

## Cockle (*Anadara granosa*) Tolerance to Ammonia Exposed to Various Concentrations

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**Abstract :** Cockles with sizes ranging from 30 -38 mm were exposed to various concentrations of ammonia to determine the lethal limits of the organisms. In each rectangular plastic container, 5 liter of artificial sea-water was filled containing 0.0 mg/L, 0.1 mg/L, 0.2 mg, 0.3 mg/L, 0.4 mg/L, 0.5 mg/L, 0.6 mg/L, 0.7 mg/l and 0.8 mg/L of total ammonia. The concentrations were prepared from the ammonia liquor containing 25% free ammonia. The temperature was at 29°C and salinity at 27 ppt and kept constant throughout the test. The water pH was noted between 8.3-8.5. With known temperature and pH, the values of NH<sub>3</sub> were calculated. Using SPSS's Probit analysis, the value of LC<sub>50</sub> at 48-h was 0.08 mg/L of NH<sub>3</sub> whereas at 96-h, the value was 0.04 mg/L. In the 48-h duration most cockles mortality occurred before the 32-h. The 100% mortality occurred for cockles exposed to concentrations between 0.08 mg/L– 0.13 mg/L before the 84-h. At 0.06 mg/L about 60% mortality was recorded at 92-h and the rest of the concentrations were 0.05 mg/l, 0.03 and 0.02 mg/L with 30%, 23% and 20% mortality respectively. The LC<sub>100</sub> at 96-h and at 48-h exposure was 0.16 mg/L and 0.3 mg/L respectively.

**Keywords:** cockles, ammonia, tolerance, mortality, Probit analysis

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### I. Introduction

The *in situ* study on the relationship between ammonia concentrations and cockles (*Anadara granosa*, blood cockles) in Sg Buluh, Selangor farm areas recorded high ammonia concentration causing the declining production of cockles [1]. The common location of cockles farming areas were closed to the river mouth where upstream water discharged into the sea was mostly polluted by effluents from factories, farms, plantations, houses and towns. Between Kuala Selangor estuary and Sg Tambak Jawa estuary, there were 50 lots of cockles farm each measuring approximately 50 hectares of area. It was the project initiated by state government to increase cockles' productivity simultaneously providing opportunity for some fishermen to improve their livelihood [2]. At present, a total of 5,263 hectares of coastal land are being utilized as cockle farms in the state of Selangor [3]. Although the farm areas nationwide have been expanded in recent years, the production in term of per unit area showed a declining trend from year 2000 to 2010 indicating a significant environmental threat to cockles [4].

The ammonia contained in water is in the form of un-ionized ammonia NH<sub>3</sub> (or simply NH<sub>3</sub>) and ammonium ions NH<sub>4</sub> [5]. The combination of both NH<sub>3</sub> and ammonium is commonly indicated as total ammonia which most methods of ammonia measurement are giving. The NH<sub>3</sub> is the most toxic substance than the NH<sub>4</sub> and its value increases with the increase in temperature and pH [6]. It is therefore a mandatory procedure to record the changes in temperature and pH if the interest is to find out the value of NH<sub>3</sub>. It can be obtained by using the Table 1.0 with known temperature and pH using the formula NH<sub>3</sub> = total ammonia x percent un-ionized [7]. The NH<sub>3</sub> is so toxic that at 0.02 mg/L it will kill some marine organisms [8].

The task to identify the main reason causing cockles declining production in Sg Buluh farm areas is challenging. Cockle depletion can be triggered by many factors such as pollutions [9], bad farm management [10] or predators [11] [12] [13]. Lately, it was found that most cockle beds are infertile and need to be replenished with fertilizers or detritus [14].

