Various avenues of cadmium exposure have been documented over the last century, with human activities introducing cadmium into the environment (Rahimzadeh *et al*., 2017). The persistent causes of cadmium pollution are associated with its use in industry as a corrosive reagent and its incorporation as a stabilizer in PVC products, color pigments, and Ni-Cd batteries (Genchi *et al*., 2020). Additionally, cadmium is prevalent as a pollutant in non-ferrous metal smelting and the recycling of electronic waste. The absorption of Cd predominantly occurs through the respiratory system, with minor absorption through the gastrointestinal tract, while skin absorption is relatively infrequent. Upon entry into the body, cadmium is transported through the bloodstream by erythrocytes and albumin and subsequently accumulates in the kidneys (Satarug *et al*., 2018), liver, and intestines (Tinkov *et al*., 2018). Numerous negative consequences in the organisms, including pulmonary edema, testicular injury, osteomalacia, hepatic and renal dysfunction, and harm to the adrenal glands and hemopoietic system, can be brought on by exposure to cadmium. Additionally, a correlation was found between peripheral artery disease, coronary heart disease, stroke, and atherogenic lipid profile alterations and Cd exposure indicators (blood and urine) (Tinkov *et al*., 2018).

The bivalve species known as *Paphia undulata*, commonly called the clam, holds considerable importance in the aquaculture industry in Asia, as emphasized by Leethochavalit *et al*. (2004). These hard clams, which are a significant component of the infaunal bivalve mollusk population, are typically found residing on the seafloor within layers of fine sand, silt, or sandy silt (Lin *et al*., 2018). Due to their sedentary nature, hard clam are frequently utilized as indicators of water pollution, specifically for substances like heavy metals (Amiard *et al*., 2006), and microplastics (Zhang *et al*., 2021).

Salinity exerts a pivotal influence on aquatic ecosystems, shaping a range of physiological and ecological phenomena. Fluctuations in salinity have the potential to modify the solubility and accessibility of heavy metals, impacting the interaction of microplastics with these metals as highlighted by Barus *et al*. (2021). An in-depth understanding of salinity's repercussions is indispensable for understanding the transfer of heavy metals from microplastics to hard clams.

Previous studies have documented the accumulation of microplastics and their impacts on hard clams (Prokic *et al*., 2019. The detrimental effects become more pronounced when microplastic pollutants accumulate in the body of the clam alongside heavy metals that have been absorbed onto the surface layer of the microplastics. Notably, there is a lack of research exploring different salinity levels in this context, despite the limited number of studies that have investigated how microplastics serve as carriers for heavy metals within hard clam bodies. This study is designed to investigate the role of microplastic particles in transporting heavy metal cadmium in *P. undulata* hard clams under varying treatment and salinity conditions, employing different types of microplastic particles.

MATERIAL AND METHODS

For this research, we utilized microplastic particles with a diameter of 150 µm, consisting of polypropylene (PP), polyethylene (PE), and polyethylene terephthalate (PET), as the representative model for microplastics. These microplastics were acquired from Tersulan Chemical Co., Ltd. located in Guangdong, China. The microplastic particles utilized in this research are consistently uniform in size and appear as spherical granules. All three types of microplastics are characterized by their pure white color, and all the particles used in our investigation have been confirmed to be devoid of detectable metal content. We introduced cadmium (Cd) as the heavy metal element procured from Merck KGaA, headquartered in Darmstadt, Germany. To determine the density of each microplastic type, we conducted a density analysis using the method outlined by Li *et al*. (2018). This particle density assessment corresponds to the quantity of particles employed in the evaluation of each microplastic type.

Adult *P. undulata*, with a weight of 20.42 ± 0.04 grams, was procured from a local market. The hard clams were subsequently held in 1000 liters of seawater with a salinity of 30‰ for 5 days. The source of the seawater was coastal, and this quarantine period was implemented to facilitate the adaptation of the clams and to ensure the removal of any plastic contaminants through the depuration process. Following this initial phase, a subset of the hard clams was transitioned to environments with salinities of 25 and 20‰. It is noteworthy that the optimal salinity range for clams falls within the estimated range of 20 to 30‰ (Malouf & Bricelj, 1989). To facilitate a smooth adjustment, the salinity was gradually reduced by 1‰ per day. This categorization was conducted to facilitate testing in varying salinity conditions. Throughout this period, the organisms were consistently maintained and acclimated at a constant temperature of 22 ± 0.5 °C with continuous aeration. During the maintenance phase, *P. undulata* received a diet of green algae. Before exposure to microplastic particles, an analysis of heavy metal contamination in the meat of the three clams was performed after the acclimation process.

A beaker, containing 1 gram of microplastic (MP) particles, was filled with 100 milliliters of a solution containing heavy metal Cd at a concentration of 1 milligram per liter and a pH of 6.8. The mixture underwent agitation for 24 hours at 180 revolutions per minute using a magnetic stirrer. The chosen concentration of MPs and heavy metals was based on prior tests, indicating its representation of the absorption capacity of heavy metals by microplastics in aquatic ecosystems (Barus *et al*., 2021). Following the 24-hour exposure, the particles were filtered using a 0.45 µm filter paper. The microplastic particles that absorbed heavy metals were subsequently utilized in experiments involving hard clams.

After the 24-hour interaction with heavy metals, measurements were taken to determine the adsorption value of heavy metal Cd on the MP particles. Each sample's particles were separated via sonication (at 120 watts for 10 minutes, using 2% nitric acid). An extract solution was collected and its composition was determined using a flame atomic absorption spectrophotometer (SpeactAA 240-FS, VARIAN, USA). Three replicates of the assay were conducted.

Hard clams, previously raised and adapted to different salinities, were introduced into a glass container containing 1 liter of seawater with varying salinities (20, 25, and 30‰), and the efficacy of the treatment was then evaluated.

P. undulata specimens, acclimated to different salinity levels, were introduced into a glass container filled with 1 liter of seawater having varying salinities (20, 25, and 30‰), followed by the experimental treatment. The exposure treatment involved five groups: Group A (seawater with 0 mg/L of Cd + MPs without exposure to any heavy metals); Group B (seawater with 0 mg/L of Cd + MPs exposed to Cd at 1 mg/L for 24 hours); Group C (seawater with 0.1 mg/L of Cd and MPs without exposure to Cd at 1 mg/L); Group D (seawater with 0.1 mg/L of Cd without any particles); Group E (seawater with 0.1 mg/L of Cd + MPs exposed to Cd at 1 mg/L for 24 hours). Each glass tube contained ten hard clams, aerated continuously to ensure the clams' viability and to keep the particles suspended in the tubes. The hard clams were harvested at 6, 12, 18, and 24 hours after initial contact, with three repetitions for each trial.

