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Original paper

ACCUMULATION OF ALUMINIUM IN THE TISSUE OF GIANT FRESH WATER PRAWN (*Macrobrachium rosenbergii* de Man) EXPOSED TO ACIDIC WATER CONTAMINATED WITH ALUMINIUM SALT

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ABSTRACT

Aluminium is known as competitive trivalent and its occurance in an acidic environment causes ionic disturbances in aquatic invertebrates and crustaceans. An investigation was conducted to determine the accumulation of aluminium in the tissue of giant fresh water prawn (M. rosenbergii de Man) exposed to acidic water (pH 5.0) and more alkaline media (pH 6.5) contaminated by aluminium salt (0.3 mg/l of nominal concentration of Al). A static test with regular water exchange was employed during the experiment.

The first moulting was recorded in all treatments at the first week of the investigation. Normal moulting period, i.e. 6 – 8 days after the first moulting was observed in 55% of prawns in the media with normal pH (pH 6.5). A longer period, more than 10 days, was needed by prawn in the media at pH 6.5 with 0.3-mg/l aluminium, pH 5.0 and at pH 5.0 with 0.3 mg/l aluminiun. The third moulting was only recorded at prawn in media at pH 6.5. The elevated aluminium in the acidic media caused the highest mortality rate and there was no mortality recorded at normal pH. Most of the mortality was observed before and soon after moulting.

The elevation of 0.3 mg/l aluminium in the more acidic water (pH 5.0) increased the aluminium and decreased the calcium concentrations in the prawn tissue. However, the magnesium in the prawn's tissue showed its highest consentration at pH 5.0 with 0.3-mg/l aluminium.

The decrease of calcium concentration in the prawn's tissue was always followed by the increase of concentration of aluminium significantly ($P < 0.01$). This suggests that the aluminium interfers the intake of calcium from the media by the prawn. However the magnesium intake was not affected. As a conclusion, the elevated level of aluminium in the acidic media increased the accumulation of aluminium in the prawn's tissue and influenced the moulting behaviour of the tested prawn by interfering the absortion of calsium and magnesium, i.e. decreasing the calcium and increasing the magnesium concentrations in the prawn tissue.

Key words: water acidification; aluminium toxicity; fresh water prawn; accumulation of aluminium

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INTRODUCTION

Acid deposition, a form of several kinds of air pollution, induces serious damages to the environment as a whole (Elsom, 1992). The acidity of the water body influences the leaching of some metals such as calcium, magnesium and aluminium.

Aluminium is the third common metal, accounting for 8% of the earth's crust

(Cross, 1998). One of the most important acidification of soil water system is mobilization of aluminium from the edahapic to the aquatic environment (Cronan and Schofield, 1979; Seip *et al*, 1989 *In* Lydersen *et al*, 2002). It has been found to be toxic to a wide range of fish and invertebrate species (O'Donell *et al*, 1984 *In* Cross, 1998).

Acid water creates the reduction of species diversity, leads to the decrease of biomass and production in freshwater environment, and the production of fish and other organisms are substantially reduced (Mason, 1991; Laws, 1993). In addition, the effects of acid water have been reported to cause the depletion of fish population in temperate water bodies (Wellburn, 1988; Howells, 1990; Mason, 1991; Elsom, 1992). Although the decrease of the fish population may be caused by several factors, such as food availability and other water pollution, however, in relation to low pH of water, acid deposition could be considered to be the main cause.

Several studies have been done on the acute and chronic effects of aluminium on temperate fish. The concentration of aluminium beyond tolerable limit to most fishes was reported to be in the range of 0.2 – 0.5 mg/l approximately (Driscoll *et al*, 1980). The aluminium concentration of 0.42 mg/l at pH 5.0 was found to kill 50% of brook trout after 115 hours exposure (Driscoll *et al*, 1980).

The acute toxic action of aluminium is thought to be mainly due to the precipitation of aluminium hydroxide in the gill membrane, resulting gill damage, disturbance of sodium regulation mechanisms at the fish gills. In addition, it increases mucus production of the gill results in gill clogging, anoxia, impaired ion exchange across the gill membrane which cause a rapid loss of body sodium (McWilliam, 1982; Howells, 1990). At low concentration, it inhibited the fish growth and damaged the reproductive system (Howell, 1990).

