

CHARACTERISTICS OF DIARRHETIC SHELLFISH POISONS IN LAIZHOU BAY, BOHAI SEA, CHINA

XU, R.* – ZHAO, X.Y.

College of Food Science and Technology, Hebei Normal University of Science and Technology, Changli, Qinhuangdao 066600, China

**Corresponding author*

e-mail: robust100@163.com; phone: +86-33-5203-9074; fax: +86-33-5203-9074

(Received 24th May 2022; accepted 2nd Sep 2022)

Abstract. Laizhou Bay in the Bohai Sea is an important shellfish mariculture area in China. The research aims to ascertain compositions and contents of shellfish toxins inducing diarrhetic shellfish poisoning (DSP) in the sea area with a method known as solid phase adsorption toxin tracking. The results showed that seawater in Dongying and Laizhou sea areas presents the same composition of DSP toxins (including OA, DTX-1 and PTX-2). The content of DSP toxins in the Dongying sea area was generally higher than that in the Laizhou sea area. The study addresses the gap in the knowledge of DSP toxin compositions and changes in shellfish mariculture areas within the sea area and provides basic data enabling strengthened supervision, thus guaranteeing the food safety of shellfish.

Keywords: *diarrhetic shellfish toxin, distribution, toxin tracking, okadaic acid, algal bloom*

Introduction

Laizhou Bay, located to the south of the Yellow River Estuary–Longkou line and covering an area of 6060 km², is one of three bays in Bohai Sea and also the largest bay in Shandong Province, China. *Scapharca subcrenata* ranks the top among marine shellfish in Shandong Province in terms of the areal distribution and quantity of resources. Besides, the sea area is abundant with *Maetra veneriformis*, *Meretrix meretrix*, *Solen strictus*, and *Mytilus edulis*. As one of the most important fishing grounds in China, the sea area is located in an excellent geographical position with abundant fish, shrimp, and shellfish species.

Laizhou Bay, where red tides frequently occur, contains 90 red tide organisms and eight dinoflagellates according to data collected during the monitoring of red tide events from 2010 to 2015. Here, red tides occurring in 2010 to 2014 were typically induced by dinoflagellates and diatoms. Multiple dinoflagellates can produce diarrhetic shellfish poisoning (DSP) toxins, which greatly influence the marine environment, marine organisms, and human health. Shellfish samples collected in coastal areas in China are heavily contaminated by DSP toxins. DSP toxins have been detected in Laizhou Bay, the North Yellow Sea, south coastal areas in Zhejiang Province and the Fujian sea area. However, research on related shellfish toxins in shellfish mariculture areas in Laizhou Bay, Bohai Sea remains sparse.

The occurrence of harmful algae and phycotoxins in coastal areas of many countries has been monitored in relation to three poisoning syndromes: diarrhea, amnesia, and paralysis. The poisoning of shellfish is one of the greatest concerns for the public's health because phycotoxins have an extremely stable non-proteic structure and do not affect the taste or appearance of raw or processed bivalve mollusks. Therefore, shellfish poisoning can occur even when seafood has been dried, smoked, cooked or salted. In shellfish, OA and its derivatives (DTX1, DTX2, DTX3) accumulate in adipose tissue.

Cellular biochemical reactions can be altered by these molecules, because they are able to traverse the phospholipid bilayer of the cell membrane. These compounds could inhibit phosphatase, which regulate numerous cellular processes, including neuronal activity, cell differentiation and muscle contraction (Twiner et al., 2016). An animal study found that a low dose of OA (80 mg/kg body weight) had effects on the colonic microbiota and gastrointestinal tract in rats (Liu et al., 2020). It has also been proposed that the capacity of DSP toxins to cause tumor progression has been attributed to their ability to increase TNF- α secretion (Delcampo et al., 2017). So many countries have developed monitoring programs for harmful algae and shellfish due to the risk associated with their consumption.

