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Behaviour of environmental iron, manganese and aluminium in relation to brown/black gill syndrome in *Penaeus monodon* cultured in ponds on acid sulphate soils

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Abstract

Acid sulphate soils are marginally acceptable soils for aquaculture and brown/black gill syndrome is one of the common problems associated with shrimp culture in acid sulphate soils. Unusually high concentrations of iron (up to 1588 $\mu g\ g^{-1}$ dry wt) and manganese (up to 93.2 $\mu g\ g^{-1}$ dry wt) were observed in *Penaeus monodon* cultured on acid sulphate soils in the west coast of Sri Lanka exhibiting concomitant colour changes in gills. A steady increase in iron and manganese levels was observed in gills of the cultured shrimp with culture time. Statistically significant differences were observed for levels of iron ($p < .001$) and manganese ($p < .05$) in the gills of shrimp showing normal gill colour (2 to 5 weeks) and shrimp showing high incidence of brown gills (6 to 10 weeks). Iron in the gills of the shrimp appears to be in the form of hydrated oxides while manganese appears to be in a co-precipitated form with iron oxides.

Introduction

It is estimated that 1,002,300 ha of land are used for shrimp farming in the eastern hemisphere (World Shrimp Farming 1990). The high demand for coastal land for shrimp culture has resulted in many shrimp farms being constructed in South East Asia in areas with potential acid sulphate or acid sulphate soils.

Pyrite (FeS_2) is the main chemical compound responsible for acid sulphate soil conditions and has a high concentration of iron (Dent 1986). In

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addition acid sulphate soils also contain high concentrations of manganese and aluminium compounds. All these metal compounds undergo several oxidation and reduction processes under the influence of the redox potential and pH conditions of their immediate environment (Ponnamperuma et al. 1967; Collins and Buol 1970a; Breeman 1976). Iron and manganese compounds in an acid sulphate environment can vary from soluble Fe^{2+} and Mn^{2+} compounds under relatively low redox potential and acidic pH conditions to complex insoluble hydrated ferric and manganese oxides under relatively high Eh and basic pH conditions (Collins and Buol 1970a and 1970 b; Breeman 1976; Dent 1986).

The management practices employed in shrimp farms can affect the stability of different iron and manganese compounds through their effects on the Eh-pH relationships in the culture environment. The behaviour of penaeid shrimp includes resting on or partially burying in soft sediments, and they are very sensitive to the presence of different iron and manganese compounds in their immediate environment. Iron encrustation on the cuticle of shrimp has been reported by Simpson et al. (1983) and Lightner (1988) in acidic culture conditions. Several pathological changes in shrimps in relation to the presence of environmental iron compounds have been described by Nash et al. (1988) and Nash (1990). A brown or black discolouration of the gills is the most common symptom, a condition which in severe cases leads to death, probably from asphyxiation (Nash et al. 1988). A parallel study (Jayasinghe and Machintosh 1993) showed the morphological and histopathological changes in gills associated with brown gill syndrome of the cultured shrimps on acid sulphate soil ponds and demonstrated the presence of iron deposits on the gills of cultured shrimps with brown/ black-gill syndrome.

Although previous studies have stressed the importance of environmental metal compounds, particularly iron, as one possible cause of gill colour changes and associated histopathological affects, no attempt has been yet made to correlate these changes to the behaviour of metals in the acid sulphate soil environment. The present paper describes the relationship observed between brown and black gill syndrome in shrimp reared in ponds in an acid sulphate soil area in Sri Lanka and the stability relationships of the different iron, manganese and aluminium compounds present in this environment.

Materials and Methods

As a part of a major study of the problems associated with shrimp farming in acid sulphate soil environments, black tiger shrimp (*Penaeus monodon*) were reared for 10 weeks in three randomly selected earthen ponds at a 30 ha commercial shrimp farm situated on the west coast of Sri Lanka. A prior survey has revealed the presence of unripe sulphidic clay soil in the study area according to the International Institute for Land Reclamation and Improvement (ILRI) classification of acid sulphate soils (National Aquatic Resources Agency 1988).

