Acute Toxicity of DDT on Embryo and Larval Growth of the Pacific Oyster, *Crassostrea gigas* (Thunberg, 1793): A Case Study in the Saigon–Dongnai River, Vietnam

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ABSTRACT

Dichloro-Diphenyl-Trichloroethane (DDTs) are potent organic toxins widely used in agriculture and malaria prevention. The extensive use of DDTs has raised concerns about their adverse impacts. This study aimed to evaluate the toxicity of DDTs on Pacific oyster (*Crassostrea gigas*) larvae and embryos in the lower Sai Gon-Dong Nai River. We exposed oyster larvae and embryos to sediment samples containing DDT concentrations of 0.01, 0.05, 0.1, 0.5, and 1 mg·kg^{-1}, as well as artificial seawater samples with DDT levels of 0, 0.1, 1, 10, and 100 µg·L^{-1}. The analysis results revealed a significant adverse impact of DDTs on the survival ratio of larvae and the cell division of embryos in both water and sediment samples compared to control samples. The Effective Concentrations in embryo cell division capacity (EC_{50}) after 2 and 24 h of exposure were 66.88 and 4.62 µg·L^{-1} in artificial seawater and 1.1 and 0.3 mg·kg^{-1} in sediment, respectively. Lethal Concentrations (LC_{50}) were 4.62 µg·L^{-1} and 0.3 mg·kg^{-1}, respectively. Transmission Electron Microscopy (TEM) and Scanning Electron Microscope (SEM) images further confirmed the significant morphological disruptions caused by DDTs after 24 h of exposure. These findings indicate that DDT toxicity poses a threat to oyster farming in coastal areas, where chronic pesticide exposure is likely.

Keywords: Artificial seawater, Ecotoxicity, DDTs, Pacific oyster (*Crassostrea gigas*), Sediment samples, Toxicity

INTRODUCTION

Plant protection chemicals and persistent organic pollutants (POPs) are typically organic compounds that are highly resistant to decomposition. They originate from various sources, including agricultural and non-agricultural activities, insect control, antifouling biocides, residues from chemical factories, and the management of invasive weeds (Banks *et al*., 2005; Colas, 2011). Upon entering the environment, these compounds can disperse into all environmental components, such as soil, water, air, and sediment, with the potential to cause significant and lasting impacts on ecosystems and aquatic life, especially in coastal ecosystems (Renault, 2010).

Among these compounds, DDT stands out as the most toxic substance within the organochlorine pesticide (OCP) category. It has been widely used worldwide for malaria control and as a pesticide in agriculture. DDT is a persistent pollutant, with

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a half-life in soil of up to 30 years and up to 150 years in aquatic environments (Buisson *et al*., 2008). While DDT has been banned in many countries since 1972, it is still employed in certain regions for limited medical purposes, such as malaria and disease vector control (ATSDR, 2002). Studies have documented the accumulation of DDT and its metabolites in various body tissues, including adipose tissue, serum or plasma, liver, and hair (ATSDR, 2002). DDT is rapidly metabolized and transformed into dichlorodiphenyldichloro ethylene (DDE), a more toxic compound than DDT itself. Forgotten DDT stockpiles have emerged as sources of groundwater pollution, leading to the emergence of "cancer villages" in Vietnam (His *et al*., 1999). Additionally, research has indicated the presence of total DDT concentrations in air samples collected from urban and estuarine areas of Vietnam (His *et al*., 1999; VASEP report, 2019).

Bivalve mollusk species are primarily benthic organisms widely distributed in estuarine and coastal regions, including the green mussel and the Pacific oyster. Due to their habitat and behavior, they tend to accumulate pollutants more readily than other aquatic species. Consequently, they are frequently utilized as model organisms in both chemical toxicity and water quality assessments (His *et al*., 1999; Tanabe and Subramanian, 2006). Some authors have emphasized the suitability of the early stages of bivalve mollusks for ecotoxicological testing. During this developmental phase, which varies in duration depending on the species, they exhibit rapid proliferation, often occurring within just a few hours or days (His *et al*., 1999).

To date, numerous studies have investigated the impact of pesticide toxicity on the development of aquatic species (Gormley and Teather, 2003; Akcha *et al*., 2012; Mai *et al*., 2014). Akcha *et al*. (2012) reported that diuron and glyphosate adversely affected sperm spermatogenesis and the DNA morphological structure of Pacific oysters even at low testing concentrations (0.05 $\mu g \cdot L^{-1}$). Mai *et al*. (2014) observed significant impacts on spermatozoa fertilizing capacity following exposure to irgarol, diuron (1 $\mu g \cdot L^{-1}$), and metolachlor (10 $\mu g \cdot L^{-1}$) on Pacific oyster gametes and embryos. According to Gormley and Teather (2003), exposure to endosulfan concentrations for 24 h (0.01, 0.10, and 1.0 $\mu g \cdot L^{-1}$) resulted in various changes in Japanese Medaka (*Oryzias latipes*) such as alterations in hatching time, growth, mobility, foraging ability, and reproduction, across all tested concentrations.

It is well-known that DDT has long been banned in many countries due to its toxicity to humans and the environment. However, it is still utilized in a limited capacity. Despite its restrictions, it remains the most cost-effective and efficient means of malaria control in many countries (Bate and Tren, 2005). In Vietnam, DDT maintains its position as the most widely employed pesticide, followed by Lindane (gamma 666), with only a handful of other pesticides like Aldrin and Dieldrin in use (Minh *et al*., 2007; Hoai *et al*., 2010). Certainly, illegal usage occurred; nonetheless, these findings suggest that the unlawfully applied DDTs were released and accumulated in the environment within the study area.

The Saigon-Dongnai estuary is renowned for oyster farming, yielding approximately 2,000 tons annually and providing 10 million juvenile oyster shells (Tong *et al.*, 2018; VASEP report, 2019). Unfortunately, the water quality in the river has significantly deteriorated over the years, resulting in impaired reproduction, increased mortality, and developmental abnormalities in oyster embryos, larvae, and juveniles (Phuong *et al*., 2017; Tong *et al*., 2018; Nam *et al*., 2019; VASEP report, 2019). The escalating population, intensified productive activities, and indiscriminate pesticide use in agriculture have contributed to elevated pesticide residues in the river.

Remarkably, no prior studies have explored the impact of DDT in both sediment and water on oysters, especially in the Sai Gon-Dong Nai River region, Vietnam. Hence, the objectives of the present study are: to assess the toxicity of DDT on the growth of Pacific oyster embryos and larvae in both artificial seawater and sediment, and to investigate any their morphological and structural alterations. This endeavor is imperative for promoting sustainable development and ensuring the safety of seafood in the region.

MATERIALS AND METHODS

Experimental methods

Collection of sediment samples and Pacific oyster

In this study, we gathered sediment samples from the Soai Rap estuary, a part of the Sai Gon-Dong Nai River system, as shown in Figure 1. These sediment samples exhibited a weakly acidic to moderately alkaline nature, with pH values ranging from 5.6 to 7.8 during the dry season and 5.7 to 7.4 in the rainy season. The physicochemical characteristics of the sediment, including pH, Total Organic Carbon (TOC), and mechanical composition, play a pivotal role in the distribution of contaminants. Predominantly, the sediment was characterized by clay and fine sand as the dominant texture components. TOC content varied between 2.9 and 5.2%, while the mechanical composition revealed that raw sand and fine sand constituted the lowest proportions, ranging from 0.1 to 5.2% and 9.0 to 39.2%, respectively. Conversely, clay content constituted the highest proportion, ranging from 36.7 to 78.0% (Nguyen, 2021).