Solid phase adsorption toxin tracking (SPATT) is a technology developed in recent years for collecting and monitoring shellfish toxins. Used in concert with an appropriate detection method, the technique can provide early predictions of blooms of toxin-producing algae and contamination of shellfish by toxins by using a passive sampler with adsorbents to enrich shellfish toxins in seawater (Fux et al., 2008). The technique is simple, convenient, accurate, sensitive, and allows continuous monitoring unlike some traditional methods such as shellfish detection and algal monitoring. The laboratory and field tests verified that non-polar aromatic resin HP-20 can be used to adsorb various lipophilic shellfish toxins, including okadaic acid (OA), dinophysistoxins (DTXs), pectenotoxins (PTXs), yessotoxins (YTXs), and azaspiracids (AZAs) (Fux et al., 2008, 2009; MacKenzie et al., 2004; Li et al., 2011; Rundberget et al., 2009). The toxins inducing diarrhea mainly involve OA, DTX-1 and DTX-2 and the other several toxins (PTXs, YTXs and AZAs) show a slight or no influence on diarrhea. By separately selecting a developing sea area as the sampling point, five DSP toxins (including OA, DTX-1, DTX-2, and some PTX-2 and YTX) in seawater of the sea area were regularly monitored from May to October, 2020 by employing the SPATT technique. The composition and amounts of DSP toxins in seawater in the sea area were determined and the differences in contents of DSP toxins in adjacent mariculture areas in the same sea area and under different environmental conditions were compared. Furthermore, the change in amount of DSP toxins in seawater within the sea area was measured. The data may provide a scientific basis for studying DSP toxins in Laizhou Bay, aiming at an economical and practical temporary environment for purification of shellfish toxins and ensuring the food safety of shellfish.

Materials and methods

Sample collection and processing

Dongying and Laizhou are two marine sampling spots and Shouguang and Changyi are two offshore shellfish mariculture areas with ponds, each with a water depth of about 1 m (*Fig. 1*). The seawater in these ponds is changed every fortnight. We collect samples on the 10th of each month. According to the sequence in *Figure 1*, the coordinates of each sampling point are 118°07'-119°10' E, 36°55'-38°10' N, 118°32' E, 36°41' N, 119°13' E, 36°25' N and 119°33' E, 36°59' N.

The adsorption bags were sewed by separately packing 10 g of HP-20 resin into a polyester mesh with the pore size of 48 μ m to adsorb toxins. In terms of the marine sampling, the adsorption bag was separately tied onto a lifting cage and allowed to sink to a depth of 3.5 m underwater; as for the pond sampling, a rod was fixed at the center of the pond, on which an adsorption bag was tied, and a heavy object was added so that

the adsorption bag was completely immersed in the water. The sampling lasted for 10 d from May to July while it was extended to 20 d from August to October. The adsorption bags were processed in the laboratory. The resin in the adsorption bags was transferred to a sand filter column. Thereafter, salt was washed away with the aid of 200 mL of deionized water and the moisture in the column was removed by blow-drying under positive pressure. Subsequently, 25 mL of methanol was slowly added to the column to soak the resin. The mixture was allowed to stand for 2.5 h and then filtered into a heart-shaped bottle. Then, 25 mL of methanol was added again to repeat the extraction. The extracting solution was heated at 40 °C to evaporate the methanol. About 0.5 mL of extracting solution was retained, and 5 mL of dichloromethane was added before vortex extraction for 1.5 min. After standing for 15 min, a dichloromethane layer was extracted. Subsequently, 5 mL of dichloromethane was added and the extraction operation repeated. The dichloromethane layers were combined and dried by nitrogen-blowing at 40 °C. Then, 1 mL of 80% methanol aqueous solution was used to reach a certain volume and the resultant solution was filtered through a 0.22-mm membrane for organic phase assay. The filtered mixture was analyzed by using liquid chromatography–tandem mass spectrometry (LC–MS/MS). The detection result was divided by the mass (g) of adsorbents and days of adsorption and the toxin content in seawater is expressed using the toxin content (ng) in per gram of the adsorbent (g resin) per day (day), that is, ng/(g resin·d) (Fux et al., 2009; Li et al., 2010).