As in all crustaceans, the growth *M. rosenbergii* is only possible by periodic shedding of exosceleton, moulting. In juvenile stage, moulting most commonly occurs every 4 – 6 days (Bardach *et al*, 1973). Immediately before and after moulting, water is absorbed into the body of animals to expand the soft new cutical. This allows the absorbtion of dissolved substances in the water into the body (Robertson, 1937; Lockwood, 1958). This means that the animals are particularly susceptible to pollutant during moulting as shown by the results of Fingerman and Fingerman (1974) that an industrial pollutant, Archlor 1242, had a moulting inhibitory effect on fidler crab (*Uca pugilator*). Dethletsen (1978) also found that the brown shimp (*Crangon crangon*) increased its susceptability to mercury and cadmium at the moult, and acute toxicity of copper on cray fish resulted the death of this animal at the act of ecdyses.

It has also been oberved that prawns in ponds such as *P. monodon*, *P. merguensis*, *Metapenaeus monoceros*, and *M. brevicornis* grown in the brackishwater pond in a potential acid sulphate soil areas have a low yield with a large number of soft shell animals (Poernomo, 1978; Simpson and Pedini, 1985). It was thought that the cause of those incident was the acidic condition of the water, the lack of essential minerals for shell formation and the presence of metal, such as aluminium as the most common metal in water draining acid sulphate soil.

Many studies on the toxicity of aluminium on temperate fish and crustaceans have been done (Dethletsen, 1978; Fingerman and Fingerman 1974; Driscoll *et al*, 1980; McWilliam 1982; Appelberg 1985; Howells, 1990). However, very little has been known about the effect of aluminium on warm water crustaceans, especially giant fresh water prawn.

The objective of this study was to find out the effect of aluminium on giant fresh water prawn (*M. rosenbergii*) exposed to an acidic water contaminated by aluminium, and the acummulation of this metal in the prawn tissue.

MATERIALS AND METHODS

Experimental animals

Juveniles of fresh water prawn (*M. rosenbergii*) from the same batch obtained from local hatchery near Semarang with mean body weight of 0.075 gram (± SD 0.03) were reared in the laboratory with a static water exchange. Prawns were adapted to the laboratory condition for two weeks prior to experiment. A limited feed (prawn pellet) was given during the study.

Experimental conditions

In the laboratory, the tested prawn was kept individually in the happa of 7.5 cm in diameter and 20 cm height. The happas were kept in the plastic tank of 35 x 23-x 20 cm. Each plastic tank contained 12 happas that accommodated 12 juveniles. Aeration was given during the experiment.

A standard dilution water of a known hardness (30 mg/l total hardness as $CaCO_3$) and pH (pH 5.0 and 6.0) was used to dilute the test material. This water was prepared following the recipe from HMSO, 1969 *In* Stirling (1985). Three kinds of stock solution (Table 1) were prepared and each was made up to 1 (one) litre by adding distilled water.

Tabel 1. The stock solution used for dilution water (HMSO, 1969 *In* Stirling (1985)

Stock Solution Number	Chemicals components	Amount (g)
1.	$CaCl_2$. 6 H_2O	320
	NaCl	29
	$NaNO_3$	9
2.	$MgSO_4$	151
	Na_2 SO_4	79
3.	$NaHCO_3$	27.5

To make up the dilution water of 30 mg/l total hardness as $CaCO_3$, 0.75 ml solution 1 + 0.75 ml solution 2 + 0.75 ml solution 3 were added in to 10 liters deionized water.

Analar Grade aluminium nitrate, Al $(NO_3)_3$. 9 H_2O, was used as aluminium source. The stock solution was prepared by diluting 13.9 mg Al $(NO_3)_3$. 9 H_2O in 1000 ml distilled water, so that 1 ml of stock solution contained 1-mg aluminium. The amount of aluminium stock solution added in the media, as treatment was known as nominal concentration. After adding aluminium in the water, a sample water was taken and filtered using 0.45 µm GF / C in a milllipore filter. The concentration of aluminium in the filtered water was the soluble aluminium, whereas the concentration of aluminium in the unfiltered water was the total of aluminium. The total and soluble concentration of aluminium in the water were analyzed by using Colorimetric Dougan and Wilson (1974) method. The absorbance was measured at 585 nm against reagent blank in dual beam spectrophotometer.

