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INVESTIGATION OF PHYTOTOXIN (MAINLY PSP, DSP) AT CUA BE (NHA TRANG BAY) DURING 1998

Do Tuyet Nga, Luu Thi Ha, Pham Xuan Ky, Cao Phuong Dung, Dao Viet Ha
Institute of Oceanography (Nha Trang)

ABSTRACT Four species of bivalves including Saccostrea cucullata, Isognomon ephippium, Amussium pleuronectes and Katalysia hiantina were collected from January to October/1998 at Cua Be area (Nha Trang bay) to investigate PSP and DSP.

Saccostrea cucullata, Isognomon ephippium accumulated PSP toxin at the quite low concentrations, basing on the death time of mice lengthened hours, out of the legally toxic level.

A. pleuronectes accumulated DSP toxin more than PSP toxin. The DSP accumulation of A. pleuronectes happened in three months (July, September and October), highest in July (the death time was 6 hours) when the water temperature and Nitrogen were the highest and NH_3 was also high.

KHAO SAT ÑOC TO TAO (PSP, DSP) TAI CÖA BE (VÒNH NHA TRANG) NAM 1998

Ño Tuyet Nga, Löu Thò Ha, Pham Xuan Ky, Cao Phöông Dung, Ñao Viet Ha
Vien Hai Döông Hoc (Nha Trang)

TOM TAT 4 loai Hai Manh Vo gom: Saccostrea cucullata, Isognomon ephippium, Amussium pleuronectes va Katalysia hiantina ña ñöôc thu tö thang 1 ñen thang 10/ 1998 tai Cöa Be (vònh Nha Trang) ñe phan tích ñoc to PSP va DSP.

Saccostrea cucullata, Isognomon ephippium tích luy ñoc to PSP ô nong ño thap, can cö vao thôi gian chet cua chuot keo dai hang giô va khong nam trong möc ño ñoc qui ñònh.

A. pleuronectes tích luy ñoc to DSP nhieu hôn la vôi ñoc to PSP. Sö tích luy ñoc to DSP cua A. pleuronectes xay ra trong 3 thang (7, 9 va 10), cao nhat vao thang 7 (thôi gian chet la 6 giô sau khi tiem); vao thôi ñiem ño nhiet ño nöôc, nitô co nong ño cao nhat va NH_3 cung co nong ño cao.

I. INTRODUCTION

Cua Be located at $12^0$09'54"–$12^0$12'25" North and $109^0$11'00"–$109^0$12'10" East of the south of Nha Trang bay, receiving the tributary of Cua Be river. It is also the area receiving the urban wastewater and sewage from the ship repairing factory, the sea product and the textile

factories, which easily led to eutrophication of waters and the bloom of algae.

In April 1997, there was the appearance of micro algae, primary Gonyaulax sp. (Ho Van The, 1997) which increased the concentration of PSP in some species of bivalves (Do Tuyet Nga et al., 1999a). With the aim to know PSP and DSP more clearly, we were keeping on studying these toxins and some environmental factors at Cua Be area in 1998.

II. MATERIALS AND METHODS:

1. Materials

- Four species of bivalves including Saccostrea cucullata, Isognomon ephippium, Amussium pleuronectes and Katalysia hiantina were collected at Cua Be area from January to October.

- In parallel, the seawater sample at this area was also collected in the same time (about 8h00 AM) in order to have the physical and chemical factors analyzed.

2. Methods

Determinating PSP by the method of AOAC (1990).

The conversion factor (CF 3/3) = 0.20.

The observing time: essentially in 60 minutes, but due to the low PSP concentration, we were keeping on observing up to 24 hours.

Results were accepted if 2/3 mice were died which had the characteristic symptoms of these toxins.

Determinating DSP by the method of Yasumoto et al., 1984.

The observing time of mice reaction: 24 hours.

Results were accepted if 2/3 mice were died.

III. RESULTS

1. PSP toxin

- Saccostrea cucullata:

Mostly assayed mice were died in all our samplings. However, the death time lengthened. The shortest death time of mice was 3 hours with the ratio of 3/3 (April 1998), and the longest death time of mice was 22 hours and 30 minutes with the ratio of 2/3 (October 1998) (Table 1).

- Isognomon ephippium:

Mice which were assayed by the extracts of this species collected in February, July and October 1998 were not died. The shortest death time of mice was 12 hours 30 minutes with the ratio of 3/3 (September 1998), and the longest death time was 24 hours with the ratio of 2/3 (June 1998) (Table 2).