Figure 1. Location of sample collection. 1: Dongying, 2: Shouguang, 3: Changyi, 4: Laizhou

Instruments, reagents, and materials

The instruments included a TSQ Quantum Access liquid chromatograph-tandem mass spectrometer (Thermo Fisher Scientific, USA), a rotary evaporator (Buchi Corporation, Switzerland) and a NVAP112 nitrogen blower (Organomation, USA). An HP-20 resin (Mitsubishi Chemical Corporation, Japan) was used throughout.

The reagents involved standard samples of OA, DTX-1, DTX-2, PTX-2, and YTX (National Research Council, Canada); methanol and acetonitrile (chromatographically pure, Merck Corporation); dichloromethane, formic acid, and ammonium formate (chromatographically pure, Fluka); Milli-Q ultrapure water was used throughout.

LC–MS/MS analysis

The OA, DTX-1, DTX-2, PTX-2, and YTX in seawater were synchronously assayed by applying LC–MS/MS analysis (Wood et al., 2011). Sample numbers of three were used to establish repeatability.

Data analysis

The Kolmogorov Smirnov test was used to determine whether SPATT data were normal. Non-normal data were transformed using square root and log transformations, but skewed distributions could not be normalized. As a result, non-parametric tests were used for data analysis. The relationship between the occurrence of each of the toxins over time was determined using Spearman's Rank Order Correlation. Analyses of the data were carried out using SPSS (Version 26).

Results and discussion

DSP toxin compositions in seawater in shellfish mariculture areas in Laizhou Bay

The seawater in Dongying and Laizhou sea areas within Laizhou Bay harbored the same DSP toxin compositions (Table 1, mainly including OA, DTX-1 and PTX-2). OA was the principal component, accounting for 46.3% and 54.6% in the total toxin contents in Dongying and Laizhou sea areas. DTX-2 and YTX were not found in these two sea areas. The DSP toxin compositions (OA and PTX-2) in ponds in Shouguang were the same as those in Changyi, Laizhou Bay, without DTX-1, DTX-2, or YTX.

Table 1. DSP toxins in seawater in Laizhou Bay

Toxin	Dongying		Laizhou		Shouguang		Changyi	
	Content ^a	Percentage ^b	Content	Percentage	Content	Percentage	Content	Percentage
OA	2.09~18.58	46.3	0.28~13.51	54.6	0.02~0.11	90.2	0.09~0.35	76.5
PTX-2	0.59~22.66	40.5	0.14~19.78	8.3	0~0.03	9.8	0.02~0.14	23.5
DTX-1	1.1~3.49	13.2	0.22~2.04	37.1	NF	NF	NF	NF
DTX-2	NF	NF	NF	NF	NF	NF	NF	NF
YTX	NF	NF	NF	NF	NF	NF	NF	NF

^a and ^b refer to the concentration range (ng·g⁻¹ resin·d⁻¹) of toxins and percentage (%) of the content of a toxin in the total toxin content; NF represents no toxins detected

Changes in amounts of DSP toxins in seawater in shellfish mariculture areas in Laizhou Bay

Figure 2 shows the changes in contents of DSP toxins in seawater in shellfish mariculture areas of Dongying, Laizhou Bay. The contents of OA and PTX-2 fluctuated significantly from May to October, 2020 with high contents measured from May to July, with maxima of 18.12 and 22.53 ng/(g resin·d), respectively. The DTX-1 content fluctuated slightly and remained below than 3.5 ng/(g resin·d). After July, PTX-2 and DTX-1 contents remained low, while the OA content was high and fluctuated to a significant extent. The OA content dropped to a low level and the three followed a consistent trend in September. No large-area tides occurred between May and October, 2020, while toxic algae at low concentrations might still be present. Some genera of DSP-toxin-producing algae (*Dinophysis* spp. and *Prorocentrum* spp.) were distributed within the sea area. At seawater temperatures of 20 to 30 °C, the algal abundance in seawater was high and toxin-producing algal cysts were reproduced in significant number; the seawater in Laizhou Bay was severely eutrophicated, resulting in poor exchange capacity. Therefore, the nutrients in seawater were difficult to dilute; the