Treatments

A 21 day investigation of accumulation of aluminium in the prawn tissue at exposed to acidic water was performed. A Completely Randomized Design with four treatments and three replicates was applied in this experiment. The four treatments were:

Table 2. The four treatments applied in the experiment

TREATMENTS				
Code	**pH Levels**	**Nominal Concentration of Aluminium (mg/l)**	**Total Concentration of Aluminium (mg/l)**	**Soluble Concentration of Aluminium (mg/l)**
A	5.0	0	0.0078	0.0046
B	5.0	0.3	0.0970	0.0730
C	6.5	0	0.0037	0.0025
D	6.5	0.3	0.0780	0.0340

The nominal concentration of aluminium (0. 3 mg/l) was the sub lethal concentration obtained from a preliminary finding test. In this case, $LC_{50\text{-}96\ hours}$ of aluminium on *M. rosenbergii* at pH 5.0 at the water hardness of 30 mg/l $CaCO_3$ was 0.253 mg/l (total aluminium) and 0.155 mg/l (soluble aluminium) (Rejeki, Unpublished data).

Data Collection

The prawn behaviour, mortality and moulting were observed daily during the experiment. The accumulation of aluminium in the prawn body from each treatment was collected at the end of the investigation, i.e. after 21 days, similarly for the calcium and magnesium concentration.

At the end of the investigation living prawns from each treatment were killed, washed and frozen. All prawns were weighed, freeze dried and reweighed to determine the constant dry weight prior to the digestion. The dry prawn from each treatment was kept in the glass tube and digested by adding 5 ml of Aristar Nitric Acid, left overnight and heated until fully digested. The digested solution was made up to 25 ml distilled water.

The aluminium concentration in the prawn body was analysed by using Graphite Furnace Atomic Absorbtion Spectrophotometer. While the calsium and magnesium concentration in the tissue were analysed following Stirling (1985)

The concentration of aluminium, calsium and magnesium in the prawn tissue were calculated as follows:

$$\text{Concentration in the prawn tissue} = \frac{C \times d}{1000 \times W}$$

C = Concentration in the digested sample (mg/ml)
d = Volume of digested sample (ml);
W = Dry weight of prawn (mg)

The water quality parameters, i.e. pH, temperature, dissolved oxygen, total hardness and aluminium concentration in the tested media were collected daily before and after water changes.

Data Analyses

The data of aluminium, calsium and magnesium concentrations in the prawn tissue were analyzed by using Analysis of Variance (ANOVA) to find out the differences in the treatments. To find out the differences between treatments, a Duncan Multiple Range Test was used.
The prawn behaviour, mortality and moulting were descriptively analyzed.

RESULTS

The Aluminium, Calcium and Magnesium Concentrations in The Prawn's Tissue

The following results (Table 3) show that low pH and elevated level of aluminium (0.3 mg/l nominal concentration) in the media gave an opposing effects on the aluminium (Al^{3+}), calcium (Ca^{2+}) and magnesium (Mg^{2+}) concentrations in the prawn tissue with a decrease of calcium and increase of magnesium concentration at pH 5.0 with and without aluminium (treatment A and B). The accumulation of aluminium in the prawn tissue was also found to be affected by pH level and aluminium in the media.

Table 3. The Mean Value of Aluminium (Al^{3+}), Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) Concentrations in the tissue of *M. rosenbergii* de Man (mg/g dry body weight) After 21 Days Investigation (mean value ± SD)

Treatments	Al^{3+}	Ca^{2+}	Mg^{2+}
A (pH 5.0)	0.085 ± 0.010	29.39 ± 0.383	1.87 ± 0.232
B (pH 5.5 + 0.3 Al)	1.800 ± 0.121	24.14 ± 0.359	2.06 ± 0.070
C (pH 6.5)	0.050 ± 0.014	47.10 ± 0.143	1.60 ± 0.174
D (pH (6.5 + 0.3 Al)	1.160 ± 0.062	41.55 ± 0.181	1.80 ± 0.081

The Aluminium (Al^{3+}) Accumulation In The Prawn's Tissue

The aluminium (Al^{3+}) accumulation in the prawn tissue showed its highest at treatment B (pH 5.0 + 0.3 mg/l nominal concentration of Aluminium), i.e. 1.8 mg/g dry body weight and the lowest was found at treatment C (pH 6.5), i.e. 0.05 mg/g dry body weight (Table 4)