Three hard clams, immersed for an extended duration, were chosen and frozen at -20 °C. Subsequently, these clams underwent lyophilization at -60°C using the FD-20A2D equipment (H.C.S. Taipei, Taiwan). After 3 days, the samples were treated with concentrated nitric acid (70% concentration) and digested in a high-speed microwave reactor (MARS Xpress, CEM, USA). The levels of heavy metals present in the clam bodies were then analyzed using a flame atomic absorption spectrophotometer (SpeactAA 240-FS, VARIAN, USA).

Additionally, we examined the persisting concentrations of heavy metals in both microplastic (MP) particles and seawater at different time intervals to understand the contribution of heavy metals adsorbed onto MP particles to the elevated levels of heavy metals in hard clams. Within a 24-hour timeframe, the particles were isolated using a 0.45 µm filter paper. Ultrasound treatment (120 W, 10 minutes, 2% nitric acid) was used to eliminate particles from each sample. The composition of the extract solution was analyzed using an atomic flame adsorption spectrophotometer (SpeactAA 240-FS, VARIAN, USA).

After specified contact durations, three resilient clams were extracted and their meat isolated. To decompose organic material, 200 ml of 30% H_2O_2 solution was added to each beaker with the clam meat and incubated at 60°C for 24 hours. The remaining solution was then filtered through a 0.45 µm membrane. Microplastics captured by the filter were examined using a stereo microscope at 40x magnification, identified based on color, shape, and surface texture.

Both one-way and two-way ANOVA were used for data analysis. The Duncan test identified significant distinctions between treatments, focusing on the percentage increase of heavy metals in clam bodies at different salinities. Statistical significance was set at $p<0.05$. Additionally, a general linear model assessed the linear relationships among the type of microplastic particles, salinity, the quantity of accumulated microplastic particles, and the heavy metal content in clam bodies.

RESULTS AND DISCUSSION

Microplastics made of PET exhibited the highest particle density (1.36 g/cm^3), followed by PE (1.09 g/cm^3) and PP (0.87 g/cm^3). After 24-hour exposure, distinct levels of Cd adsorption were observed, with PP showing the highest adsorption and PE and PET displaying the lowest. Specifically, Cd adsorption was 0.547 ± 0.005 µg/g in PP, 0.507 ± 0.012 µg/g in PE, and 0.483 ± 0.009 µg/g in PET (Fig. 1). The polarity and physicochemical properties of MPs significantly influence heavy metal adsorption. The mechanism likely involves direct adsorption of cations or complexes onto charged or neutral sites on the microplastic's surface, highlighting the importance of surface characteristics and porosity in this process (Li *et al*., 2018; Gao *et al*., 2021).

To establish a baseline Cd concentration, the initial amount of heavy metals in the hard clams'

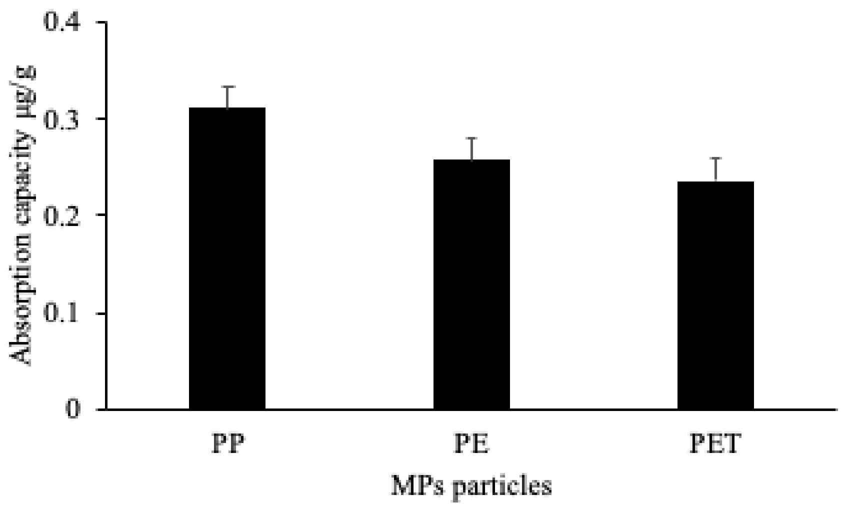

Fig. 1. Cd adsorption (µg/g) of 1 g MPs particles after 24 hours of exposure to the heavy metal Cd at a concentration of 1 mg/L and a pH of 6.8.

bodies was measured before the exposure experiment, revealing very low Cd contamination (0.032 ± 0.005 µg/g) in *Paphia undulata*. The increase in Cd content within the hard clams' bodies varied depending on the treatment groups. In Group A, Cd levels in hard clams did not increase during tests with three different salinity values (Figs. 2, 3, and 4), indicating that Cd metal was not present in the microplastic particles used in this study.

In Group B, there was an evident increase in the Cd content within the firm clams. After monitoring for six hours, a substantial spike in salinity to 20‰ was observed, followed by a more modest increase in the subsequent six hours (Fig. 2). During the initial six hours, Cd-absorbing microplastic particles (MPs) entered the hard clams' bodies before eventually being released into Cd-free saltwater, explaining this phenomenon. The Cd concentration in the MPs particles consistently decreased until the final observation, indicating a release of Cd into the solution (Table 1).

Group C's hard clams showed a more pronounced increase in Cd metal concentration compared to Group B, likely due to the Cd content in the seawater. During the six-hour observation period, there was a significant improvement, potentially because of a substantial decrease in lead concentration in the seawater, which was absorbed by the MPs particles. Table 1 indicates that as observation time increased, Cd concentration within MPs particles rose while it decreased in the surrounding seawater. Group D showed different findings, lacking MPs particles to absorb heavy metals, resulting in a higher Cd increase in clam bodies compared to Group C after 24 hours, though the initial increase was lower. This suggests that MPs particles in Group C acted as carriers for pre-absorbed Cd (Fig. 2). Similar results were observed in all tests conducted in the study (Figs. 3 and 4).