The elevated values of these parameters during the dry season are primarily attributed to reduced water flow, resulting in heightened sedimentation rates. It is noteworthy that sediments collected from tributaries exhibited higher physicochemical parameters compared to those collected from the main channel of the Saigon-Dong Nai estuary. The collection process at the Soai Rap estuary involved taking the top 3 to 5 cm of sediment layer from the downstream of the Sai Gon-Dong Nai River, sealing it in clean polyethylene bags, and transporting it to the laboratory in freezer containers. To ensure accuracy, samples were collected three times at each sampling station and thoroughly homogenized. Subsequently, in the laboratory, the samples were stored in dark conditions at -20 °C before analysis.

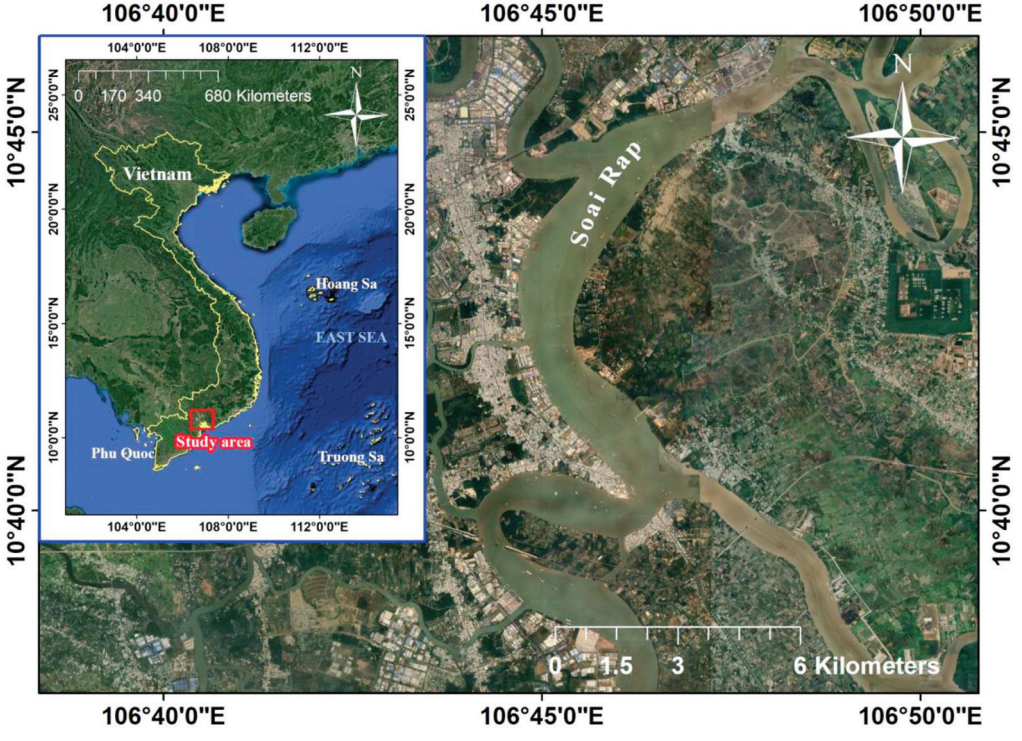

Figure 1. Map of the study area.

Preparation of Pacific oyster embryo and larvae

Pacific oyster embryo and larvae were obtained from the National Breeding Center for Southern Marine Aquaculture, Vung Tau city (Ba Ria-Vung Tau Province, Vietnam. They were cultivated in artificial seawater, prepared in accordance with the BS ISO 17244 guidelines.

Upon arrival at the laboratory, the embryo and larvae oysters underwent a meticulous inspection to remove any deceased specimens, followed by a swift rinse under running water and gentle drying on paper towels. Subsequently, the oysters were acclimated to artificial seawater at 20 °C for a duration of 4 h before the commencement of the experiments. It's worth noting that all embryo and larval oysters were utilized within the same day.

Preparation of DDT solutions

Analytical grade DDT was procured from Sigma-Aldrich (France). The stock concentration of DDT was 1235 ppm (p = 97.8%) diluted in 0.1% organic solvent DMSO (dimethyl sulfoxide) and subsequently preserved in dark glass tubes at -20 °C. The working solutions required for subsequent experiments were derived through appropriate dilutions of these stock solutions.

Preparation of clean sediment and specific concentration of DDT solution

Clean sediment samples were carefully collected from the Soai Rap estuary, precisely at coordinates 10°25'56.2"N and 106°46'48.1"E. These samples were sieved through a 0.2 mm mesh and thoroughly rinsed with artificial seawater to eliminate any debris, impurities, or microorganisms. The experimental setup and sediment treatment followed the procedure outlined by Fathallah (2014).

To begin, a working solution of DDT at a concentration of 100 ppm was prepared by diluting the stock solution in n-hexane. Next, 20 g of the sediment were carefully weighed, moisture content determined, and then dried overnight at 103 °C in a 500 mL beaker. Subsequently, artificial seawater and the DDT 100 ppm solution were added to the dried sediment at a 1:4 ratio. The resulting mixture was thoroughly stirred for 5 min and then shaken for 8 h. Following this, the mixture was allowed to settle overnight, after which the transparent water layer was carefully removed. These prepared samples were then utilized within a single day. The steps to prepare sediment are described in Figure 2 as follows:

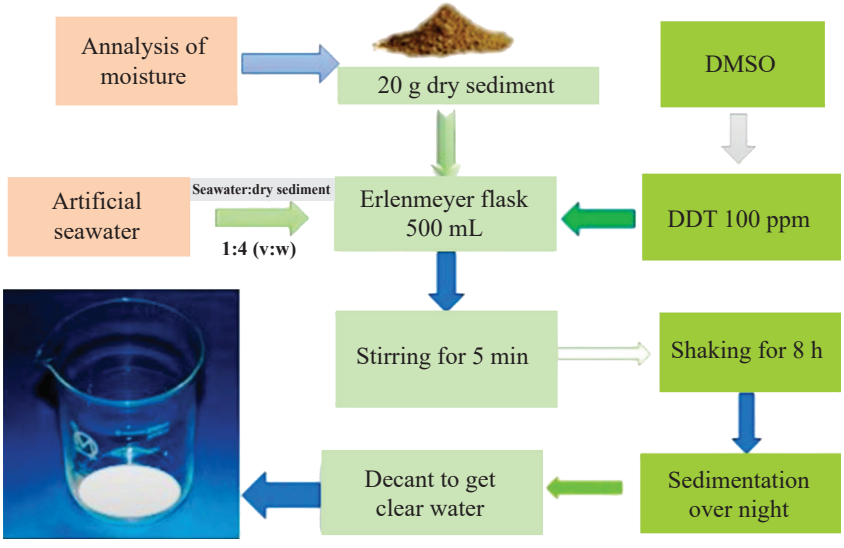

Figure 2. Process of preparing sediment for toxicity experiments.