- A. pleuronectes and K. hiantina

The PSP extracts from these species didn't cause any death of mice.

2. DSP toxin

All the extracts from Saccostrea cucullata, Isognomon ephippium, Katalysia hiantina in our analysis did not cause the death of mice, all mice recovered within 24 hours (Table 2).

Only Amussium pleuronectes gave the results as follows:

- Mice recovered within 24 hours for the extracts from viscera of A. pleuronectes collected in January, February and April 1998.

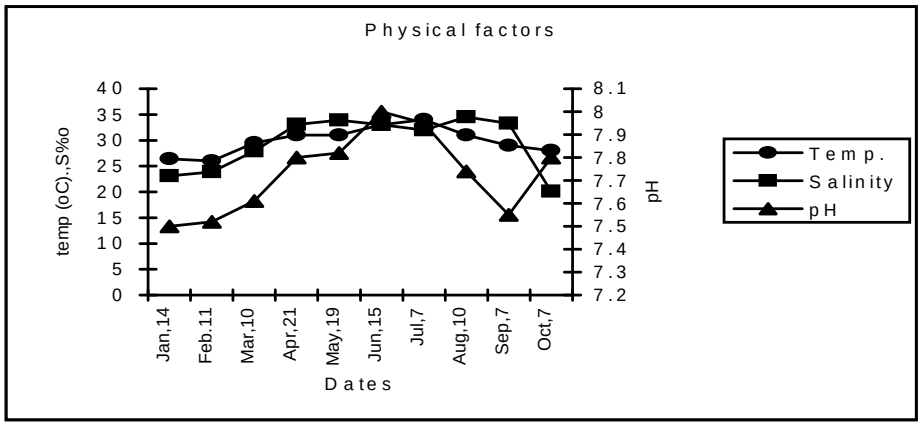
- Mice had the big abdomen and lengthened the apathy, sluggishness and weakness even after 24 hours for the extracts of A. pleuronectes collected in March, May, June and August.

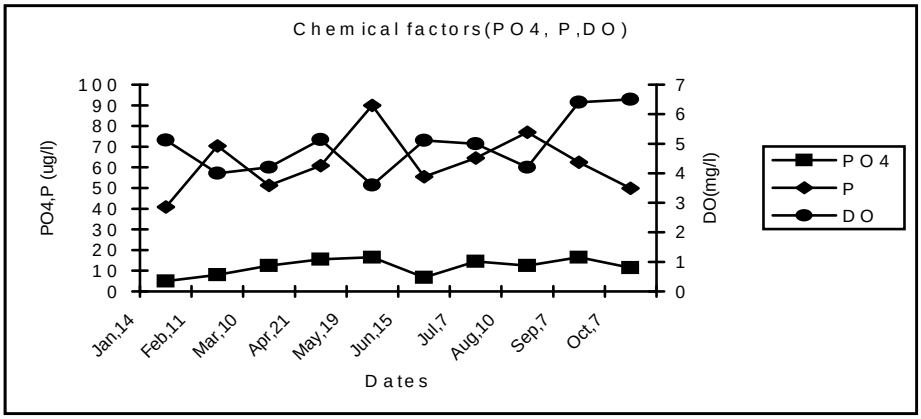
Table 1: Effects of PSP on bioassayed mice (observing in 24 hours)

Species	Saccostrea cucullata			Isognomon ephippium			Amussium pleuronectes			Katalysia hiantina		
Dates	Rates	Death time	Death ratio	Rates	Death time	Death ratio	Rates	Death time	Death ratio	Rates	Death time	Death ratio
14 Jan.		13:44	3/3		14:30	3/3	Tired		0/3	Recovery		0/3
11 Feb.		5:18	3/3	Very tired		0/3	Tired		0/3	Recovery		0/3
10 Mar.		14:32	2/3		15:30	2/3	Tired		0/3	Recovery		0/3
21 Apr.		3:00	3/3		15:15	2/3	Tired		0/3	Recovery		0/3
19 May		22:00	2/3		22:00	2/3	Tired		0/3	Tired		0/3
15 Jun.		6:16	3/3		24:00	2/3	Tired		0/3	Tired		0/3
7 Jul.		15:20	2/3	Tired		0/3	Tired		0/3	Recovery		0/3
10 Aug.		4:00	3/3		15:30	2/3	Tired		0/3	Tired		0/3
7 Sep.		4:00	3/3		12:30	3/3	Tired		0/3	Recovery		0/3
7 Oct.		22:30	2/3	Tired		0/3	Recovery		0/3	Recovery		0/3