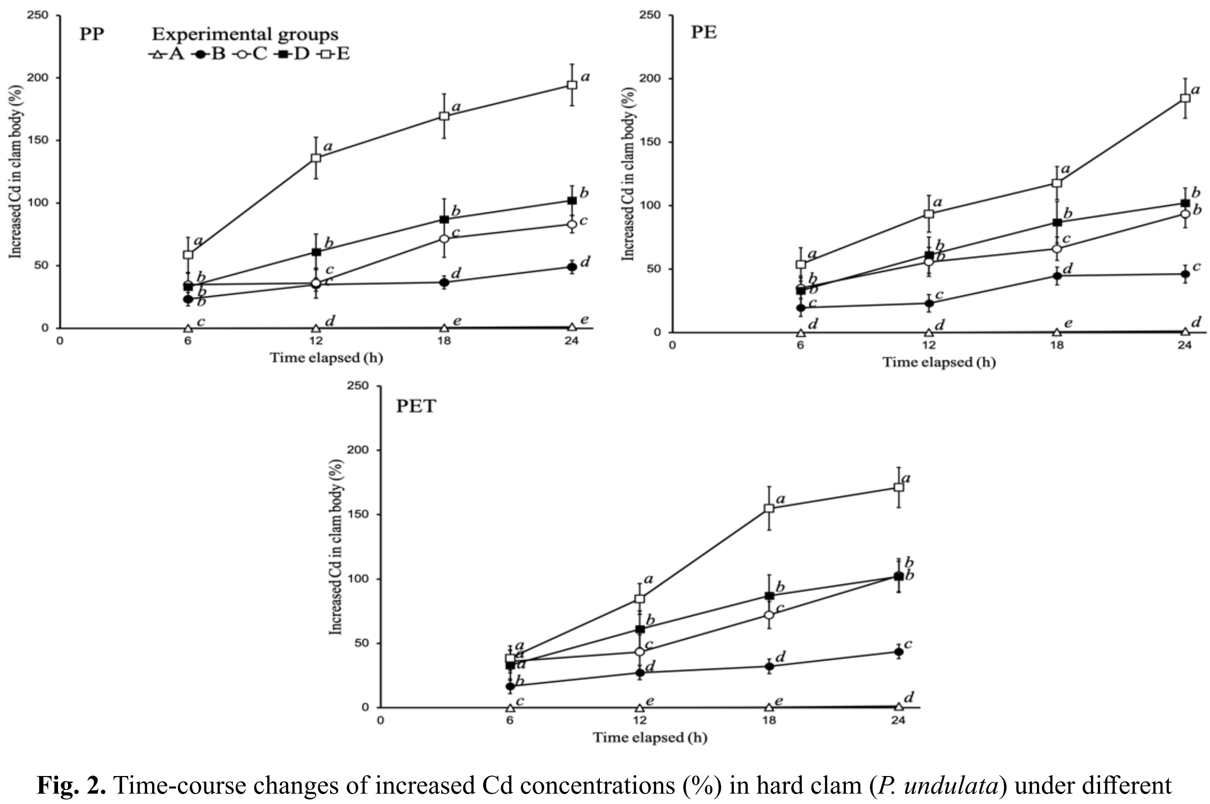

Fig. 2. Time-course changes of increased Cd concentrations (%) in hard clam (*P. undulata*) under different groups of experiment (see in the methods section) at salinity 20‰. Data for the increasing heavy metal concentration in the clam body with different letters (a, b, c, and d) are significantly different ($p<0.05$) among diverse experimental treatments at the same time.

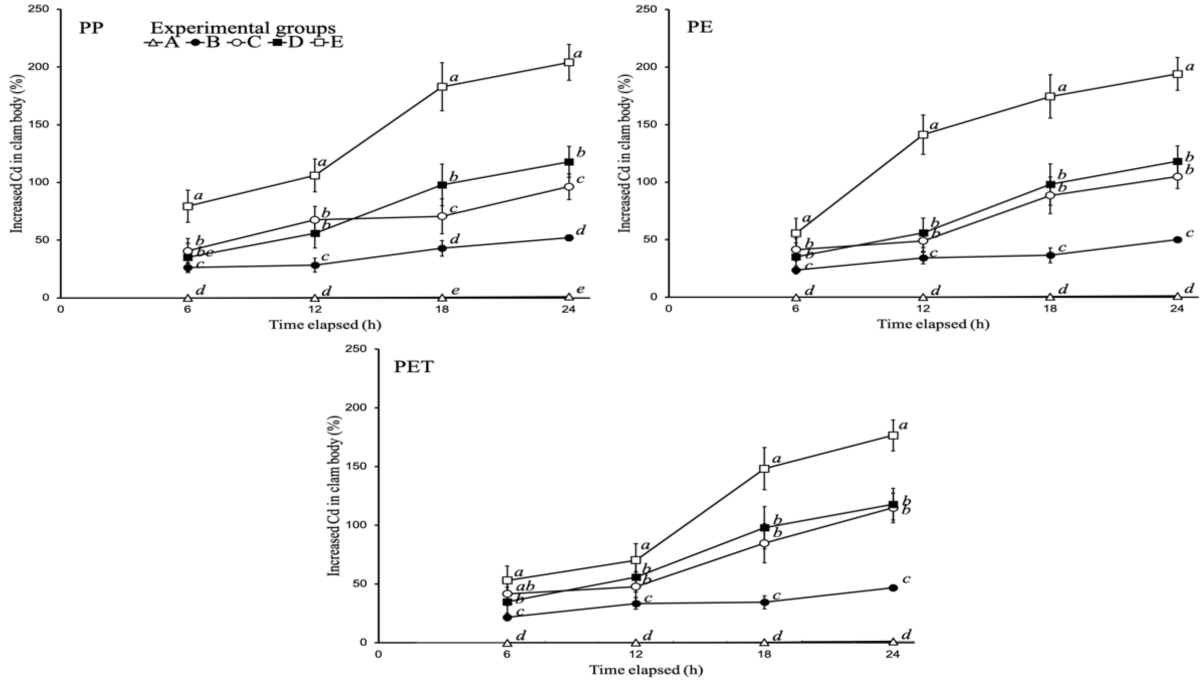

Fig. 3. Time-course changes of increased Cd concentrations (%) in hard clam (*P. undulata*) under different groups of experiment (see in the methods section) at salinity 25‰. See Fig. 2 for statistical information.