**Table 1.0:** Calculated from data in Emmerson et al [7].

pH	Temperature													
	42.0 (°F)	46.4	50.0	53.6	57.2	60.8	64.4	68.0	71.6	75.2	78.8	82.4	86.0	89.6
	6 (°C)	8	10	12	14	16	18	20	22	24	26	28	30	32
7.0	.0013	.0016	.0018	.0022	.0025	.0029	.0034	.0039	.0046	.0052	.0060	.0069	.0080	.0093
7.2	.0021	.0025	.0029	.0034	.0040	.0046	.0054	.0062	.0072	.0083	.0096	.0110	.0126	.0150
7.4	.0034	.0040	.0046	.0054	.0063	.0073	.0085	.0098	.0114	.0131	.0150	.0173	.0198	.0236
7.6	.0053	.0063	.0073	.0086	.0100	.0116	.0134	.0155	.0179	.0206	.0236	.0271	.0310	.0369
7.8	.0084	.0099	.0116	.0135	.0157	.0182	.0211	.0244	.0281	.0322	.0370	.0423	.0482	.0572
8.0	.0133	.0156	.0182	.0212	.0247	.0286	.0330	.0381	.0438	.0502	.0574	.0654	.0743	.0877
8.2	.0210	.0245	.0286	.0332	.0385	.0445	.0514	.0590	.0676	.0772	.0880	.0998	.1129	.1322
8.4	.0328	.0383	.0445	.0517	.0597	.0688	.0790	.0904	.1031	.1171	.1326	.1495	.1678	.1948
8.6	.0510	.0593	.0688	.0795	.0914	.1048	.1197	.1361	.1541	.1737	.1950	.2178	.2422	.2768
8.8	.0785	.0909	.1048	.1204	.1376	.1566	.1773	.1998	.2241	.2500	.2774	.3062	.3362	.3776
9.0	.1190	.1368	.1565	.1782	.2018	.2273	.2546	.2836	.3140	.3456	.3783	.4116	.4453	.4902
9.2	.1763	.2008	.2273	.2558	.2861	.3180	.3512	.3855	.4204	.4557	.4909	.5258	.5599	.6038
9.4	.2533	.2847	.3180	.3526	.3884	.4249	.4618	.4985	.5348	.5702	.6045	.6373	.6685	.7072
9.6	.3496	.3868	.4249	.4633	.5016	.5394	.5762	.6117	.6456	.6777	.7078	.7358	.7617	.7929
9.8	.4600	.5000	.5394	.5778	.6147	.6499	.6831	.7140	.7428	.7692	.7933	.8153	.8351	.8585
10.0	.5745	.6131	.6498	.6844	.7166	.7463	.7735	.7983	.8207	.8408	.8588	.8749	.8892	.9058
10.2	.6815	.7152	.7463	.7746	.8003	.8234	.8441	.8625	.8788	.8933	.9060	.9173	.9271	.9389

Pollution factors can be considered significant contributor towards cockles declining production since it is well known that Sg Buluh river is heavily polluted although less affected by heavy metals [15]. The ammonia concentration in Sg Buluh river was between 0.3 mg/L to 4 mg/L indicating the concentration had exceeded the cockle maximum tolerance level towards ammonia [1]. NH<sub>3</sub> is always be associated with aquatic organisms slowed growth [16] [17], reduced reproduction [18] [19] or death [20]. In this laboratory study, live cockles were exposed to concentrations of ammonia ranging from 0.1 mg/L to 0.8 mg/L to determine its values at LC<sub>50</sub> and LC<sub>100</sub> at 48-h and 96-h durations. With known pH and temperature, the values of NH<sub>3</sub> were calculated.

## II. Materials And Methods

The artificial sea water at 27 ppt was prepared by dissolving 4.2 kg of commercial sea salt in 140 liters of water. The various concentrations of ammonia for the test were prepared from the stock of 25% free ammonia solution and diluted to 10 mg/L of ammonia. The salicylate method was used to measure the ammonia using HACH DR/890 colorimeter [21]. The dilution formula to obtain the required concentrations is thus,  $V_1 = V_2 \cdot M_2 / M_1$  where M<sub>1</sub> is the initial concentration, V<sub>1</sub> is the initial volume, M<sub>2</sub> is the final concentration and V<sub>2</sub> is the final volume [22]. The ammonia concentration for the controls was 0.0 mg/L. In each replicate container, 30 healthy adult cockles with sizes ranging from 30 mm to 38 mm were used and well aerated. The test design is shown in Table 2.0 below.