number of toxin-producing algae increased, as did the content of DSP toxins at an appropriate temperature and given a sufficiency of nutrients.

The growth of algae is associated with various marine environmental factors such as water temperature, illumination, and availability of nutrients. The reproduction of algae is mainly influenced by illumination and availability of nutrients within the range of water temperatures suitable for their reproduction. The water temperature is low in winter, which is considered as a determinant for the reproduction of algae. The content of DSP toxins is high due to the higher water temperatures measured between May and July while it is low because of the low water temperatures found from August to October (Fig. 2). Next, it is necessary to explore the relationship between toxin concentrations and marine environmental factors.

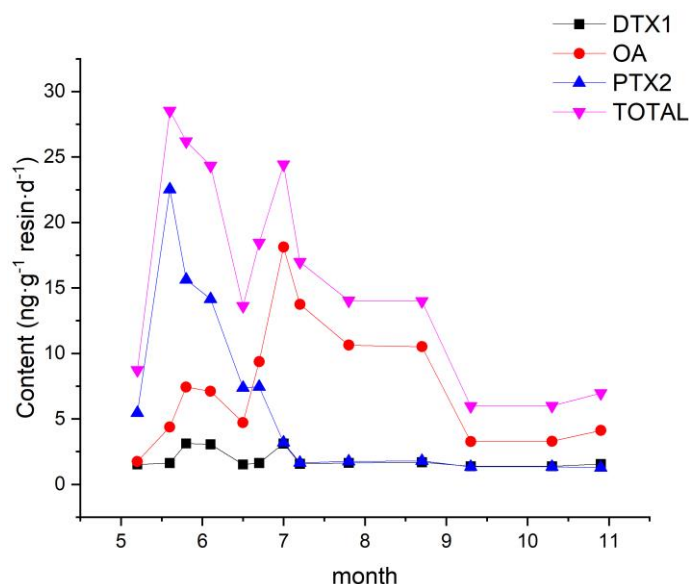


Figure 2. The variation curves of DSP content in Dongying seawater, Laizhou Bay

Figure 3 shows the change in the content of DSP toxins in seawater within shellfish mariculture areas in Laizhou sea area. The contents of OA and PTX-2 fluctuated significantly from May to October, 2020 and were high from May to July, with maxima of 12.95 and 20.51 ng/(g resin·d). In this period, the DTX-1 content did not fluctuate to any significant extent, remaining below 2.5 ng/(g resin·d). Overall, the seasonality of toxins in Laizhou sea area was similar to that in the Dongying sea area: the PTX-2 content was higher than the OA content from May to June, which was similar to that in the Dongying sea area.

Spatial differences in the amounts of DSP toxins in seawater within shellfish mariculture areas in Laizhou Bay

The difference between Dongying and Laizhou sea areas in terms of DSP toxin concentration

In the seawater within the shellfish mariculture areas in Dongying and Laizhou sea areas, Laizhou Bay presented the same compositions of DSP toxins (including OA, DTX-1, and PTX-2), without the presence of DTX-2 and YTX. The contents of the