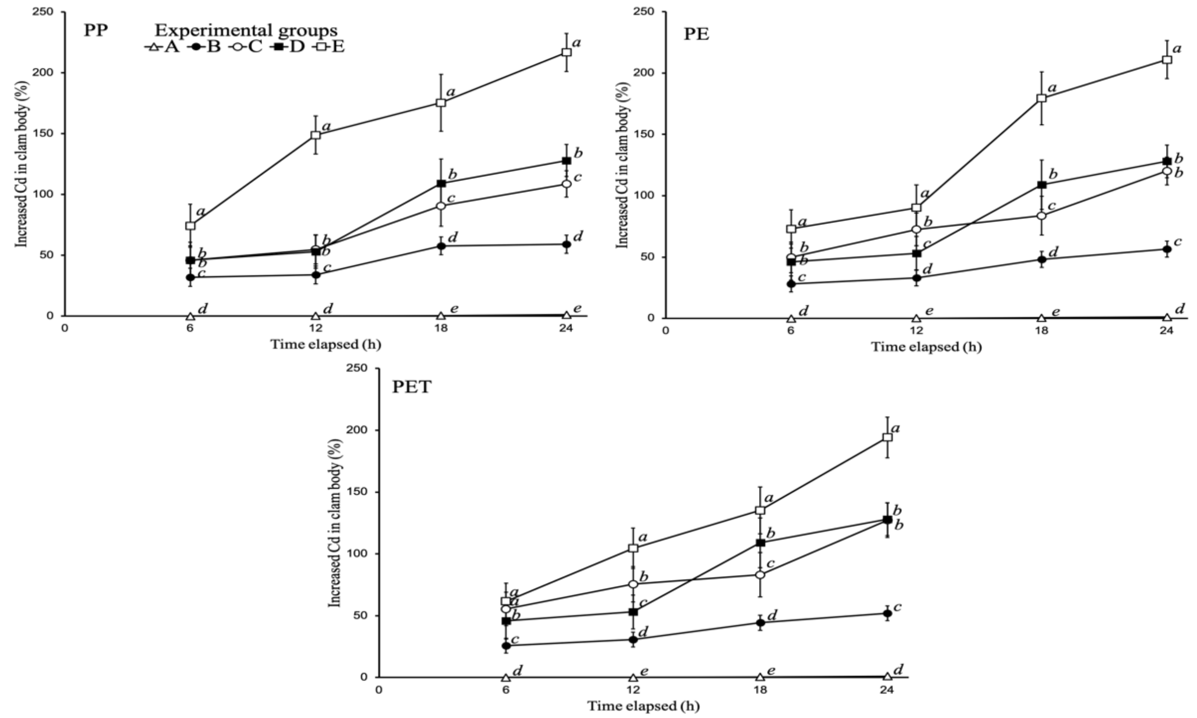

Fig. 4. Time-course changes of increased Cd concentrations (%) in hard clam (*P. undulata*) under different groups of experiment (see in the methods section) at salinity 30‰. See Fig. 2 for statistical information.

Group E exhibited the most significant rise in Cd concentration within the hard clams' bodies, consistent at every observation time. Both Cd in seawater and Cd-absorbing MPs particles contributed to this accumulation. Tests at salinities of 25‰ (Fig. 3) and 30‰ (Fig. 4) showed comparable trends.

The results indicate that the increase in Cd levels in clams due to Cd-laden MPs being absorbed by the clams was observed in all tests, except for Group A, which lacked cadmium metal in MPs and water. The acidic digestive systems of exposed species may release heavy metals from MPs, allowing these metals to be transported into the organisms' tissues (Wang *et al*., 2020), highlighting the role of MPs as carriers of Cd pollution. Vieira *et al*. (2021) proposed a correlation between heavy metal concentration and microplastic presence in oysters, noting elevated heavy metal levels in contaminated oysters. Over time, Cd content in MPs decreased as Cd was gradually released into saltwater, supported by remaining Cd level analysis in MPs and seawater (Tables 1, 2, and 3). At each observation point, Cd concentration in MPs decreased, and the increase in Cd levels in seawater was not significantly pronounced due to the low Cd concentrations released from MPs.

Similar findings across various MP types and salinities were consistent with previous studies (Brennecke *et al*., 2016; Barus *et al*., 2021). Barus *et al*. (2021) noted that MP particles can release and absorb heavy metals in solutions with varying salinities. Even low Cd concentrations injected into saltwater increased Cd levels in hard clams, but this was less significant after 6 hours. Group C's results contrasted with Group B's, as MPs absorbed Cd from seawater, reducing its concentration, with PP particles showing the most significant decrease. This aligns with previous findings that Cd is more readily absorbed by PP particles (Holmes *et al*., 2014;

Table 1. Time-course changes of heavy metal Cd concentrations remaining in seawater (mg/L) and MPs particles (µg/g) in diverse experimental groups and salinity 20‰.

Remaining Cd	Experimental group	MPs type	0 hour	6 hours	12 hours	18 hours	24 hours
In seawater (mg/L)	A	PP	n.d	n.d	n.d	n.d	n.d
		PE	n.d	n.d	n.d	n.d	n.d
		PET	n.d	n.d	n.d	n.d	n.d
	B	PP	n.d	n.d	n.d	n.d	0.010
		PE	n.d	n.d	n.d	n.d	0.010
		PET	n.d	n.d	n.d	n.d	n.d
	C	PP	0.100	0.047	0.027	0.017	0.010
		PE	0.100	0.050	0.047	0.023	0.017
		PET	0.100	0.057	0.043	0.027	0.013
	D		0.100	0.088	0.072	0,057	0,046
	E	PP	0.100	0.113	0.103	0.094	0.071
		PE	0.100	0.104	0.089	0.076	0.064
		PET	0.100	0.084	0.076	0.068	0.058
MPs particle (µg/g)	A	PP	n.d	n.d	n.d	n.d	n.d
		PE	n.d	n.d	n.d	n.d	n.d
		PET	n.d	n.d	n.d	n.d	n.d
	B	PP	0.347	0.108	0.018	n.d	n.d
		PE	0.307	0.087	0.026	n.d	n.d
		PET	0.283	0.077	0.026	n.d	n.d
	C	PP	0.031	0.039	0.041	0.042	0.043
		PE	0.031	0.032	0.037	0.041	0.037
		PET	0.030	0.031	0.032	0.033	0.033
	E	PP	0.347	0.263	0.109	0.054	0.043
		PE	0.307	0.243	0.078	0.049	0.040
		PET	0.283	0.203	0.053	0.039	0.017

n.d = not detected (<0.01 mg/L)

Table 2. Time-course changes of heavy metal Cd concentrations remaining in seawater (mg/L) and MPs particles (µg/g) in diverse experimental groups and salinity 25‰.