Toxicological evaluation methods

Experimental procedures for DDT toxicity assessment

The procedures for adding and extracting DDTs from sediment were conducted in accordance with the protocol outlined by Fathallah (2014). Additionally, biological experiments and chemical exposure of Pacific oysters to DDT followed the methodology described by Leverett and Thain (2013). Working solutions were subsequently prepared by diluting the stock solution with artificial seawater to attain the final tested concentrations required for further experiments. In each culture well, 20 mL of Pacific oyster embryo and larvae liquid was introduced, containing varying concentrations of DDT: 0, 0.1, 1, 10, and 100 $\mu g \cdot L^{-1}$ in contaminated seawater, and 0, 0.01, 0.05, 0.1, 0.5, and 1 $mg \cdot kg^{-1}$ in contaminated sediment. Healthy embryos (embryos with transparent structures, intact embryonic membranes, and evenly thick yolk masses) were selected and transferred to experimental wells according to the corresponding concentrations of DDTs. Each experiment was repeated three times, each well had 10 embryos/concentration. After 2 and 24 h of exposure to DDT, the embryo-larvae were examined for mortality under a microscope with magnification from 4 to 100 times (Figure 3).

To assess the cytotoxic effects of DDTs on the embryo and larvae, measurements were made of mortality ratios, cell division ability, and EC_{50} and LC_{50} values at both 2 and 24 h. The calculations for EC_{50} and LC_{50} values involved employing the Probit method and the y = ax+b regression equation, using Sigmaplot 14 and JMP 10 software, following the procedures outlined by Yu *et al*. (2014) and the Probit regression table by Finney (1971). Actual mortality rates of Pacific oyster embryo and larvae after 2 and 24 h in the experimental concentrations were computed using the Abbott (1987) and WHO (2009) formulas:

$$\text{Actual mortality rate} = \frac{\text{Control survival ratio-Experiment survival ratio}}{\text{Control survival ratio}} \times 100$$

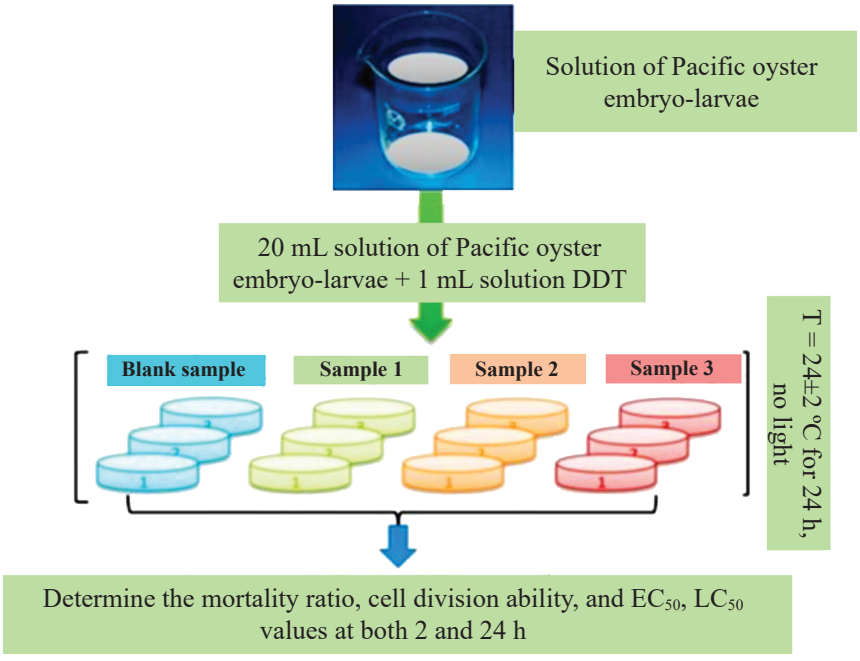

Figure 3. Process for preparation of Pacific oyster embryo and larvae for toxicity experiments.

Microscopic analysis of oyster tissue

The analysis of the surface structure and morphology of oyster tissue in this study was conducted at the National Institute of Hygiene and Epidemiology in Vietnam. The surface structure was examined using Scanning Electron Microscopy (SEM), specifically the S-4800 model from HITACHI. To prepare the oyster cells for SEM analysis, samples from both water and sediment, which had been exposed to DDT concentrations of 1 $mg \cdot L^{-1}$ and 1 $mg \cdot kg^{-1}$, respectively, for 24 h, were collected via centrifugation. These cells were then fixed in a solution containing 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 and stored overnight at 4 °C. Subsequently, the samples underwent multiple rinses with 0.1 M cacodylate buffer at pH 7.2. The fixed samples, now in pellet form, were treated with 1% OsO_4 in the same buffer. Finally, the dehydration process involved a series of ethanol solutions, progressing from 30 to 100% in 10% increments, following repeated washing with 0.1 M cacodylate buffer. The dried samples were then subjected to a critical point dryer (Emitech, K850, Quorum Technologies) and coated with Pt-Pd using ion sputtering before SEM imaging.

Similar to the SEM analysis procedure, the ultrastructural changes within the interior of the oyster cells and sediment were examined using Transmission Electron Microscopy (TEM) at 80 kV, specifically the JEM 1010 model from JEOL. The subsequent steps after the oyster cell fixed with 1% OsO_4 in the same buffer involved dehydration in graded ethanol (50 to 100%) and washing in propylene oxide. The samples were then infiltrated for 6 h with a 1:1 mixture of propylene oxide and epoxidic resin (Epon). Finally, the samples were embedded in Epon 812, and thin sections of 60–80 nm were collected using an ultra-microtome (Leica, UC6) with collodion-coated copper grids (300 meshes).

Experimental replication and data analysis

To ensure robustness and reliability, all experiments were meticulously conducted in triplicate. Subsequently, the data obtained were processed using the software JMP 10 and Sigmaplot 14, and the results were presented as the mean±SE (standard error). In this context, EC50 value, which is defined as the concentration of the toxicant causing a 50% reduction in embryonic development, was calculated using Sigmaplot 14. To establish statistical significance, a threshold of $p<0.05$ was employed.

RESULTS AND DISCUSSION

Impacts of DDT toxicity on pacific oyster embryo and larvae in artificial seawater environment

Many studies on the effects of pesticides in the water environment on bivalve embryos and larvae have been chosen to study for 24 h (Lindsay *et al*., 2010; Mai *et al*., 2012). However, most studies only focused on evaluating the effects of pesticides on the D-shaped stage of Pacific oyster larvae. The literature lacks data regarding the toxicity of DDT in a water environment concerning the growth and development of Pacific oyster embryo and larvae within a 2 h timeframe. Notably, oyster embryos typically initiate cell division around the 2 h mark following fertilization (Miossec *et al*., 2009). Consequently, in this study, we selected two time points, 2 and 24 h, to evaluate the impact of DDT in an artificial seawater environment.

The results observed after 2 h of exposure to DDT unveiled a significant influence on Pacific oyster embryos within the artificial seawater environment (Figure 4a). The proportion of abnormal embryos and undivided embryos exhibited a linear increase with rising DDT concentrations, ranging from 28 to 70% in the experimental samples, corresponding to concentrations escalating from 0.1 to 100 $\mu g \cdot L^{-1}$. This was in stark contrast to the control sample, which exhibited only a 14% abnormality rate ($p<0.05$). These results underscore that even brief exposure to DDT hindered the growth and cell division of Pacific oyster embryos, elucidating this inhibition as a contributing factor to the elevated mortality rates observed after 24 h of exposure.