From the calculation of Table 4 was also noted that the addition of 0.3 mg/l aluminium in the more acidic water (pH 5.0) increased the aluminium concentration in the prawn tissue by 95 %. However, the elevation of 0.3 mg aluminium in the water increased the concentration of aluminium in the prawn tissue by 35 % when water pH dropped from 6.5 to 5.0

The Analyses of Variance of aluminium (Al^{3+}) accumulation in the prawn tissue (Table 5) shows a highly significant difference in the treatments ($P < 0.01$). The Duncan Multiple Range Test (Table 6) also shows that elevation of aluminium by 0.3 mg/l in the media at pH 5.0 and at pH 6.5 affected the accumulation in the prawn tissue significantly (treatment B is highly significant to treatment A and C; D is highly significant to C; treatment B showed a significant difference to treatment D ($P < 0.01$).

Table 4. Aluminium (Al^{3+}) Accumulation in the Tissue of *M. rosenbergii* de Man (mg/g dry body weight) After 21 Days Investigation

REPLICATES	TREATMENTS			
	A	B	C	D
1	0.093	1.93	0.046	1.14
2	0.087	1.78	0.085	1.23
3	0.074	1.69	0.038	1.11
Average ± SD	0.085 ± 0.010	1.800 ± 0.121	0.050 ± 0.014	1.16 ± 0.062

Table 5. Analyses of Variance Aluminium (Al^{3+}) Concentration in the Tissue of *M. rosenbergii* de Man

HS	FD	ST	MS	F Calc.	F Tables	
					0.05	0.01
Treatments	3	6.605	2.202	463.509**	4.07	7.59
Error	8	0.038	0.005			
Total	11	6.642				

****)** $F_{Calc.} > F_{Tables}$ (0.01) → highly significant

Table 6. Multiple Range Duncan Test of Aluminium (Al^{3+}) Concentration in the Tissue of *M. rosenbergii* de Man

Treatments	Mean Value	Difference			
D	1.18	B			
B	1.16	0.64**	D		
A	0.085	1.715**	1.075**	A	
C	0.05	1.75 **	1.11**	0.035 **	C

The Aluminium concentration in the prawn's tissue shows a highly significant different between treatments ($P < 0.01$)

The Calcium (Ca^{2+}) Accumulation In The Prawn's Tissue

The calcium (Ca^{2+}) concentration shows its highest at treatment C whereas the lowest was at treatment B (Table 7). Table 8 shows that there are highly significant differences at the level of 0.01 in the treatments.

The different pH level and elevation of 0.3 mg/l aluminium in the media at different pH levels also affected the concentration of calcium in the prawn's tissue (Table 9).

It was found that the elevated aluminium in the more acidic media (pH 5.0) decreased the calcium concentration by 41.55 % compared to similar aluminium concentration in the higher pH level (pH 6.5).

Table 7. Calcium Concentration (Ca^{2+}) in the Tissue of *M. rosenbergii* de Man (mg/g dry body weight) After 21 Days Investigation

REPLICATES	TREATMENTS			
	A	B	C	D
1	29.5	22.84	47.07	41.57
2	28.96	22.71	46.98	41.36
3	29.7	26.86	47.26	41.72
Average ± SD	29.39 ± 0.383	24.14 ± 0.359	47.10 ± 0.143	41.55 ± 0.181

Table 8. Analyses of Variance Calcium (Ca^{2+}) Concentration in the Tissue of *M. rosenbergii* de Man

HS	FD	ST	MS	F Calc.	F Tables	
					0.05	0.01
Treatments	3	1013.191	337.73	234.278 **	4.07	7.59
Error	8	11.5326	1.44			
Total	11	1024.723				

****)** $F_{Calc.} > F_{Tables}$ (0.01) → highly significant

Table 9. Multiple Range Duncan Test of Calcium (Ca^{2+}) Concentration in the tissue of *M. rosenbergii* de Man

Treatments	Mean Value	Difference			
C	47.1	C			
D	41.55	5.55 **	D		
A	29.39	17.71 **	12.16 **	A	
B	24.14	22.96 **	17.41 **	5.25 **	B

The Calcium concentration in the prawn tissue shows a highly significant difference between treatments ($P < 0.01$)

The Magnesium (Mg^{2+}) Accumulation in The Prawn's Tissue

The Magnesium (Mg^{2+}) concentration shows its highest at treatment B followed by treatment D, A and C as shown in the Table 10. From the calculation in the Table 10, it seemed that the elevation of aluminium in the more acidic media (pH 5.0) increased the magnesium in the prawn's tissue by 12 %.