Remaining Cd	Experimental group	MPs type	0 hour	6 hours	12 hours	18 hours	24 hours
In seawater (m/L)	A	PP	n.d	n.d	n.d	n.d	n.d
		PE	n.d	n.d	n.d	n.d	n.d
		PET	n.d	n.d	n.d	n.d	n.d
	B	PP	n.d	n.d	n.d	n.d	n.d
		PE	n.d	n.d	n.d	n.d	n.d
		PET	n.d	n.d	n.d	n.d	n.d
	C	PP	0.100	0.063	0.047	0.033	0.023
		PE	0.100	0.070	0.067	0.043	0.037
		PET	0.100	0.077	0.063	0.047	0.033
	D		0.100	0.078	0.063	0.045	0.039
	E	PP	0.100	0.105	0.087	0.068	0.047
		PE	0.100	0.098	0.077	0.058	0.033
		PET	0.100	0.079	0.574	0.045	0.032
MPs particle (µg/g)	A	PP	n.d	n.d	n.d	n.d	n.d
		PE	n.d	n.d	n.d	n.d	n.d
		PET	n.d	n.d	n.d	n.d	n.d
	B	PP	0.347	0.146	0.047	0.01	n.d
		PE	0.307	0.113	0.037	n.d	n.d
		PET	0.283	0.102	0.026	n.d	n.d
	C	PP	0.021	0.029	0.031	0.032	0.033
		PE	0.021	0.022	0.027	0.031	0.027
		PET	0.020	0.021	0.022	0.023	0.023
	D	PP	0.347	0.208	0.155	0.099	0.047
		PE	0.307	0.189	0.108	0.084	0.030
		PET	0.283	0.173	0.104	0.063	0.027

n.d = not detected (<0.01 mg/L)

Gao *et al*., 2019). After six hours, Cd content increase in clams slowed due to MPs' capacity to absorb heavy metals. Group E showed a substantial Cd level increase in clams when both MPs and seawater contained Cd, indicating that the interaction between MPs and Cd significantly affects Cd accumulation in clams.

The type of microplastic (MP) polymer has a discernible effect on the elevated cadmium (Cd) levels within clam bodies. This phenomenon is demonstrated by the outcomes observed in each group during the study. Specifically, in Group B, the use of polypropylene (PP) particles in the treatment led to a more substantial increase in Cd concentration within clams compared to treatments involving other types of particles. This difference is attributed to the higher Cd absorption capacity of PP particles, a fact supported by previous studies (Gao *et al*., 2019; Barus *et al*., 2021).

Statistical analysis indicated that at a salinity of 20‰, there were significant differences ($p<0.05$) in the increase of Cd metal concentration among all experimental groups over various observation times, with consistent findings using PE particles. In the PET test, after 6 hours of monitoring, there was no significant difference ($p>0.05$) in Cd increase between Groups C and D. Similar results were observed at salinities of 25 and 30‰, except that at 25‰, no significant difference ($p>0.05$) in Cd increase was found between Groups C and D after 12 hours (Fig. 3). At 30‰, the Cd increase over 6 hours also showed no significant difference ($p>0.05$).

Different MP particles influenced Cd level increases in clams, with PP particles generally causing higher Cd increases except in Group C (Fig. 4), where virgin MPs absorbed Cd from seawater. PP particles absorbed Cd more readily than the other types, leading to lower Cd

Table 3. Time-course changes of heavy metal Cd concentrations remaining in seawater (mg/L) and MPs particles (µg/g) in diverse experimental groups and salinity 30‰ at each observation time.

Remaining Cd	Experimental group	MPs type	0 hour	6 hours	12 hours	18 hours	24 hours
In seawater (mg/L)	A	PP	n.d	n.d	n.d	n.d	n.d
		PE	n.d	n.d	n.d	n.d	n.d
		PET	n.d	n.d	n.d	n.d	n.d
	B	PP	n.d	n.d	n.d	n.d	n.d
		PE	n.d	n.d	n.d	n.d	n.d
		PET	n.d	n.d	n.d	n.d	n.d
	C	PP	0.100	0.083	0.063	0.057	0.467
		PE	0.100	0.087	0.077	0.063	0.533
		PET	0.100	0.090	0.080	0.073	0.057
	D		0.100	0.071	0.054	0.039	0.031
	E	PP	0.100	0.088	0.069	0.046	0.039
		PE	0.100	0.068	0.057	0.038	0.028
		PET	0.100	0.057	0.046	0.029	0.028
MPs particle (µg/g)	A	PP	n.d	n.d	n.d	n.d	n.d
		PE	n.d	n.d	n.d	n.d	n.d
		PET	n.d	n.d	n.d	n.d	n.d
	B	PP	0.347	0.169	0.067	0.01	n.d
		PE	0.307	0.129	0.056	0.01	n.d
		PET	0.283	0.115	0.047	n.d	n.d
	C	PP	0.011	0.019	0.021	0.022	0.023
		PE	0.010	0.018	0.017	0.021	0.017
		PET	0.010	0.011	0.012	0.013	0.013
	D	PP	0.347	0.277	0.189	0.126	0.077
		PE	0.307	0.222	0.126	0.113	0.067
		PET	0.283	0.287	0.084	0.108	0.040

n.d = not detected (<0.01 mg/L)

concentrations in seawater. Figure 4 shows Cd concentration increases in clams across all test groups using different MPs and salinities, with Groups B and D showing the highest Cd increases with PP particles, and Group C showing the highest increase with PET particles (Fig. 5).

This result indicated that the type of microplastic (MP) polymer has a discernible effect on the elevated cadmium (Cd) levels within clam bodies. This phenomenon is demonstrated by the outcomes observed in each group during the study. Specifically, in Group B, the use of polypropylene (PP) particles in the treatment led to a more substantial increase in Cd concentration within clams compared to treatments involving other types of particles. This difference is attributed to the higher Cd absorption capacity of PP particles, a fact supported by previous studies (Gao *et al*., 2019; Barus *et al*., 2021).

In lab-scale experiments, Gao *et al*. (2019) found that PP particles exhibited superior absorption of heavy metals, including Cd, when compared to polyethylene (PE), polyamide (PA), and polyoxymethylene (POM) particles. Furthermore, studies conducted in open ocean conditions indicate that PP outperforms polyvinyl chloride (PVC) in absorbing heavy metals. This is primarily due to the smoother surface of PP particles in comparison to PVC. Virgin PP particles display large protrusions and a uniform surface, while the surface of PVC particles is relatively flat with fewer, smaller depressions, which facilitates greater metal element adsorption. PP consistently outperformed other particles in absorbing heavy metals across trials with varying salinities. Additionally, PP particles release Cd metals into the solution at a slower rate than other particles (Barus *et al*., 2021). The distinct surface morphologies of different microplastic particle types had a significant impact on their adsorption behavior concerning metal ions, as emphasized by Brennecke *et al*. (2016).

Variations in salinity levels significantly influence Cd accumulation in clam bodies. The study found a direct correlation between Cd content in clams and salinity, with the highest increase observed at 30‰ and the smallest at 25 and 20‰. For instance, at salinities of 20, 25, and 30‰, Cd increases in Group D (PP particles) were 273.26%, 283.45%, and 316.25%, respectively. Statistical analysis showed significant differences ($p<0.05$) between the 30‰ test and the other two salinities, and between 25‰ and 20‰ (Fig. 6). These trends were consistent across tests with different MP particles.