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The ponds (0.5 ha) were prepared according to generally accepted procedures which included drying, liming, then filling the pond, followed by fertilization with inorganic fertilizers as recommended for intensive shrimp culture (Apud et al. 1989). Shrimps were introduced at stocking densities of 20 post larvae m^{-2}. Artificial aeration commenced after the 4^{th} week of culture using two paddle wheel aerators per pond. Water exchange rates were 4% daily initially; this was increased progressively to reach a level of 10% at the end of week 10.

Water samples were obtained from the pond bottom and pond surface at weekly intervals from 2^{nd} week to 6^{th} week and once in two weeks thereafter. The parameters measured were salinity (refractometer); pH and Eh (Bobby model smp 1 Eh-pH meter); turbidity (Hach model Portlab HH 16800), levels of dissolved oxygen (Jenway Portlab 9070 oxygen meter), phosphates, nitrates, nitrites and sulphide, chlorophyll a concentration, total suspended solids, and temperature using standard methods specified by American Public Health Association (1981) and Stirling (1985).

Shrimps were sampled from each pond weekly from week 2 to 6 and bi-weekly thereafter. A sample of 80 to 120 shrimps was removed by cast netting; gross observations were made to differentiate colour and other changes in their gills and to group them into three categories, viz. Category 1: Normal gills: gills of uniformly transparent colour, without sign of any accumulated material; Category 2: Brown-gills: light brown coloured gills with a somewhat opaque appearance when compared to normal gills; Category 3: Black-gills: opaque, dark brown or black gills, showing with very clear signs of foreign particle accumulation.

A randomly selected sub-sample of shrimp (20 to 30) was removed from the 3 ponds for the determination of total iron, manganese and aluminium levels in their gills. Wild shrimps collected from an adjoining lagoon and cultured shrimps from the same farm exposed to acid sulphate soil conditions for an 18 week period were also analyzed for comparative purposes. In the laboratory dissected gill samples were oven dried at 60°C and sub-samples in triplicate were then digested in a concentrated nitric and sulphuric acid 1:1 mixture for 48 hours at 70°C. The remaining solution was filtered and total iron, manganese and aluminium concentrations were measured using atomic absorption spectrometry (Perkin Elmer 2280).

Redox potential (Bobby model smp 1 Eh-pH meter, Russel type cept II/ rod/280 /1.5 m electrode) and pH values (Bobby model smp 1) of the surface sediments of the culture ponds were also monitored weekly throughout the culture period by immersing the respective electrodes directly into the surface 3 cm layer of the pond bottom sediment.

Analysis of variance were performed to test if there are any significant differences of iron, manganese and aluminium levels in the gills among different shrimp groups followed by Duncan's multiple range test to determine the group pairs with significant differences. The correlation (Pearson correlation coefficient) between iron and manganese levels in the gills was tested to find out whether there was a co-precipitation of manganese with iron.

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Results

The data on pond water quality are summarized in Table 1. The variations in total iron, total manganese and extractable aluminium concentration in the pond sediments and dissolved iron, manganese and aluminium concentrations recorded in the pond water are summarized in Table 2. The variation in iron levels in the sediments was between 17.4 g kg^{-1} and 61.5 g kg^{-1}. The total manganese and extractable aluminium concentrations in soil were relatively low and fluctuated between 74.0 to 227.0 mg kg^{-1} and 265 to 338 mg kg^{-1} respectively.

Table 1. Water quality of the culture ponds during the study period

Parameter	Surface layers		Bottom layers	
	Range	Mean±SD	Range	Mean±SD
Salinity (g L^{-1})	15-21	17.4±1.90	16-21	17.8±1.57
pH	7.7-8.4	8.08±0.20	7.4-8.2	7.9±0.25
Eh (mV)	58-118	97.7±23.7	68-111	92.6±18.7
Dissolved oxygen (mg L^{-1})	6.8-7.8	7.31±0.35	6.2-7.4	6.64±0.42
Phosphate (mg L^{-1})	0.01-0.06	0.04±0.01	0.01-0.06	0.03±0.02
Nitrates (mg L^{-1})	0.56-2.52	1.23±0.61	0.26-2.34	1.42±0.69
Nitrites (mg L^{-1})	0.01-0.06	0.07±0.08	0.01-0.28	0.07±0.09
Sulphides (mg L^{-1})	-	-	0.02-0.04	0.03±0.01
Chlorophyll a (µg L^{-1})	1.70-13.3	7.73±4.07	-	-
				-
Temperature ºC	25.5-29.5	28.4±1.3	25.0-29.5	27.9±1.48
Turbidity (NTU)	6.5-27.0	17.8±6.68	7.0-30.0	19.3±7.5
Total suspended solids (mg L^{-1})	26.0-93.2	49.7±25.93	-	- -