Table 2: Effects of DSP on bioassayed mice (observing in 24 hours)

Species	Saccostrea cucullata			Isognomon ephippium			Amussium pleuronectes			Katalysia hiantina		
Dates	Rates	Death time	Death ratio	Rates	Death time	Death ratio	Rates	Death time	Death ratio	Rates	Death time	Death ratio
14 Jan.	Recovery		0/3	Recovery		0/3	Recovery		0/3	Recovery		0/3
11 Feb.	Recovery		0/3	Recovery		0/3	Recovery		0/3	Recovery		0/3
10 Mar.	Recovery		0/3	Recovery		0/3	Tired		0/3	Recovery		0/3
21 Apr.	Recovery		0/3	Recovery		0/3	Recovery		0/3	Recovery		0/3
19 May	Recovery		0/3	Recovery		0/3	Tired		0/3	Recovery		0/3
15 Jun.	Recovery		0/3	Recovery		0/3	Tired		0/3	Recovery		0/3
7 Jul.	Recovery		0/3	Recovery		0/3		6:00	3/3	Recovery		0/3
10 Aug.	Recovery		0/3	Recovery		0/3	Tired		0/3	Recovery		0/3
7 Sep.	Recovery		0/3	Tired		0/3		18:00	3/3	Recovery		0/3
7 Oct.	Tired		0/3	Tired		0/3		23:30	2/3	Tired		0/3





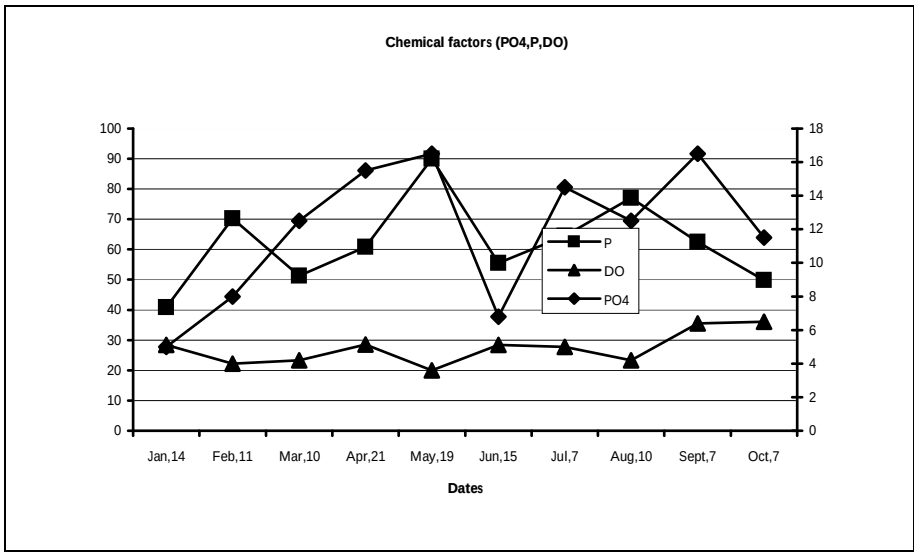


Fig. 1: The chemical and physical factors of the seawater collected at Cua Be area in 1998

- Mice were died within 6 hours with the ratio of 3/3 (3 mice survival among 3 mice assayed) in July, within 18 hours with the ratio of 3/3 in September and within 23 hours 30 minutes with the ratio of 2/3 in October.

3. The environmental factors (listed in table 3)

Table 3: The environmental factors of the seawater collected at Cua Be area (Vinh Truong- Nha Trang), 1998