Higher salinity in seawater slowed Cd release from Cd particles, as shown in Tables 1, 2, and 3. In Group B, all MP types significantly increased Cd concentration in clams at 30‰. Salinity also affected Cd adsorption by MP particles, with higher salinity reducing Cd absorption by MPs in Group C, thus increasing Cd levels in clams. Tables 1, 2, and 3 detail these variations in Cd adsorption and release by MP particles at different salinities.

Increased salinity levels in the ocean lead to a higher tendency for microplastic particles to accumulate within clam bodies, consistent across all groups using three types of microplastics. Statistical analysis shows that particle accumulation in clams at 30‰ salinity significantly exceeded ($p<0.05$) that at lower salinities (Fig. 7). This phenomenon is directly correlated with increased Cd concentration in clams, with heavy metal content rising in proportion to water salinity. This reflects the clams' osmoregulation mechanism, where hard clams, as

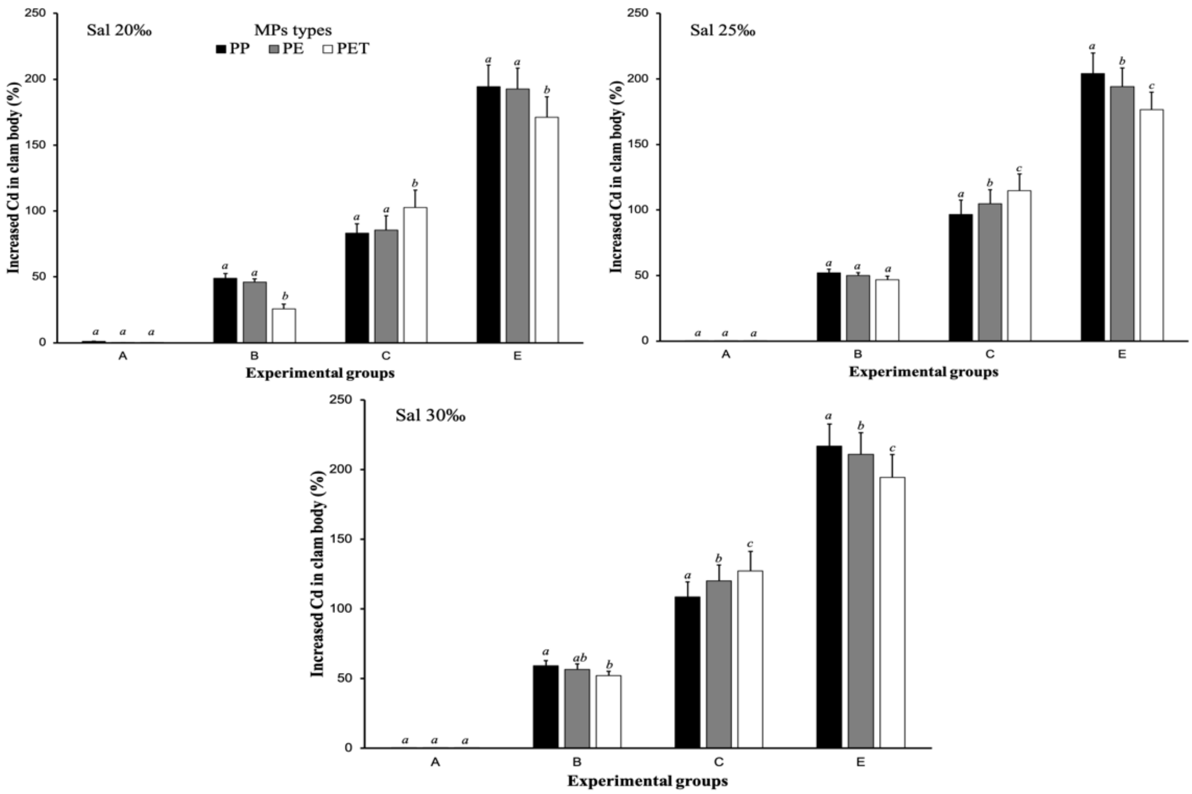

Fig. 5. The increased concentration of Cd in the hard clam (*P. undulata*) body in each experimental group with different types of MP particles after 24 hours of exposure time at various salinities. Data for the increasing concentration of Cd in clam body with different letters (a, b, and c) are significantly different ($p<0.05$) among diverse types of MPs particles at the same time.

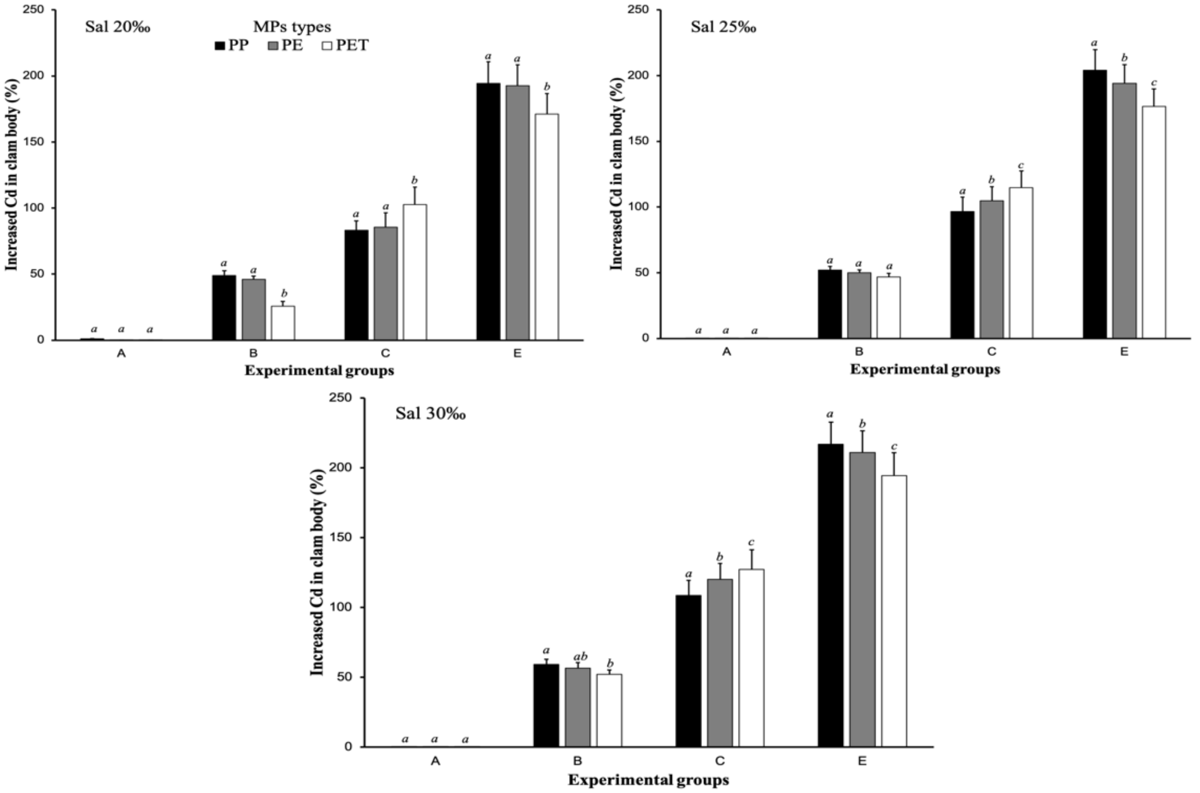

Fig. 6. The increased concentration of Cd in the hard clam (*P. undulata*) body in each experimental group used MPs particles with different salinities after 24 hours of exposure time. Data for the increasing concentration of Cd in clam bodies with different letters (a, b, and c) are significantly different ($p<0.05$) among diverse salinities at the same time.