The observed fluctuations in the percentage of brown-gill / black-gilled shrimps in the culture ponds are shown in Figure 1. Brown gill syndrome was first observed during the 5th week of culture. The percentage of shrimp with brown-gills gradually increased from week 5 to 7, and then increased rapidly to reach a peak of 40% during the 8th week. At this stage, the shrimp became lethargic and migrated towards the peripheral areas of the ponds and a considerable number of dead shrimp were observed. Dark brown or black gilled shrimps were observed after the 7th week and they increased progressively in percentage until the 10th week.

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Table 2. Total iron, total manganese and extractable aluminium in pond sediments and total iron, total manganese and total aluminium in pond water over a ten week shrimp culture cycle.

Parameter	Range	Mean ± SD
Water		
Total iron (mg L^{-1})	0.49 – 1.75	1.08 ± 0.47
Total manganese (mg L^{-1})	0.16 – 0.27	0.18 ± 0.04
Total aluminium (mg L^{-1})	0.05 – 0.07	0.06 ± 0.01
Soil		
Total iron (g kg^{-1})	17.4 – 61.5	35.7 ± 18.6
Total manganese (mg kg^{-1})	74.0 – 227.0	125.8 ± 59.1
Total aluminium (mg kg^{-1})	265.8 – 338.2	296.7 ± 25.14

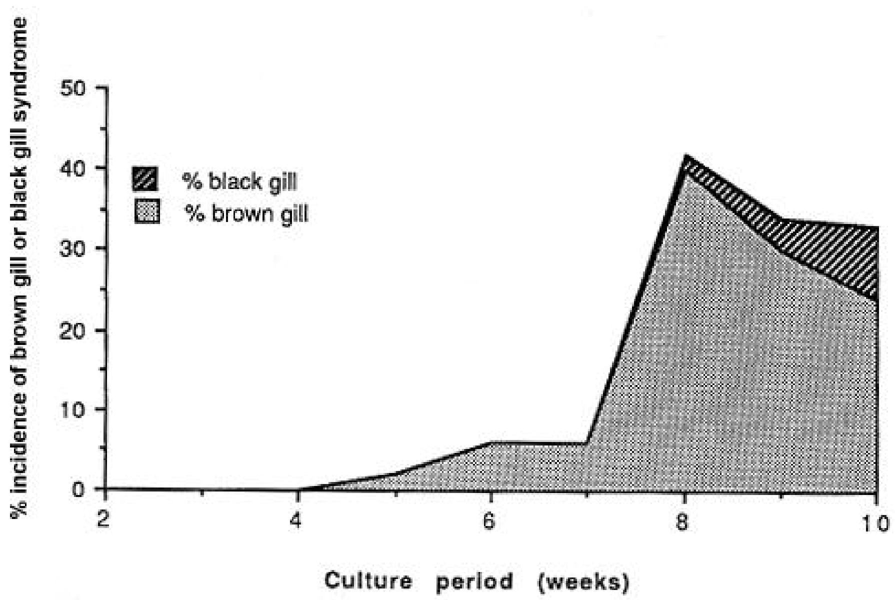

Figure 1. Incidence of brown gill and black gill syndrome in shrimps in culture ponds