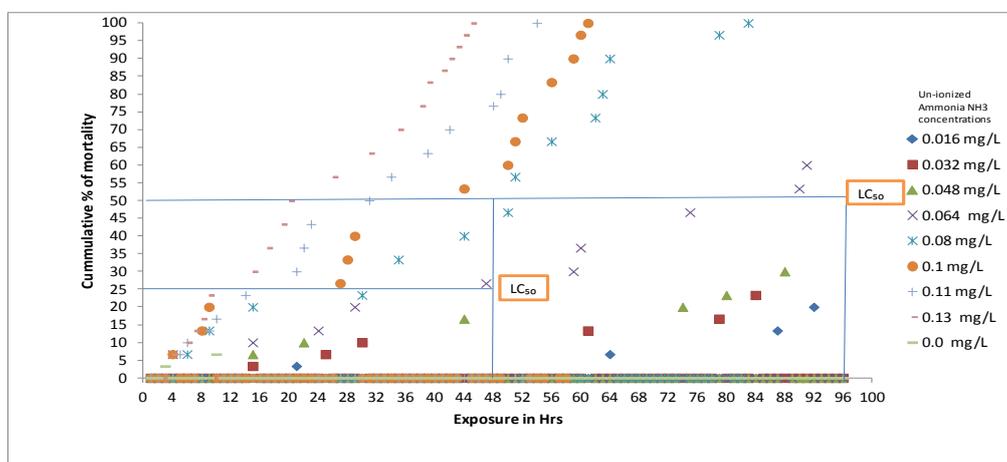
**Table 2.0:** Test design

V <sub>1</sub> (ml)	Volume of salt water added (ml)	V <sub>2</sub> (ml)	M <sub>2</sub> (mg/L)	Number of replicates	Number of cockles in each replicate
50	4950	5000	0.1	3	30
100	4900	5000	0.2	3	30
150	4850	5000	0.3	3	30
200	4800	5000	0.4	3	30
250	4750	5000	0.5	3	30
300	4700	5000	0.6	3	30
350	4650	5000	0.7	3	30
400	4600	5000	0.8	3	30
0	5000	5000	0	3	30

The duration of exposure to ammonia was 96 hours and observation recorded hourly. Cockles were not fed during the observation to reduce ammonia production/interference [23]. The death of the cockles was confirmed by its inability to close the valves upon mechanical stimulus such as touching it with glass rod [24] and immediately removed from the container. The essential water parameters such as pH, dissolved oxygen, temperature and salinity were recorded and whenever possible kept constant. Suitable water parameters for cockles were referred; salinity 25-30 ppt [25], temperature 25<sup>0</sup>C-32<sup>0</sup>C [12], pH 7.1-8.5 [26] and oxygen above 7 mg/L [27]. Probit analysis as in IBM SPSS Statistics 20 was performed to obtain the values of ammonia for LC<sub>50</sub> and LC<sub>100</sub> at 48-h and 96-h.

### III. Results And Discussion

During the 96-h observation, the recorded pH values ranged from 8.3 to 8.5, average dissolved oxygen was 7.9 mg/L, temperature at 29°C and salinity at 27 ppt. For the estimation of NH<sub>3</sub>, the pH value was taken as an average value of 8.4. Using % NH<sub>3</sub> table as provided, at 29°C and pH 8.4, the % NH<sub>3</sub> is 15.9. Fig. 1.0 shows the cumulative % of mortality against hourly observation and NH<sub>3</sub> concentrations.



**Figure 1.0:** Cumulative cockles mortality exposed to various NH<sub>3</sub> concentrations at 29°C and pH 8.4

Using Probit analysis in the SPSS, the LC<sub>50</sub> at 48-h exposure was 0.08 mg/L of NH<sub>3</sub>, whereas at 96-h, the value was 0.04 mg/L. In the 48-h duration most cockles mortality occurred before the 32-h. The 100% mortality occurred for cockles exposed to concentrations between 0.08 mg/L– 0.13 mg/L before the 84-h. At 0.06 mg/L about 60% mortality was recorded at 92-h and the rest of the concentrations were 0.05 mg/L, 0.03 mg/L and 0.02 mg/L with 30 %, 23% and 20% mortality respectively. The LC<sub>100</sub> at 96-h and at 48-h exposure was 0.16 mg/L and 0.3 mg/L respectively.

The temperature of the coastal water of Kuala Selangor had been recorded between 29.21°C and 30.9°C and pH between 7.14 and 8.05 [28]. On the other hand, Ramli, Abu Hassan and Saadon [1] in their study on the cockles production in Sg Buluh (about 6.5 nm south of Kuala Selangor), had recorded the average ammonia between 0.3 mg/L and 4.0 mg/L during the months of January till June , 2013. If the temperature is taken as an average of 30°C and pH 7.7, the lowest NH<sub>3</sub> is estimated at 0.012 mg/L and the highest is 0.16 mg/L in the Selangor’s waters. To illustrate the water quality status in relation to its uses, the Department of Environment Malaysia has published two standards; the Interim National Water Quality Standards (INWQS) which categorized river water into five classes based on the nutrient values (see Table 3.0 and Table 4.0) and Water Quality Index (WQI) which is an opinion-poll formula [29] summarizing water quality into three groups namely ‘clean, slightly polluted and polluted’ (see Table 5.0). With known total ammonia/un-ionized ammonia NH<sub>3</sub> values of Selangor’s water, it can be inferred that in general, the quality is in the poor state and with limited uses (see Table 6.0). Except in March (slightly polluted), the rest of the months were considered polluted with SIAN less than 41.