osmoconformers, adjust their bodily fluid osmolality in response to environmental salinity changes, a trait shared by various marine mollusks (Lin *et al*., 2016).

Hard clams can tolerate blood salinity variations but must maintain constant ion concentrations in cells for metabolic enzyme function, increasing the likelihood of MP particle penetration and heavy metal concentration rise. However, maintaining consistent cell salinity has drawbacks. When salinity decreases, blood salinity falls below cell salinity, causing water, not ions, to move through osmosis into the cell (Lin *et al*., 2016).

An earlier study by Jones (1975) provided additional support for this conclusion. It revealed that concentrations of Cd, Cd, and Zn were higher in *Idotea neglecta*, *Idotea baltica*, *Jaera albifrons*, and *Idotea emarginata* grown in higher salinity seawater compared to lower salinity seawater. The mercury concentration in Jaera albifrons' body showed similar trends.

Furthermore, the technique by which heavy metals are released into the water varies with water salinity. Higher salinity leads to a slower release of heavy metals compared to lower salinity (Barus *et al*., 2021). This can be ascribed to the ionic competition within the water, where solutions with elevated salt content experience heightened competition and ion density (Holmes *et al*., 2014; Liu *et al*., 2018), making it more challenging for heavy metal ions absorbed from plastic surfaces to be released.

Supporting this observation, data on variations in water depletion displayed an increasing impact of salinity on the osmoregulation process, particularly in high salinity environments, where hard clams lost water more rapidly. Throughout the exposure period, water depletion differed significantly between salinities. In comparison to the other two salinities, the experiment

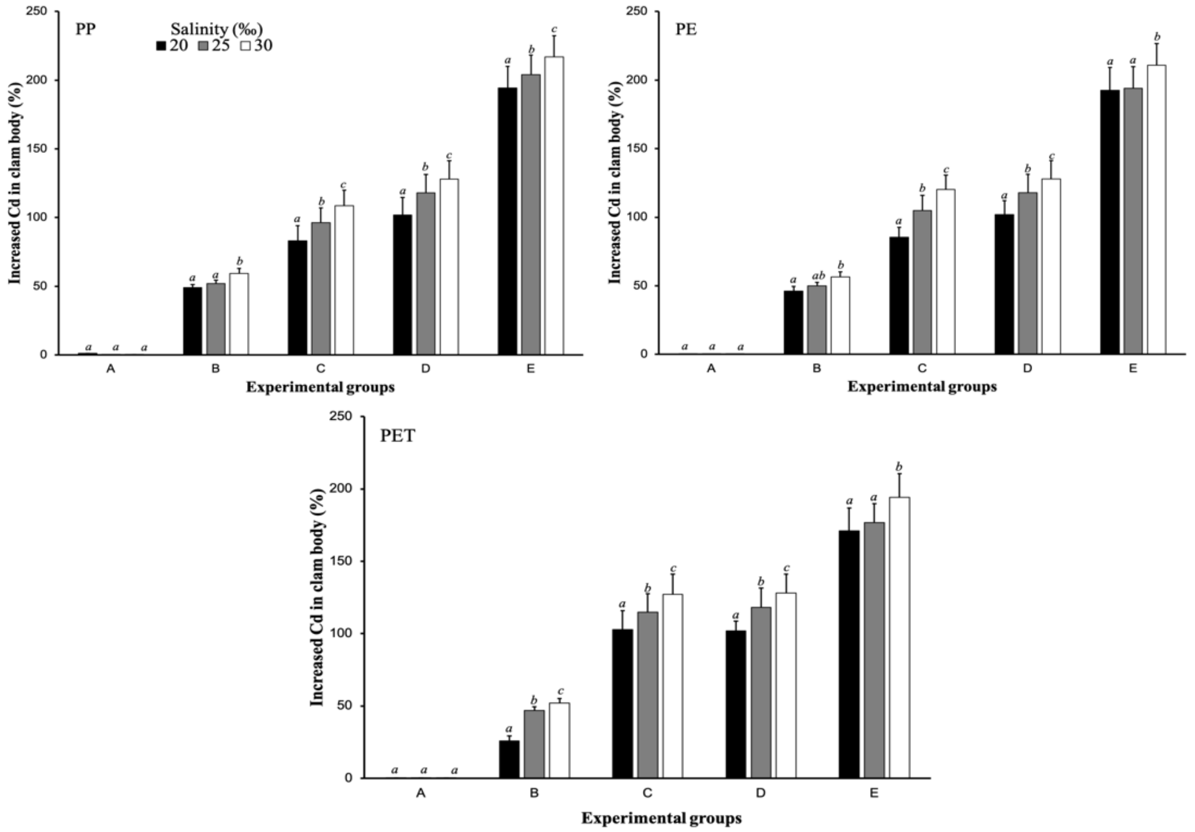

Fig. 7. The number of MP particles accumulated in the hard clam (*P. undulata*) body in each experimental group after 24 hours of exposure time at various salinity 20, 25, and 30‰. Data for the number of particles in the clam body with different letters (a, b, and c) are significantly different ($p<0.05$) among diverse salinities at the same time.

with a salinity of 30‰ exhibited significantly higher water depletion ($p<0.05$), as depicted in Figure 8.