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The total iron, manganese and aluminium concentrations measured in the gills of shrimp sampled during the 10 week culture period are given in Figure 2. This figure also includes the concentrations of these metals in wild shrimps collected from the lagoon adjoining the culture site, and in shrimps kept for an 18 week period in other culture ponds on the same farm. Very high concentrations of total iron were present in the gills of the cultured shrimps. The iron concentration fluctuated from 738 µg g^{-1} dry wt (on week 2) to 1588 µg g^{-1} dry wt (on week 6). The iron concentration in the gills showed a sharp increase between weeks 5 and 6, and then remained high for the remaining weeks of the culture period. The wild shrimps had a comparatively very low concentration of iron (347 µg g^{-1} dry wt) while the shrimps sampled after 18 weeks had an extremely high concentration (4545 µg g^{-1} dry wt). The manganese concentration in the gills of the shrimps cultured for 10 weeks fluctuated between 26.0 and 93.2 µg g^{-1} dry wt. The shrimps cultured for 18 weeks contained much more manganese (436 µg g^{-1} dry wt), while the wild stock showed only a trace level (19 µg g^{-1} dry wt). Gill aluminium concentrations were extremely low in all the sampled shrimp, the range of values being only 6.9 to 11.3 µg g^{-1} dry wt.

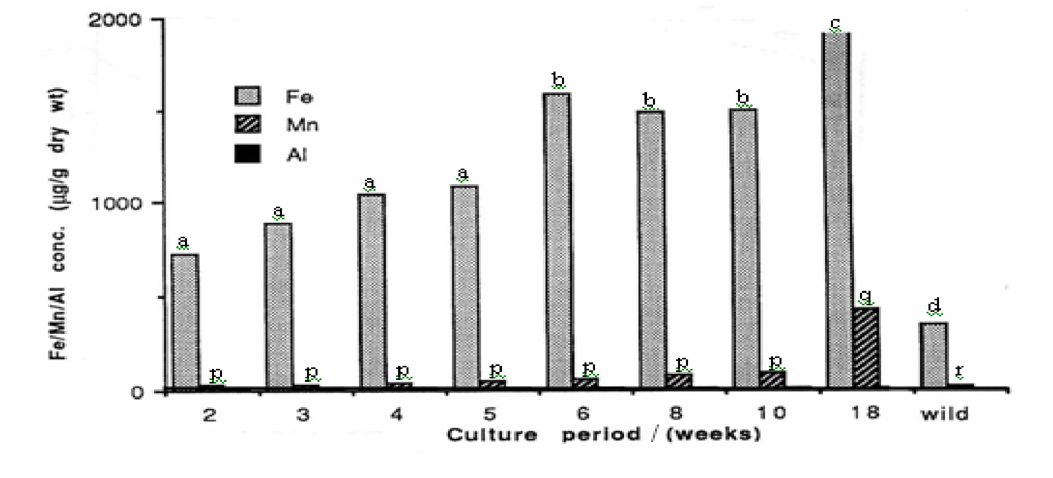

Figure 2. Comparison of total iron, manganese and aluminium concentrations in gills of the cultured shrimps cultured in ponds on acid sulphate soils and shrimps collected from wild. In each category, bars with same letters are not significantly different from each other.

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The results of one-way analysis of variance and multiple range test indicted that there were statistically significant differences ($p \leq 0.001$) among iron concentrations of the gills of different shrimp groups (2 to 5 weeks in ponds, 6 to 10 weeks in ponds, 18 week in ponds and wild stock) and among manganese concentrations in gills of different groups (2 to10 weeks in pond, 18 weeks in pond and wild stock). There were also statistically significant differences in gill iron ($p < 0.001$) and gill manganese ($p < 0.05$) concentrations of shrimp cultured up to 5 week period and shrimps culture for more than 5 weeks, where there was high incidence of brown gills. However, no statistically significant difference ($p \geq 0.05$) was observed in gill aluminium concentrations in these shrimp groups.

The stability relations of the different iron compounds (pyrite, jarosite, ferric oxides, goethite) in the acid sulphate soil environment in relation to Eh-pH conditions are given in Figure 3. The observed redox potential and pH values for the water and sediments of the culture ponds throughout the culture period are also included in this figure. In addition, highly significant correlation between gill manganese and iron was evident ($r = 0.981$, $p < 0.001$).