Indeed, the mortality of oyster embryo and larvae after 24 h of exposure to DDT in the artificial seawater environment was notably high (Figure 4b). Mortality rates ranged from 44 to

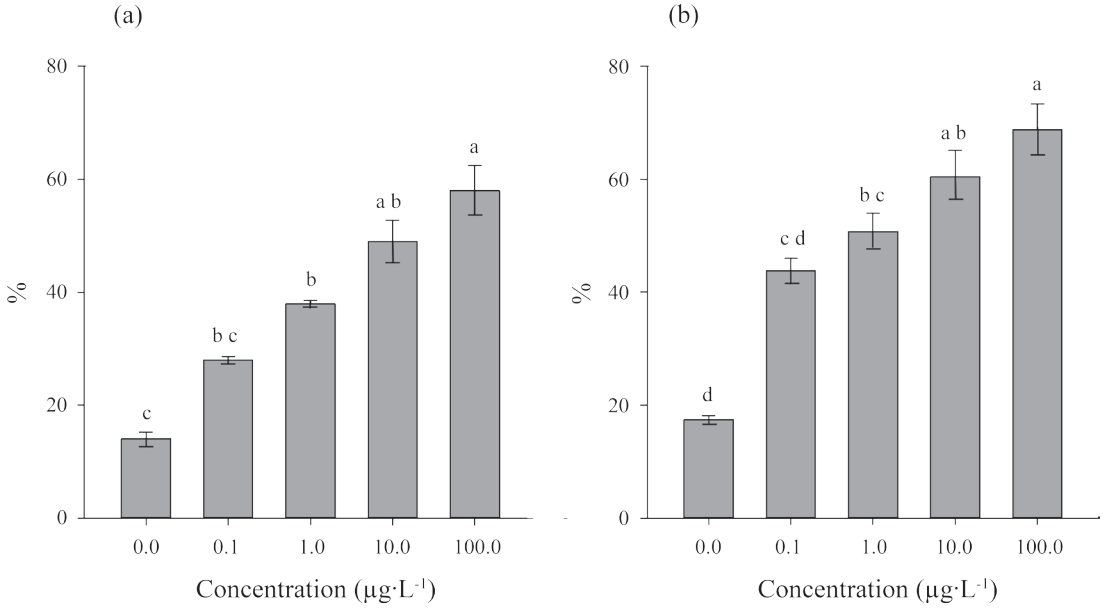

Figure 4. Percentages abnormality and mortality of Pacific oyster embryos exposed to various concentrations of DDT in artificial sea water: (a) percentages of abnormal embryos at 2 h; (b) mortality at 24 h; Bars represent mean values and error bars represent standard deviation; different lowercase letters above bars denote significant differences ($p<0.05$).

85% in the experimental samples, while the control sample recorded a mere 17% mortality rate ($p<0.05$).

Effective Concentration (EC_{50}) and Lethal Concentration (LC_{50}) values were determined based on the actual data from experimental concentrations compared to the control sample. Figure 5 illustrates the correlation between Probit coefficients for abnormal embryos (Figure 5a) and mortality (Figure 5b). Logarithmic transformations of the experimental concentrations at both 2 and 24 h revealed a linear increase in Probit coefficients with rising DDT concentrations.

Through regression analysis, EC_{50} and LC_{50} values were calculated, indicating that DDT in an artificial seawater environment significantly impacts the early development of Pacific oyster embryos. Specifically, exposure to a concentration of 66.88 µg·L^{-1} DDT (EC_{50}) results in a retardation of 50% of oyster embryos. Similarly, DDT affects the survival of oyster embryo and larvae in an artificial seawater environment, with 50% mortality occurring at a concentration of 4.62 µg·L^{-1} DDT (LC_{50}).

The presence of retardation and undivided embryos highlights that DDT not only impacts the survival rate but also hinders the development of oyster embryo and larvae. In this study, the calculated LC_{50} value stood at 4.62 µg·L^{-1}, which is lower than LC_{50} values reported in certain studies on the toxicity of pesticides concerning other bivalve mollusks, such as clams (*Mercenaria mercenaria*) (Chung *et al*., 2007) and green mussels (*Perna viridis*) (Song *et al*., 2016). However, it's worth noting that the LC_{50} value for Mediterranean mussel larvae (*Mercenaria galloprovincialis*) exposed to TBT pesticides was found to be less than 1 µg·L^{-1}, significantly lower than the LD_{50}-DDT in our study (Robert and His, 1981).

A similar outcome was reported by Mai *et al*. (2014), where the DDT-LC_{50} value for S-metolachlor exposure to Pacific oyster embryo and larvae after 24 h was less than 10 µg·L^{-1}, in line with our findings (Mai *et al*., 2014). These findings suggest that Pacific oyster embryo and larvae exhibit greater sensitivity to DDT in an artificial seawater environment compared to clams. The

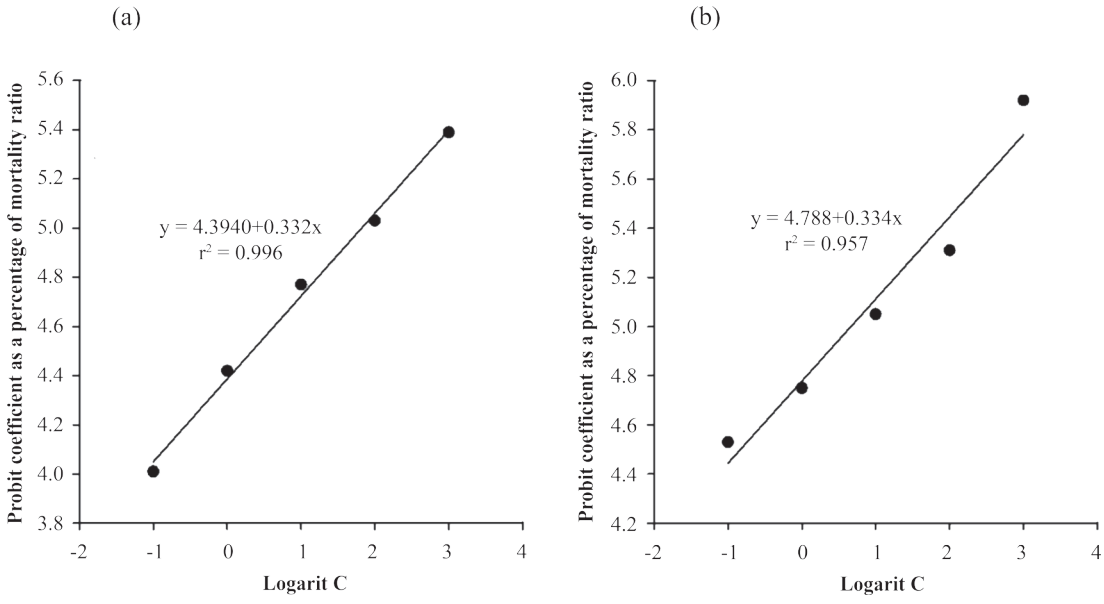

Figure 5. Regression of concentrations of DDT on: (a) percentage of abnormal embryos at 2 h, and (b) mortality rates at 24 h, of Pacific oyster in artificial seawater environment ($p<0.0001$).

clam embryo and larvae used in the experiments by Chung *et al*. (2007) had a longer growth period and more developed inner organs, making them more resilient than newly fertilized Pacific oyster embryos. The absence of a protective wall in the latter allows toxins to penetrate more easily, causing greater cellular damage.

These results underscore the varying responses of different bivalve mollusk species to plant protection chemicals, influenced by factors like experimental conditions, exposure duration, and the age of the embryo and larvae used in the experiments (Song *et al*., 2016).