The statistical analysis also revealed a significant influence of microplastic type, salinity, and the number of collected microplastic particles on the increase in heavy metal accumulation in hard clam (*P. undulata*). According to this study, a relationship exists between the rate of increase (%) in each group and the salinity (S) and the number of accumulated particles (P), as follows:

Group A: no relationship between all variables

Group B:

(PP) Cd increasing rate (%) = 18.837 + 36.783 S + 8.367 P + 0.386 SP ($R^2 = 0.818$)

(PE) Cd increasing rate (%) = 18.736 + 76.736 S + 1.486 P + 0.983 SP ($R^2 = 0.699$)

(PET) Cd increasing rate (%) = 23.676 + 68.956 S + 1.637 P + 0.678 SP ($R^2 = 0.764$)

Group C:

(PP) Cd increasing rate (%) = 16.836+ 23.847 S + 7.836 P + 6.378 SP ($R^2 = 0.837$)

(PE) Cd increasing rate (%) = 49.883 + 26.836 S + 3.829 P + 5.998 SP ($R^2 = 0.927$)

(PET) Cd increasing rate (%) = 43.826 + 26.837 S + 7.883 P + 5.286 SP ($R^2 = 0.923$)

Group D:

Cd increased rate (%) = 8.836 + 13.772 S ($R^2 = 0.835$)

Group E:

(PP) Cd increasing rate (%) = 79.837 + 21.837 S + 7.837 P + 4.927 SP ($R^2 = 0.944$)

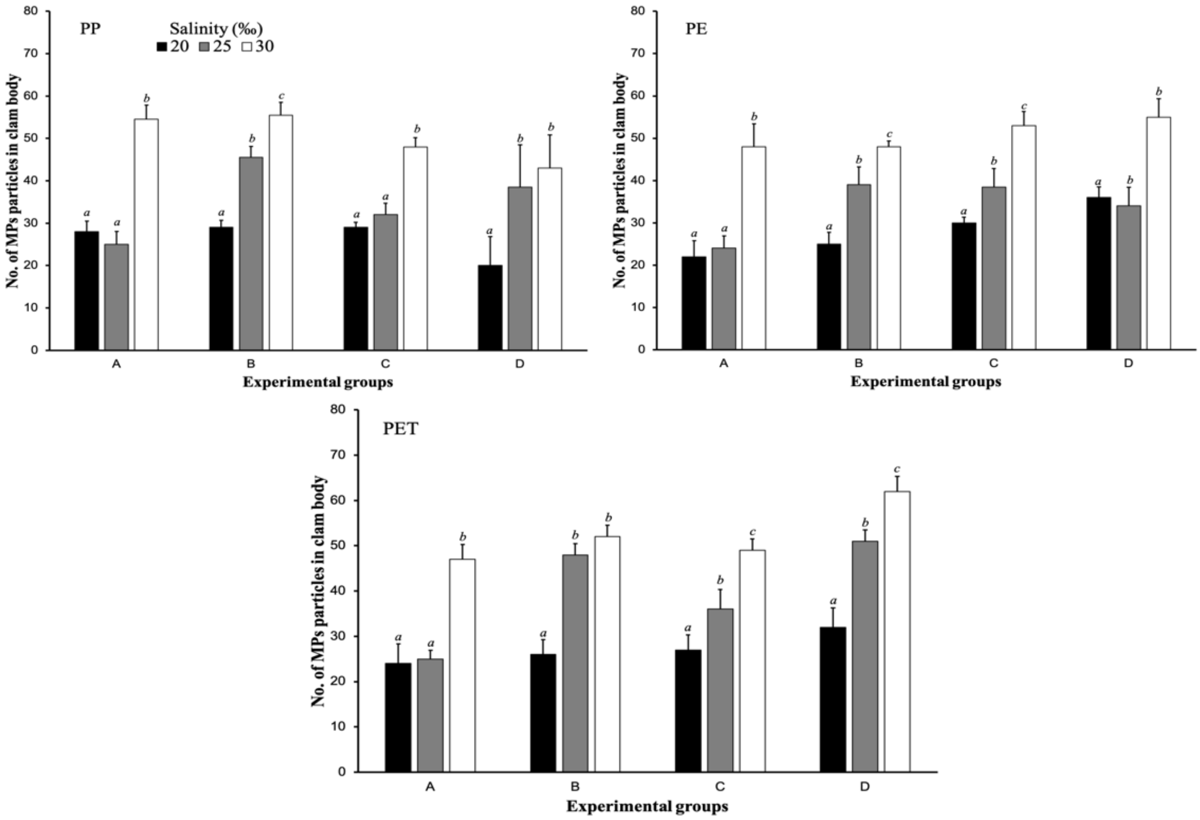

Fig. 8. Water depletion (%) after exposure to hard clam (*P. undulata*) (n=10) at different times dan salinities (20, 25, and 30‰). The data for water depletion (%) with different letters (a, b, and c) differ significantly (p<0.05) among diverse salinities at the same time.

(PE) Cd increasing rate (%) = 34.726 + 45.837 S + 8.735 P + 1.773 SP (R^2 = 0.937)
(PET) Cd increasing rate (%) = 38.864 + 62.776S + 2.767 P + 0.629 SP (R^2 = 0.879)

The data indicates that microplastic (MP) particles function as carriers for transporting heavy metal pollution into hard clams. The amount of heavy metals transferred into the clams was significantly influenced by factors such as salinity, the specific type of MPs, and their interactions with heavy metals. Microplastics are a primary source of pollution in marine environments with the capacity to absorb and transport metals, thus acting as vectors for the ingestion or release of chemicals by marine organisms into the water column, ultimately contaminating them with heavy metals. The kinds and concentrations of contaminants that build up in organisms will increase as a result. Constant buildup will have extremely harmful consequences on living things, including metabolic disorders, immunological response, DNA damage, oxidative stress, organ dysfunction, toxicity to reproduction, and even death (Prokic *et al*. 2019).

CONCLUSION

The interactions between microplastic (MP) particles and heavy metals play a critical role in determining the concentration of metals in clam bodies. The presence of heavy metal Cd in hard clam bodies can increase due to the capacity of MP particles to absorb Cd. Furthermore, heavy metals adsorbed onto MPs particles may leach into the water, leading to higher levels of heavy metal contamination in the surrounding water. The type of MPs used in the study similarly affected the degree of Cd accumulation in clam bodies. Notably, the experiment employing PP particles consistently resulted in the most significant increase in Cd levels across all experimental groups. Variations in salinity also influence the rate of Cd accumulation in clam bodies. Higher salinity in saltwater necessitates increased water intake by hard clams to maintain their internal balance compared to clams in lower salinity seawater. Consequently, more MP particles containing Cd, along with Cd-tainted seawater, enter the hard clam's body in high-salinity environments. This situation leads to a more pronounced accumulation of the toxic heavy metal Cd in the hard clam's body. These findings suggest that in various treatment conditions and salinity levels, MP particles can act as carriers of Cd metal contamination in hard clams. The environment, including humans, will be at risk from this pollution process if it continues, so precautionary measures like cutting back on plastic consumption, developing recycling technologies for plastic waste, and setting up eco-friendly waste disposal systems are required.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

DATA AVAILABILITY STATEMENT

Data sharing does not apply to this article as no datasets were generated or analyzed during the current study.

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