Discussion

The principle environmental conditions of salinity (15 to 21 g L^{-1}), pH 7.2 to 8.4), dissolved oxygen concentration (6.2 to 7.8 mg L^{-1}) and water temperature (25 to 29.5 °C) during the culture cycle were within the favourable ranges for *P. monodon* (Chiu 1988a and 1988b; Boyd 1989). The levels of nitrites, nitrates, sulphides, turbidity, total suspended solids also remained within the "safe limits" for shrimps (Boyd 1989; Kongkeo 1990; Poernomo 1990). The well documented condition of brown gill syndrome in shrimps in culture systems with high iron concentrations (Simpson et al. 1983; Lightner 1988; Nash et al. 1988) were observed in the present study after five weeks of culture. By the end of week 7, the condition had led to behavioral changes in affected shrimp and high mortalities (up to 125 to 200 shrimp day^{-1}).

Iron levels predominated in gills, contributing 91 to 96% of the total metal present. The content of aluminium was very low (between 0.1 and 1.2%) while manganese contributed 3.5 to 5.9%. The Eh-pH relationships observed for the pond water and sediments fall within the ferric oxide zone of the stability diagram (Figure 3) indicating that iron oxides were the most probable form of iron under the environmental conditions operating at the study site. According to Ponnamperuma (1972), the most common forms of iron III oxide in the acid sulphate environment includes α-Fe_2O_3(hematite), τ- Fe_2O_3 (maghemite), α-FeOOH(goethite), τ –FeOOH(lepidocrsite) and $Fe(OH)_3.nH_20$ (hydrated ferric oxides).

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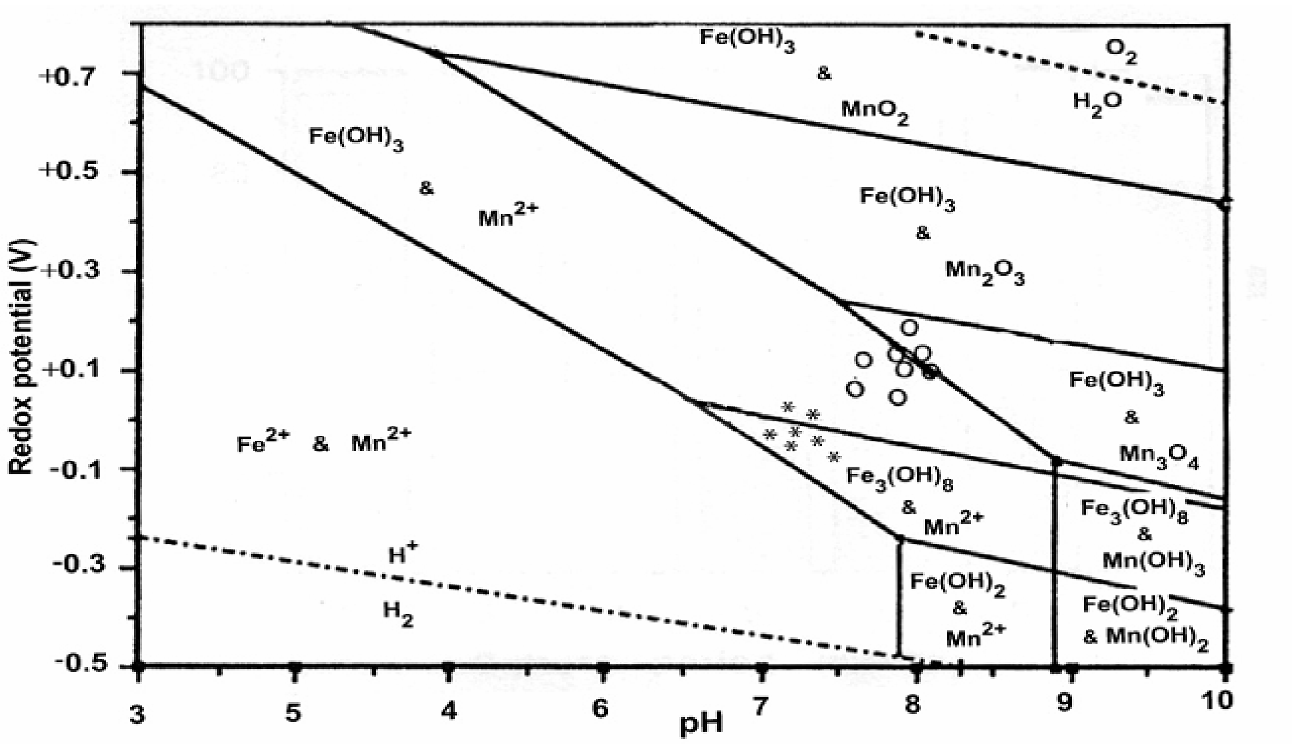

Figure 3.The stability relations of different iron and manganese compounds and the observed Eh-pH relationships for water (o) and sediments (*) of culture ponds during the culture period.