The Analyses of Variance shows a significant difference within treatments at level 0.05 (Table 11), but the Duncan Test (Table 12) shows that there was a highly significant difference between treatment B and treatment C ($P < 0.01$).

Table 10. Magnesium (Mg^{2+}) Concentration in the Tissue of *M. rosenbergii* de Man (mg/g dry body weight) After 21 Days Investigation

REPLICATES	TREATMENTS			
	A	B	C	D
1	1.61	1.98	1.40	1.79
2	2.05	2.11	1.72	1.73
3	1.96	2.09	1.68	1.98
Average ± SD	1.87 ± 0.232	2.06 ± 0.070	1.60 ± 0.174	1.80 ± 0.081

Table 11. Analyses of Variance Magnesium (Mg^{2+}) Concentration in the Tissue of *M. rosenbergii* de Man

HS	FD	ST	MS	F Calc.	F Tables	
					0.05	0.01
Treatments	3	0.325	0.1083	4.514 *	4.07	7.59
Error	8	0.192	0.024			
Total	11	0.517				

***** $F_{Calc.} > F_{Tables}$ (0.05) → significant

Table 12. Multiple Range Duncan Test of Magnesium (Mg^{2+}) Concentration in the Tissue of *M. rosenbergii* de Man

Treatments	Mean Value	Difference			
C	2.06	B			
A	1.87	0.19	A		
D	1.8	0.26	0.17	D	
C	1.6	0.46 **	0.27	0.2	C

The data shows a highly significant difference between treatment B and C ($P < 0.01$).

The Prawn's Behaviour

Prawns in treatments A and B were less active compared to the prawns in treatments C and D. The food given in the tratments C and D was immediately eaten and very a few waste food was found. In contrast, a slower feeding respond was noted in treatments A and B, and therefore, more waste food was observed. Prawns in treatment D had the most active behaviour, and often jumped out from the happa. There was no other clinical or behavioural changes recorded during 21 days of investigation.

Moulting and Mortality

The time taken from the first to the second moulting varied between treatments. First moulting in treatments A, B, C and D were recorded at the first week of the investigation. Fifty five % of prawns in treatment C moulted 6 – 8 days after first moulting. A longer period of time (more than 10 days) was needed by prawns in treatments A, B and D. The lower precentage of second time moulting was observed in treatment B (25%) followed by treatments A (40%) and D (55%). Therefore, the third moulting was only recorded in treatment C.

The highest mortality rate was found in treatment B followed by treatment A and D. No mortality was recorded in treatment C. Most of the mortalities were observed before and soon after moulting. It was found that the prawns were dead before moulting, their exosceleton still covering half of the prawn's body, i.e. around the cephalothorax. On the other hand, prawns dead after moulting had very soft exo-skeleton. It was assumed therefore, that the mortalities were possible due to the failure of shedding and/or hardening the exo-skeleton before and after moulting.

DISCUSSION

The Aluminium Concentrations in The Prawn's Tissue

The results indicated that different water pH affected the accumulation of aluminium in the prawn's tissue. The lower the pH, the higher the accumulation of aluminium in the prawn's tissue. This result may be due to the higher level of soluble aluminium present in the lower pH (Table 2), as mentioned by Cronan and Schofield (1979); Seip *et al*, (1989) *in* Lydersen *et al*, 2002*;* Mason, 1991; Laws, 1993). The higher level of soluble aluminium may be more easily absorbed by the gill and/or the muscle as shown in Table 5, where the increase of pH media from 5.0 to 6.5 reduced the accumulation of aluminium in the prawn tissue significantly ($P < 0.01$).

There are some data from fish experiments, which may be used to compare the present results. Hunter *et al* (1980) found in their investigations that the gill of brown trout (*Salmo truta)* accumulated aluminium of 15.75 mg/g wet weight at pH 4.4 and the accumulation of aluminium in its gill was only 0.275 mg/g wet weight at neutral water; while the accumulation of aluminium in the fish tissue was 2.14 mg/g in acidic media, whereas in the neutral media it was only 1.6 mg/g dry weight.