Impacts of DDT toxicity on Pacific oyster embryo and larvae in sediment environment

Following a 24 h exposure to DDT concentrations ranging from 0.01 to 1 mg·kg^{-1} in the sediment environment, a significant increase in the percentage of abnormal embryos was observed, climbing from 18 to 75%, which was significantly higher ($p<0.05$) than the control sample (13%) (Figure 6a). Similarly, Figure 6b illustrates comparable results after 24 h of exposure to DDT, showcasing a substantial decrease in the survival rate of the embryo and larvae. Mortality rates of Pacific oyster embryo and larvae peaked at 1 mg·kg^{-1} concentrations, ranging from 27 to 84% in the experimental samples, while the control sample reported only 17% mortality ($p<0.05$). These findings underscore the impact of DDT on Pacific oyster embryo and larvae in both artificial seawater and sediment environments.

Figure 7 demonstrates the regression of DDT concentration on percentage of abnormal embryos (Figure 7a) and mortality (Figure 7b) of Pacific oyster embryo and larvae in the sediment environment. Similar to the artificial water environment, the logarithmic transformation of experimental concentrations at both 2 and 24 h reveals a linear increase in Probit coefficients with rising DDT concentrations. The calculated EC_{50} and LC_{50} values after 24 h of exposure to DDT were 1.1 and 0.3 mg·kg^{-1}, respectively.

Remarkably, the cytotoxic effects on Pacific oyster embryo and larvae after 24 h of DDT exposure exhibited no significant difference between the sediment and water environments. Intriguingly, most of the DDT toxicity data obtained in this study, including abnormal embryo ratios

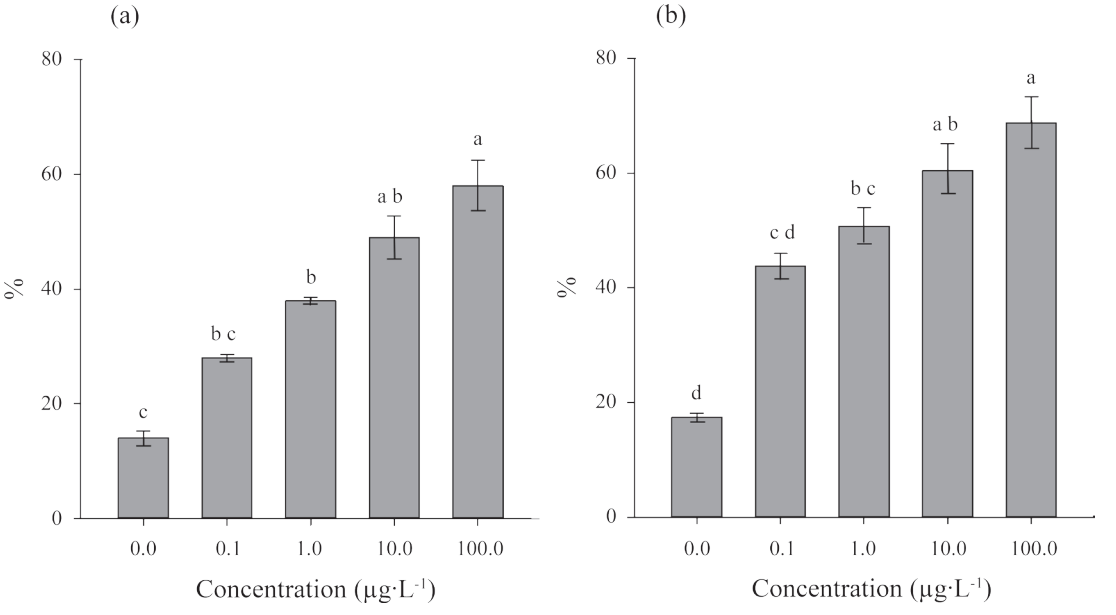

Figure 6. Net percentages of abnormal development (±SE) in *Crassostrea gigas* embryo observed after exposure to DDT in sediment environment ranging from 0.01 to mg·kg^{-1} for the abnormal embryo ratio at 2 h (a) and the mortality at 24 h, (b). The concentrations that do not share a letter are significantly different.

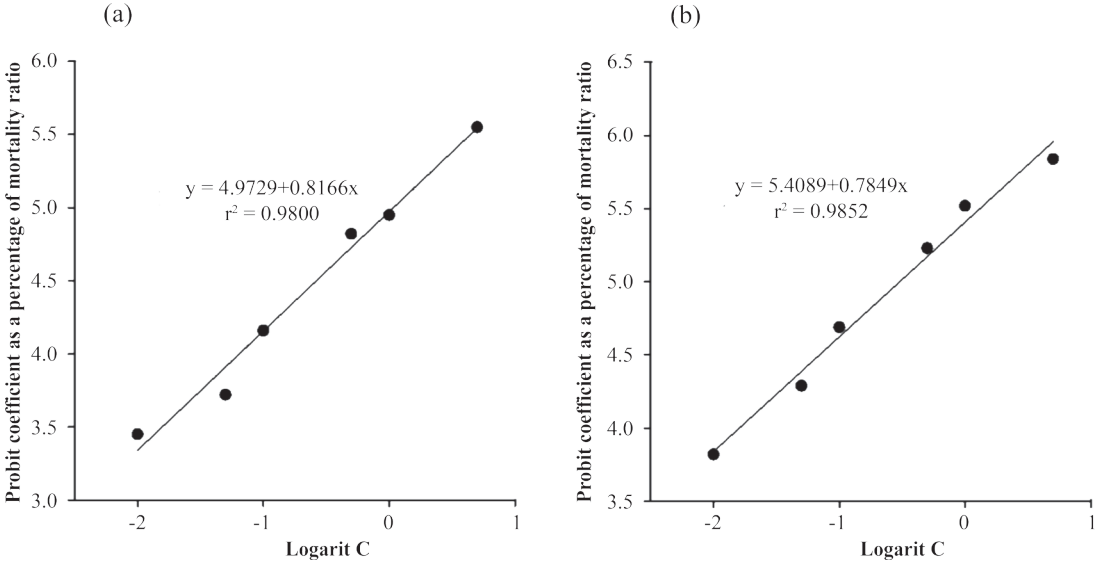

Figure 7. Regression relationships between the abnormal embryos at 2 h (a) and the mortality at 24 h (b) exposure to different concentrations of DDT in sediment environment ($p<0.0001$).

and mortality ratios, are notably lower than those reported for other pesticides such as chlorpyrifos, atrazine, and lindane. For example, Fathallah (2014) reported LC_{50} values of 2.53 and 457.4 $\mu g \cdot L^{-1}$ for chlorpyrifos and DDT exposure, respectively, in European clams (*Ruditapes decussatus*), significantly higher than the DDT-LC_{50} in this study. Lawton *et al*. (2006) found no toxic effect with atrazine concentrations up to 500 $mg \cdot L^{-1}$ after 10 days of exposure in the sediment environment for clams larvae on the American coast (Lawton *et al*., 2006).

The developmental toxicity of plant protection chemicals can vary based on various factors, including seasonal effects, aquatic species differences, and individual development stages. In the case of endosulfan, LC_{50} values for two clam species (*Lamellidens corrianus* and *L. marginalis*) in the summer season were higher than in the winter season. After 24 h of exposure to endosulfan concentrations ranging from 65 to 75 ppm, the mortality rates ranged from 10 to 70%, respectively (Mane and Muley, 1984).

DDT appears to pose a more significant threat to the early development stages of bivalve mollusks compared to the results obtained on juvenile clams by Chung *et al*. (2007). In sediment environments, LC_{50}-DDT values obtained after 10 days reached 5.8 $mg \cdot kg^{-1}$. Overall, the toxicity of embryo and larvae, both in water and sediment environments, appears to be species-dependent, sediment-dependent, and influenced by testing conditions.