**Table 3.0:** Nutrient values found in river water quality classification system

Parameters	unit	Water quality class				
		I	IIA	IIB	III	IV
Ammonia	mg/L	<0.1	0.1 – 0.3	0.1 – 0.3	0.3 – 0.9	>2.7
Nitrate	mg/L	Natural Value	<7.0	<7.0	-	<5.0
Phosphate	mg/L	Natural Value	<0.2	<0.2	<0.1	-

Source: Department of Environment, Malaysia -River Water (Surface water) quality monitoring Programme, 2005

**Table 4.0:** Classification of rivers Interim National Water Quality Standards (INWQS)

Class	Fuction	The level of water Treatment
<b>I</b>	Water supply for environmental conservation level I Fishery I - very sensitive aquatic species	No Treatment Needed
<b>IIA</b>	Water supply for environmental conservation level II Fishery II - very sensitive aquatic species	Conventional water treatment is required
<b>IIB</b>	For recreational use	Conventional water treatment is required
<b>III</b>	Water supply for environmental conservation level III Fishery III- aquatic species commonly bred and have economic value, as well as to serve as livestock drinking	extensive water treatment is required
<b>IV</b>	Irrigation	-

Source: Department of Environment -River Water (Surface water) quality monitoring Programme, 2005

**Table 5.0:** DOE Water Quality Classification Based on Water Quality Index

Parameters	index Range		
	Clean	Slightly Polluted	Polluted
SIBOD	91 – 100	80 – 90	0 – 79
SIAN	92 – 100	71 – 91	0 – 70
SISS	76 – 100	70 – 75	0 – 69
WQI	81 – 100	60 – 80	0 – 59

**Table 6.0:** Un-ionized ammonia NH<sub>3</sub> concentrations for months January till June 2013 in Sg Buluh (at average temperature 30<sup>0</sup> C and pH 7.7)

Month	Average Total Ammonia (mg/L)	Un-ionized Ammonia NH <sub>3</sub> (mg/L)	% mortality	Interim National Water Quality Standards (INWQS)	Index range, Ammoniacal nitrogen (AN) or total ammonia-SubIndex for AN (SIAN)**
Jan.	1.4	0.056 ≈0.06	60% mortality at 92-h	Class IV, Irrigation	39-polluted
Feb.	1.4	0.056 ≈0.06	60% mortality at 92-h	Class IV, Irrigation	39-polluted
Mar.	0.3	0.012 ≈0.01	Expected 100% survival	Class II- • Conservation & Recreational	71–slightly polluted
Apr.	1.4	0.056 ≈0.06	60% mortality at 92-h	Class IV, Irrigation	39-polluted
May	1.3	0.052 ≈0.05	30% mortality at 88-h	Class IV, Irrigation	41-polluted
Jun.	4.0	0.16 ≈0.2	Expected 100% mortality at 96-h	Class V, None of the other classes	-0.5-polluted

\*\* Subindex for AN : SIAN

100.5 - 105x for x ≤ 0.3

94e-0.573x - 5 x - 2 for 0.3 < x < 4

The calculation of SIAN as in Zaki [30].

#### IV. Conclusion

Water pollution in Malaysia is still a persistent problem although there are a handful of laws and regulation explicitly tailored to curb the perpetrators from polluting the environment. Unfortunately, major source of ammonia may come from the sewage treatment plants managed by Indah Water Consortium (IWK) [31], the government-owned company that its main responsibility is to ensure that wastewater is treated before being discharged into the rivers [32]. In early 2014, however, two major wastewater treatment plants in Selangor have been temporarily shut down due to high levels of ammonia in the discharged water [33]. Although the report did not specify the owner of those plants, it is believed that they belonged to IWK being the sole operator of the wastewater treatment plants in the state of Selangor. It is due time that a comprehensive study on the effect of allowable discharged ammonia to the water quality of rivers and sea to be carried out to determine new standard level that may be more tolerable to aquatic life. Currently, the water used to culture cockles in Selangor is under polluted category.

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