Dates / Factors	14 Jan.	11 Feb.	10 Mar.	21 Apr.	19 May	15 Jun.	7 Jul.	10 Aug.	7 Sep.	7 Oct.	Average
t^0	26.4	26.0	29.5	31.0	31.0	33.0	34.0	31.0	29.0	28.0	29.9
S‰	23.1	23.9	27.9	33.1	33.9	33.0	32.0	34.5	33.3	20.1	29.5
pH	7.50	7.52	7.61	7.80	7.82	8.00	7.95	7.74	7.55	7.80	7.73
NH_3^+(µg/l)	50.0	Trace	Trace	60.0	Trace	Trace	50.0	50.0	60.0	50.0	32.0
NO_2^- (µg/l)	40.0	Trace	12.0	9.0	8.5	5.6	6.8	14.2	8.6	10.2	11.5
NO_3^-(µg/l)	89.0	98.0	93.0	92.0	131.0	128.0	145.0	385.0	297.0	345.0	180.3
PO_4^{3-}(µg/l)	5.0	8.0	12.5	15.5	16.5	6.8	14.5	12.5	16.5	11.5	11.9
N (µg/l)	560.	635.0	585.0	675.0	645.0	625.0	685.0	640.0	585.0	635.0	627.0
P (µg/l)	0	70.3	51.3	60.8	90.0	55.5	64.5	77.0	62.5	49.8	62.2
DO(mgO_2/l)	40.8 5.12	4.0	4.2	5.14	3.60	5.11	5.00	4.2	6.40	6.5	4.9
Zn (µg/l)	34.1	30.8	36.6	34.6	17.1	17.6	34.5	14.3	12.9	21.7	25.4
Cu (µg/l)	6.4	7.2	8.5	27.2	4.5	2.4	1.5	3.6	1.3	5.9	6.9

IV. DISCUSSION

1. PSP

Our results showed that PSP toxins extracted from Saccostrea cucullata and Isognomon ephippium caused the death of mice more than 60 minutes, out of the readily tabled calculation. However, since the mice had the manifestation of the characteristic symptoms of PSP toxin, so they were observed up to 24 hours. Almost mice which died with the ratio of 3/3 had the death time shorter than that with ratio of 2/3.

The maximum and minimum peaks of the death times of mice caused by PSP extracts from Saccostrea cucullata fluctuated rather rhythmically (Tab. 1, Fig. 2), so it was difficult and vague to explain why it was. It needs to be studied further.

As for Isognomon ephippium, although the death times of mice were longer than those of Saccostrea cucullata, more clearly. The PSP accumulation of Isognomon ephippium could be divided into 2 periods:

- From March to July.
- From August to October.

PSP toxin of this species was accumulated highly in the second month of each period (April and September) (Tab. 1, Fig. 2). This result had the trend rather similar to that obtained in 1997. Concretely, in 1997 there were also two high peaks of PSP toxins from the extracts of Isognomon ephippium, but later than a month at each high peak (May and October) and the concentrations of PSP were rather higher compared to the results of 1998. (Do Tuyet Nga et al., 1999a).

Both Saccostrea cucullata and Isognomon ephippium accumulated high content of PSP in April, August and September. In these months, the environmental factors as NH_3, temperature and salinity were high (Tab. 3, Fig. 1).

The results obtained in this study (1998) and even in last study (1997) (Do Tuyet Nga et al., 1999a) at Cua Be area both showed that:

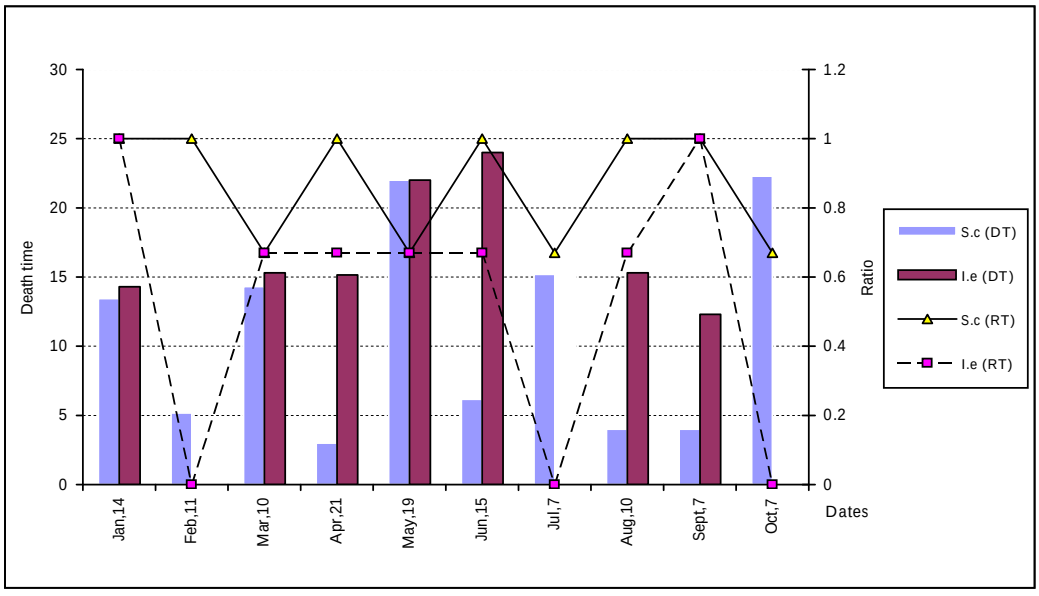
- There have been the appearances of PSP - accumulating bivalves but toxic level accumulated in them was low, so these collected bivalves could be seen relatively clean in terms of PSP infection in the time of our study.