Impacts of DDT toxicity on the morphological structure of pacific oyster embryo and larvae

Impacts of DDT toxicity in the water environment

Following a 24 h exposure to a DDT concentration of 1 $\mu g \cdot L^{-1}$ within an artificial seawater environment, a substantial alteration in the morphology of the Pacific oyster was observed under SEM. In the control sample, embryos exhibit a circular or spherical shape with a smooth surface, undergoing cell division as depicted in Figure 8a. Conversely, in the experimental samples exposed to DDT pesticides, the embryos exhibit deformities, rough surfaces, and breakage, as evident in Figures 8b, 8c, and 8d.

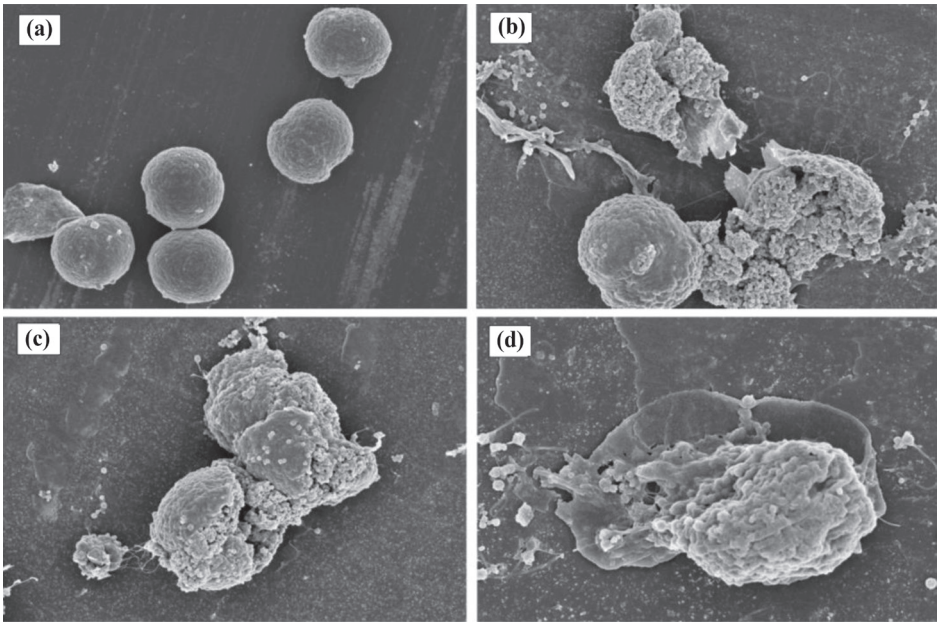

Figure 8. SEM images of Pacific oyster, before and after exposure to DDT concentration of 1 $\mu g \cdot kg^{-1}$ in artificial seawater environment at 24 h: (a) the control sample; (b, c, d) the exposed samples (bar = 50 µm).

TEM images, as depicted in Figure 9 for the control sample and Figure 10 for the experimental sample, provide insight into the morphological structure of embryos. Figure 9 illustrates that the cell structure remains intact in the control sample, with the embryos maintaining a round shape and a smooth exterior. Notably, Figure 9c reveals the presence of a typical endoplasmic reticulum (indicated by arrow 2) and particles exhibiting complete capsids (indicated by arrow 1). Additionally, the cytoplasm beneath the sub-muscular layer displays a thickened inner nucleus, and the embryo's cell wall appears distinct, measuring 610 nm (Figure 9a, 9c).

In contrast, the TEM images in Figure 10 demonstrate significant alterations in embryos exposed to DDT. These images reveal pronounced changes, including the near-complete destruction of inner organs (Figure 10b, 10c, 10d), a thinner cell wall measuring 405–440 nm (Figure 10a), the destruction and emptiness of capsids with inner nuclei (arrow in Figure 10b), and incomplete endoplasmic reticulum (arrow in Figure 10d).

Impacts of DDT toxicity in the sediment environment

The findings indicate a notable alteration in the Pacific oyster morphology due to the presence of the DDT pesticide (Figure 11). SEM images from both the control sample (Figure 11a) and the experimental sample (Figure 11b, 11c, 11d) exhibit similarities to the outcomes observed in the artificial sea environment.

Our TEM findings reveal significant differences in morphological structure between the control sample (absence of DDT) and the experimental sample (exposed to a DDT concentration of 1 $mg \cdot kg^{-1}$). In Figure 12, the cell structure remains intact, characterized by a well-defined endoplasmic reticulum (indicated by arrows 2 in Figure 12b and 4 in Figure 12c), a cytoplasm with a thickened inner nucleus beneath the sub-muscular layer (indicated by arrows 1 in Figure 12b and 3 in Figure 12c), and a relatively thickened cell wall ranging from 364 to 370 nm.

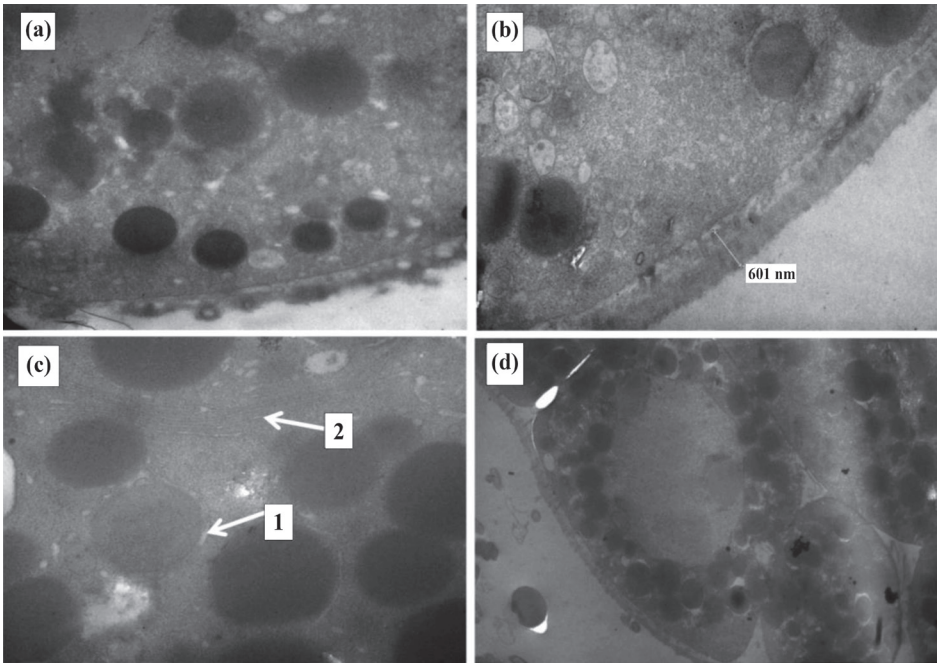

Figure 9. TEM images illustrating the ultrastructural changes in Pacific oyster in artificial seawater environment after 24 h (bar = 500 nm); arrow 1 shows particles exhibiting complete capsids and arrow 2 shows a typical endoplasmic reticulum, respectively (Figure 9c).