three DSP toxins, i.e. OA, PTX-2, and DTX-1, showed a similar seasonality (Figs. 2 and 3): they were high from May to July and then decreased; the PTX-2 content varied in a slightly different manner from the OA and DTX-1 contents. The PTX-2 content was high in May and then decreased while the OA and DTX-1 contents were high from June to August. Both Dongying and Laizhou are located in Laizhou Bay and the amounts of DSP toxins therein showed a quasi-synchronous seasonality; however, the toxin contents in the two sea areas did differ in that the content of DSP toxins in seawater in the Dongying sea area was generally higher than that in the Laizhou sea area. Owing to Dongying being adjacent to land, the seawater there is greatly contaminated and exhibits a high level of eutrophication and a weak exchange capacity. This is possibly a reason why the content of DSP toxins in the Dongying sea area is higher than that in the Laizhou sea area. Jiang et al. and Li et al. also suggested that contents of DSP toxins differ at different sampling points, even for samples taken in close proximity (Jiang et al., 2000; Li et al., 2014).

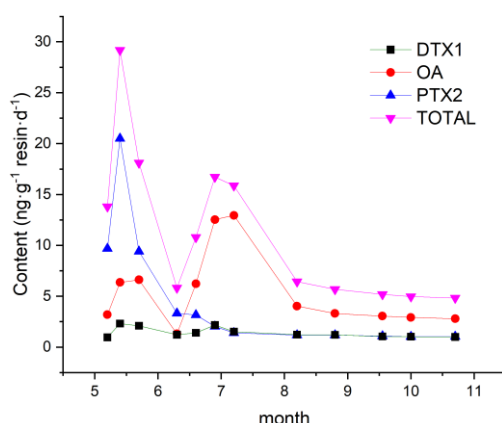


Figure 3. The variation curves of DSP content in Laizhou seawater, Laizhou Bay

The differences in toxins in shellfish aquaculture seas and culture ponds

OA, DTX-1, and PTX-2 occurred in the shellfish aquaculture seas in Dongying and Laizhou, while only low concentrations of OA and PTX-2 were detected in water taken from culture ponds in Shouguang and Changyi seas, with no DTX-1 found therein (Table 1).

Shellfish accumulate toxins by ingesting toxic algae in seawater. Shellfish toxins in ponds (at higher elevation) remain low even when the open sea areas are contaminated. A possible reason for this is that the water depth is low, or no water is contained, in ponds whereas open seawater contains many more toxic algae. Thus, the toxic algae have a low population density and furthermore, the toxin content is low in ponds. Generally, shellfish are temporarily cultured and purified in seawater without harmful plankton being present, so for some time after they are found to be poisoned, they metabolize shellfish toxins themselves. The existing techniques for shellfish purification are mainly aimed at purifying microorganisms and no engineering technique is available for purification of shellfish toxins. The main reason for this is that a large site is required to conduct large-batch, long-term purification. It is expensive and costly to build factories for shellfish purification, so ponds remain an economical and practical culture environment for purifying shellfish toxins.

Conclusions

The composition of, and changes in, DSP toxin concentrations in seawater within shellfish mariculture areas in Dongying and Laizhou, Laizhou Bay, Bohai Sea from May to October, 2020 were studied by use of the SPATT technique. The compositions of DSP toxins in seawater in the sea areas included OA, DTX-1, and PTX-2, in which the OA content was globally the highest, with no DTX-2 and YTX present; the OA and PTX-2 contents exhibited high levels from May to July and the DTX-1 content did not vary to any significant extent. A spatial difference was found in the contents of DSP toxins in seawater in Dongying and Laizhou sea areas: the content of DSP toxins in the Dongying sea area was generally higher than that in the Laizhou sea area. However, the DSP contents in seawater in the two sea areas exhibited a similar seasonality. The compositions and contents of DSP toxins in seawater of ponds within the sea areas were analyzed by using the SPATT technique. Compared with the seawater beyond ponds in the chosen sea areas, DTX-1, DTX-2, and YTX were not detected in seawater within the ponds and the contents of OA and PTX-2 therein were quite low. The existing ponds can be used as an optional culture environment for shellfish toxin purification.

Acknowledgments. This project was sponsored by the research project of Hebei Education Department, China (ZC2021230).

Conflict of interests. There are no conflicts of interests.

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