Calcium and Magnesium Concentrations in The Prawn's Tissue

It had been observed by Muniz and Leivestad (1980)**;** Havas and Hutchinson (1983)**;** Witters *et al* (1984); Appelberg (1985); Havas and Likens (1985), that aluminium is a competitive trivalent and its occurrence in an acidic environment causes ionic disturbances in aquatic invertebrates and crustaceans.

This statement supports the results of this study, in which the increased level of aluminium by 0.3 mg/l nominal concentration in the acidic water reduced the calcium concentration in the prawn's tissue significantly ($P < 0.01$) (Tables 3, 7, 8). It is well known that calcium is very important for moulting processes in crustaceans.

On the contrary, the occurrence of aluminium by 0.3 mg/l nominal concentration in the acidic water increased the magnesium concentration in the prawn's tissue significantly ($P < 0.05$) (Tables 3, 10, 11) as also found by Wickins (1984) which reported an increasing concentration of magnesium in the penaeid prawn's tissue and carapace in a low pH environment.

The results of this study indicates that the decrease of calcium concentration in the prawn's tissue always followed by the increase concentration of aluminium ($P < 0.01$) (Tables 3, 5, 6) and magnesium significantly (Tables 9, 10, 11). This suggests that the aluminium interfered with the intake of calcium from the media by the prawn. However, the magnesium intake was not, due to a stronger reaction of magnesium as mentioned by Muniz and Leivestad (1980) and Hutchinson (1983) that aluminium interfered with the gill ionic balance in fish and crustaceans.

A study by Havaz and Lickens (1985) on fresh water crustacean (*Daphnia magna)* showed that the occurrence of aluminium in an acidic media (pH 5.0) reduced the sodium influx in neutral water (pH 6.5) by 58%, and 46%, respectively. Similarly, the results of Malley and Chang (1985) on cray fish (*Orconectes virilis*) which showed that an elevated of aluminium (0.2 mg/l at pH 5.5) reduced the calcium uptake by 31%, and smaller reduction (15%) was found at a higher pH level (pH 7).

In the present experiments, elevated concentration of aluminium by 0.3 mg/l nominal concentration at pH 5.0

decreased the calcium concentration by 41.55% compared to similar aluminium concentration in the higher pH level (pH 6.5). Those results may particularly explain the occurrence of soft shelled prawn populations in the acid sulphate ponds as mentioned by Poernomo (1979); Simpson and Pedini (1985).

Moulting and Mortality

The poor moulting rate and high mortality in the treatments A, B and D over 21 days exposure time was considered due to the occurrence of aluminium in the media as well as a low pH level. Those results may explain the results of Poernomo (1979); Simpson and Pedini (1985) who found a high mortality of prawns grown in an acid sulphate soil pond after a heavy rainfall due to a sudden reduction of pH of the pond water as well as increase of aluminium leaching from the soil which entered the pond water (Cross, 1998, Ezoe *et al*, 2001; Lindersen *et al*, 2002). They also reported that such a situation was accompanied by a slow growth and soft shell harvest of the surviving prawns.

In normal condition, juvenile of *M. rosenbergii* moults every 4 to 6 days during growth **(**Bardach, 1973**)**. Similar results were found in this present study , in which, the time taken from the first to the second moulting varied between treatments. First moulting in treatments A, B, C and D were recorded at the first week of the investigation. Fifty five % of prawns in treatment C moulted 6 – 8 days after first moulting, while a longer period of time (more than 10 days) was needed by prawns in treatments A, B and D. The lower precentage of second time moulting was observed in treatment B (25%) followed by treatment A (40%) and treatment D (55%). Therefore, the third moulting was only recorded in treatment C. Those results may be due to inhibition of moulting in comparison with prawn in the normal media (treatment C). Similar report on prawn of delayed moulting behaviour was noted in acid sulphate soil pond by Poernomo (1979); Simpson and Pedini (1985).

CONCLUSION

The aluminium concentration in the acidic media increased the accumulation of aluminium in the prawn tissue of *M. rosenbergii*. It also influenced the moulting behaviour of the prawn by interfering the uptake of calcium and magnesium by decreasing the calcium and increasing the magnesium content in the prawn's tissue.

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