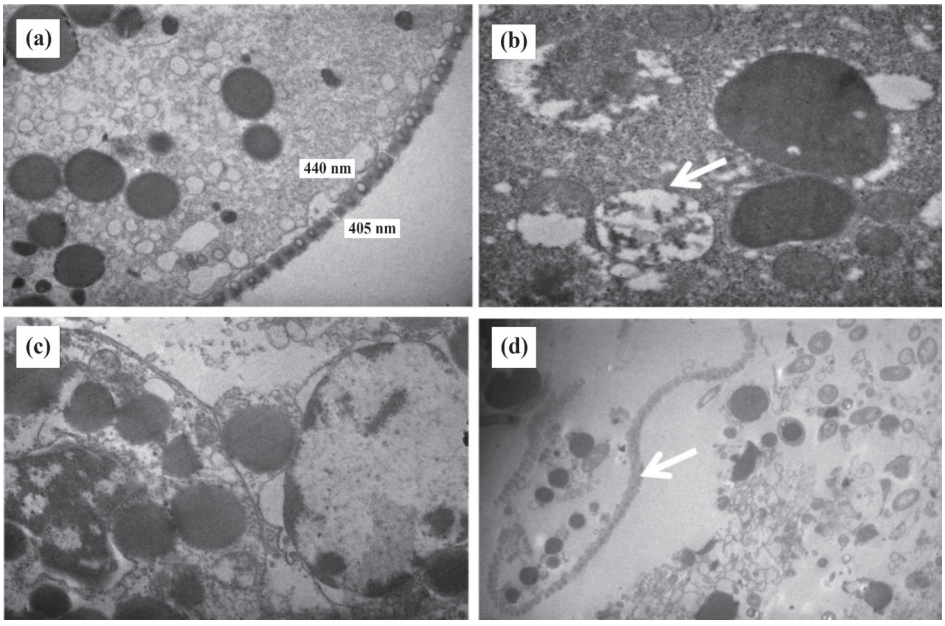

Figure 10. TEM images illustrating the ultrastructural changes of *Crassostrea gigas* embryo in artificial seawater environment caused by the exposure DDT concentration of 1 $\mu g \cdot L^{-1}$ after 24 h (bar = 500 nm); arrow in Figure 10b shows the destruction and emptiness of capsids with inner nuclei and incomplete endoplasmic reticulum (arrow in Figure 10d).

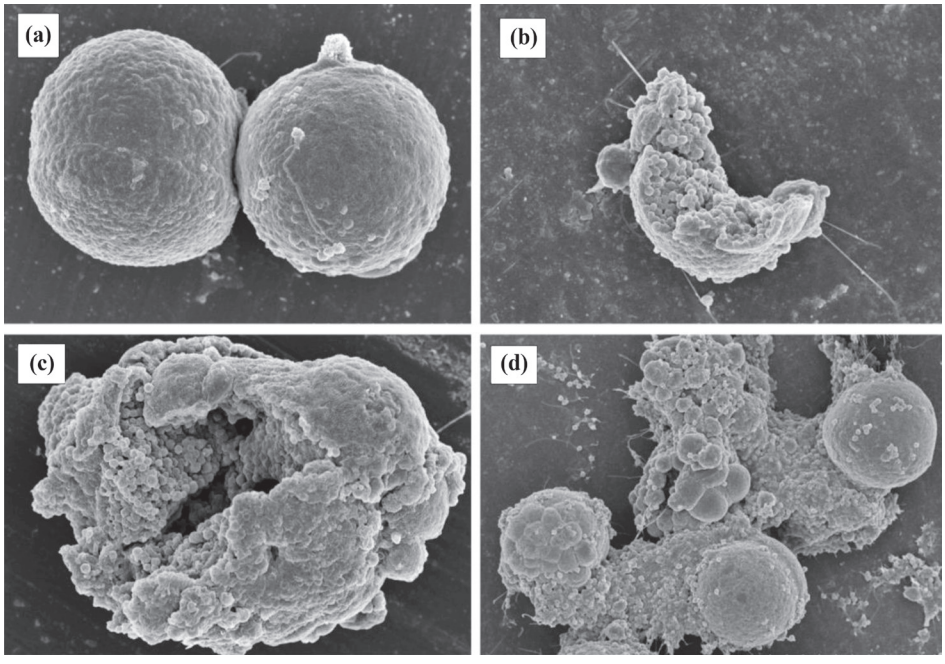

Figure 11. SEM images of *Crassostrea gigas* before and after exposure to DDT concentration of 1 $\mu g \cdot kg^{-1}$ in sediment environment at 24 h: (a) the control sample; b, c, d) the experimental samples (bar = 20 µm).

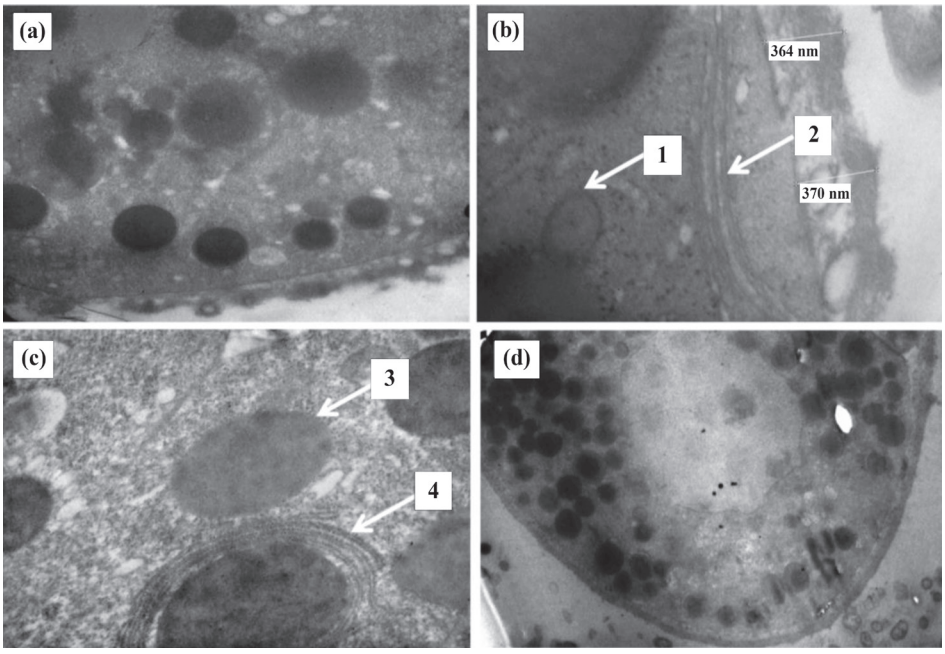

Figure 12. TEM images illustrating the ultrastructural changes *Crassostrea gigas* in artificial seawater environment (control sample, without DDT) after 24 h (bar = 500 nm); arrows 2 and 4 show a well-defined endoplasmic reticulum; arrows 1 and 3 show a cytoplasm with a thickened inner nucleus beneath the sub-muscular layer.

Conversely, when exposed to a DDT concentration of 1 $mg \cdot kg^{-1}$, TEM images in Figure 13 reveal a significant disruption in morphological structure. The endoplasmic reticulum and cytoplasm are visibly damaged (indicated by arrow 3 in Figure 13c), the cell wall becomes thinner (ranging from 293 to 315 nm, as shown in Figure 13d), and the capsids with inner nuclei appear destroyed and empty (indicated by arrows 1 and 2 in Figure 13a).

It has been reported that the early life stages of Pacific oyster embryos and larvae are more susceptible to toxicological effects and infections compared to 21-day-old larvae (Mottier *et al*., 2014). Surprisingly, only a limited number of studies have investigated the impact of plant protection chemicals on the ultrastructural changes of oyster embryos and larvae using SEM and TEM techniques, with most research focusing on the effects of viruses, bacteria, or chemicals on these early developmental stages. Typically, these studies center on assessing acute toxicity, calculating survival ratios with LC_{50} and EC_{50} values, or examining genotoxicity through RT-PCR techniques.