- S. cucullata and I. ephippium were the species, which accumulated PSP more sensitive than A. pleuronectes and K. hiantina.

Nevertheless, the PSP - accumulating level of S. cucullata and I. ephippium collected in 1998 was lower than in 1997.

2. DSP toxins

Until July, A. pleuronectes just caused the death of mice. The death time of mice in July was shortest (6 hours) with the ratio of 3/3; 18 hours with the ratio of 3/3 in September and 22 hours 30 minutes with the ratio of 2/3 in October (Tab. 2, Fig. 3). The time when Amussium pleuronectes accumulated DSP toxin coincided with the results of some researchers (Helle Ensholm et al., 1996; Shigeru Sato et al., 1996). Among 4 species which were analysed, A. pleuronectes was the most sensitive to DSP. A. pleuronectes accumulated high content of DSP toxin in July. That was the time when the water temperature, nitrogen were the highest, NH_3 was also high (50 g/l) although NH_3 was just a trace in the previous month (Tab. 3, Fig. 1).



Note: S.c.: Saccostrea cucullata, I.e.: Isognomon ephippium, DT: death time, RT: ratio

Fig. 2: Affection of PSP on bioassayed mice

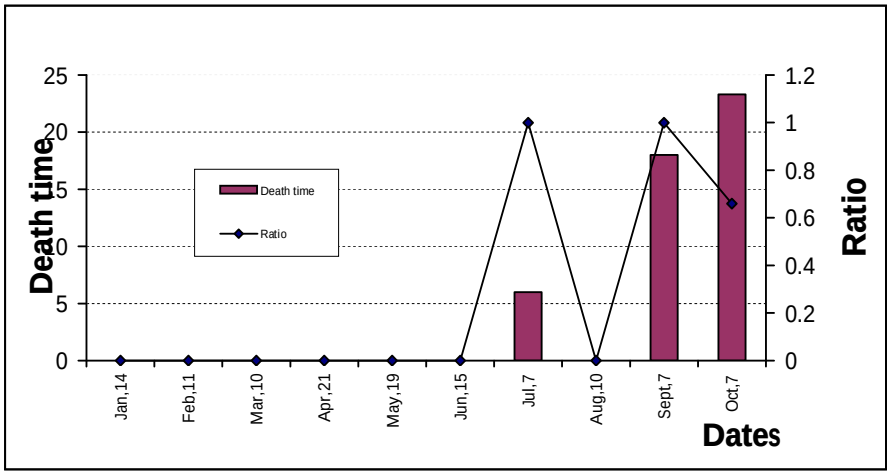


Fig. 3: Affection of DSP extracted from Amussium pleuronectes on the bioassayed mice

Results of DSP obtained in this year (1998) were similar to that in last year (1997) (Do Tuyet Nga et al., 1999b), concretely as:

- A. pleuronectes was sensitive to accumulate the DSP toxin.

- The time when A. pleuronectes accumulated the highest DSP toxin happening in late Summer- Autumn in both 1997 and 1998.

In general, our results of investigation of PSP and DSP in Bivalves collected at Cua Be area in 1997 and 1998 showed that there was already the presence of algae causing these toxins, but such a low density has not been able to cause the bloom of phytotoxin in bivalves. The toxic level of PSP toxin of 4 species of bivalve can be seen negative, but the toxic level of DSP toxin of A. pleuronectes was noticeable during the months from June to October.

V. CONCLUSION

Based on the results of collection of bivalve and seawater at Cua Be area in 10 months in combination with the obtained results in 1997, we affirmed the role of bivalves in the accumulation of PSP and DSP toxins and even in the revelation of the environmental reality in the above time as follows:

- S. cucullata and I. ephippium were sensitive to the PSP accumulation. The times for high PSP concentration were April-May and September-October every year. However, the PSP- accumulating level in S. cucullata and I. ephippium was within the safe limit for food.

- A. pleuronectes was sensitive to accumulate the DSP toxin, high DSP concentration during July- October every year. Therefore, the use of this species for food in these months should be paid more carefully.

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