Nonetheless, some authors have observed ultrastructural changes in oyster embryo and larvae that align with the findings of this study (Wassel *et al*., 2007; Buisson *et al*., 2008). The atrophies in the wall of the digestive tubules and restructuring of the connective tissue were observed at a concentration of 0.1 $\mu g \cdot L^{-1}$ of glyphosate when Pacific oyster juvenile was exposed to glyphosate for 56 days. Wassel *et al*. (2007) noted embryotoxic and genotoxic effects of 17 alpha-ethinylestradiol and the organochlorine pesticide endosulfan at concentrations ranging from 0.2 to 300 nM after 16 to 20 h of exposure. Notably, an increase in abnormal D-larvae was observed at the highest endosulfan concentration of 300 nM. Changes in reproduction (partial spawning) and histopathology (atrophy of the digestive tubule epithelium) of oyster larvae tissue when exposure to diuron and isoproturon at concentrations of 0.5 to 1 $\mu g \cdot L^{-1}$ were also documented by Buisson *et al*. (2008).

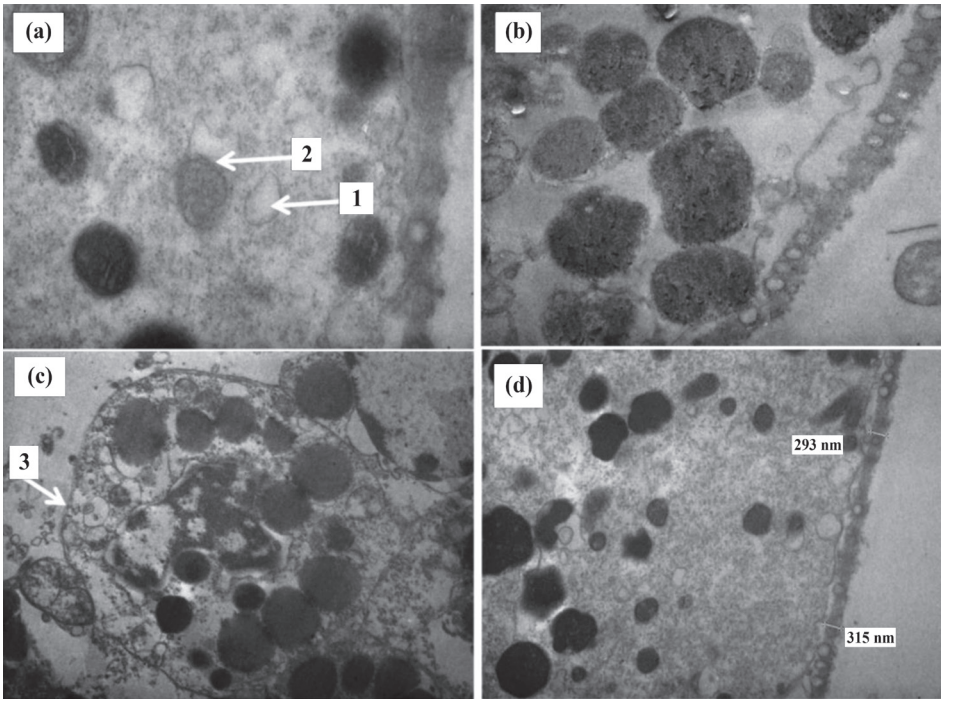

Figure 13. TEM images illustrating the ultrastructural changes of *Crassostrea gigas* embryo in artificial seawater environment caused by the exposure DDT concentration of 1 $\mu g \cdot kg^{-1}$ after 24 h (bar = 500 nm); arrows 1 and 2 show the capsids with inner nuclei appear destroyed and empty; arrow 3 shows the endoplasmic reticulum and cytoplasm are visibly damaged.

It is worth noting that lipophilic organic contaminants are easily purged during spawning, leading to gonadal necrosis in female and hermaphrodite oysters as these substances accumulate in gametic tissues (Buisson *et al*., 2008). Several authors have observed that the accumulation and detoxification of various organic and inorganic toxins in bivalve mollusks largely depend on their digestive glands (Colas, 2011) and vary among aquatic species (oysters, clams, and mussels) and geographic regions (Kim *et al*., 2008). For example, Kim *et al*. (2008) observed the presence of cestode cysts and hemocytic infiltration in *Crassostrea virginica* organelles when exposed to toxicants such as heavy metal, pepticide but did not record these effects in mussel species. It is evident that the concentrations of organochlorine pollutants in bivalve mollusks and their effects on developmental processes are influenced by sampling locations, collection seasons, test concentrations, and thespecific species under study (Solé *et al*., 2000; Buisson *et al*., 2008).

Due to the continued illegal use of DDT in Vietnam, the potential for long-term ecological impacts necessitates extensive research spanning various seasons and subjects. Assessing whether these substances can cause lasting ecological harm is challenging due to the diverse regional usage across Vietnam. However, several research and surveys by the Vietnam Ministry of Natural Resources and Environment and some other authors have recorded the effects of some toxic substances such as Lindane, Aldrin, Dieldrin, DDT (His *et al*., 1999; Minh *et al*., 2007; Hoai *et al*., 2010; VASEP report, 2019) and leading to the emergence of "cancer villages" in Vietnam (His *et al*., 1999). In the case of this study, Pacific oysters are the last filter feeders of the ecological chain that are commonly farmed in the study area, so long-term exposure to this group of toxins can cause detrimental adverse effects to their growth and development in particular as well as aquaculture productivity in general.

Therefore, to advance our understanding in this field, future research should consider these limitations and pursue several promising avenues. Firstly, conducting multi-location studies that encompass a range of environmental conditions and pollution levels would enable us to generalize our findings and gain insights into the broader impact of contaminants on Pacific oysters in diverse settings. Additionally, expanding research to explore the broader ecological implications of Pacific oyster health and reproduction in contaminated environments would contribute to more informed ecosystem management and conservation efforts. Lastly, research focusing on effective management strategies, such as contaminant mitigation or habitat restoration, would be instrumental in safeguarding Pacific oyster populations and ensuring sustainable aquaculture practices.

CONCLUSION

In conclusion, our study sheds light on the embryotoxicity and morphological changes observed in Pacific oysters cultivated in the Sai Gon-Dong Nai River downstream, Ho Chi Minh city, Vietnam. The analysis results revealed a significant adverse impact of DDTs on the survival of larvae and the cell division of embryos in both water and sediment samples compared to control samples. It is shown herein that Pacific oyster embryos and larvae are susceptible to low concentrations of DDT in both artificial seawater and sediment environment and the DDT could present a threat to the reproduction of wild or cultivated Pacific oysters. It may be of high relevance in the management of safe aquaculture areas and sustainable development at estuary Saigon–Dongnai river, Vietnam.

Because there is no data on the toxicity of DDT in the water environment on the growth and development of Pacific oyster embryo and larvae after 2 h in the literature, we selected this time period for research. However, it's important to acknowledge several limitations in our research. Firstly, our study focused on a single location, which may limit the generalizability of our findings to other regions with different environmental conditions and contaminant profiles. Additionally, our investigation primarily centered on the effects of DDT, leaving unanswered questions about potential interactions with other pollutants commonly encountered by Pacific oysters in their natural habitats. Furthermore, the relatively short 24 h exposure duration may not capture the long-term consequences of DDT exposure on Pacific oyster populations adequately. A larger sample size and more extended exposure studies could enhance the statistical robustness of our conclusions. Lastly, while we aimed to account for environmental variability, natural ecosystems exhibit complex temporal and spatial fluctuations that could influence Pacific oyster responses in ways our study might not fully capture.

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