MnO_2 (pyrolusite), Mn_2O_3(bixbyte) Mn_3O_4, (hausmannite) and $Mn(OH)_2$ (pyrochroite) are the most common oxidised compounds of manganese in acid sulphate soil environments (Collins and Buol 1970a and 1970b), but the redox potential-pH conditions in the present study appeared to be more favourable for the formation of water soluble manganese compounds (Figure 3). The solubilities of different manganese oxides and iron oxides commonly found in acid sulphate soil environments are given in Table 3. The solubilities of these manganese and iron oxides decrease in the order; hausamanite > bixbyte > pyrolusite > pyrochroite >Fe(OH)3 amorp >maghemite > lepidocrocite > hematite > goethite. The formation of iron hydroxides from pyrites, which are the main compounds responsible for acid sulphate soil conditions, can be summarised as follows (Dent 1986):

$$FeS_2 + 15/4\ O_2 + 7/2\ H_2O \longrightarrow Fe(OH)_3 + 2\ SO_4^{2-} + 4H^+$$

Table 3. Equilibrium reactions of manganese and iron oxides commonly found in acid sulphate soils at 25°C (adapted from Lindsay, 1979).

Chemical reaction			Log K°
Mn_3O_4 (Hauamannite) + $8H^+$ + 2e	--------→	$3Mn^{2+} + 4H_2O$	63.03
Mn_2O_3 (Bixbyite) + $6H^+$ + 2e	--------→	$2Mn^{2+} + 3H_2O$	51.46
β-MnO_2 (Pyrolusite) +$4H^+$+2e	--------→	$Mn^{2+} + 2H_2O$	41.89
$Mn(OH)_2$ (Pyrochroite) +$2H^+$	--------→	$Mn^{2+} + 2H_2O$	15.19
$Fe(OH)_3$ (Amorp) + $3H^+$	--------→	$Fe^{3+} + 3H_2O$	3.54
½ - Fe_2O_3 (Maghemite) + $3H^+$	--------→	$Fe^{3+} + 3/2\ H_2O$	1.59
γ- FeOOH (Lepidocrocite) + $3H^+$	--------→	$Fe^{3+} + 2\ H_2O$	1.39
½ - Fe_2O_3 (Hematate) + $3H^+$	--------→	$Fe^{3+} + 3/2\ H_2O$	0.09
α- FeOOH (Geothite) +$3H^+$	--------→	$Fe^{3+} + 2\ H_2O$	0.02

A model describing the pyrite oxidation in shrimp culture ponds on acid sulphate soils is given in Figure 4. Once shrimp ponds constructed in such environments are filled with water the redox potential of the sediments tends to decrease, resulting an increase in pH. But if artificial aeration is introduced during the culture cycle oxygen diffuses into the sediments and iron (II) oxides are oxidised to iron (III) oxides. This results in the formation in ferric oxides. It is normal in the semi-intensive and intensive pond culture methods for *P. monodon* operating in Sri Lanka and South East Asia to commence aeration after the first month of production (Kongkeo 1990).

Circumstances where reversible oxidation and reduction prevails (Ponnamperuma 1972), as in the case of shrimp culture environment hydrated iron oxide ($Fe(OH)_3.nH_2O$) is expected to be the dominant form of iron oxide. This compound is a colloidal gel which forms a reddish brown precipitate in culture ponds. This precipitate tends to settle on the bottom of the pond which is the immediate environment for the cultured shrimps. Black tiger shrimp also show diurnal behavioural changes and live half buried in the bottom sediments during the day time, which suggests the possibility of iron hydroxide precipitate to get deposited on the gills.

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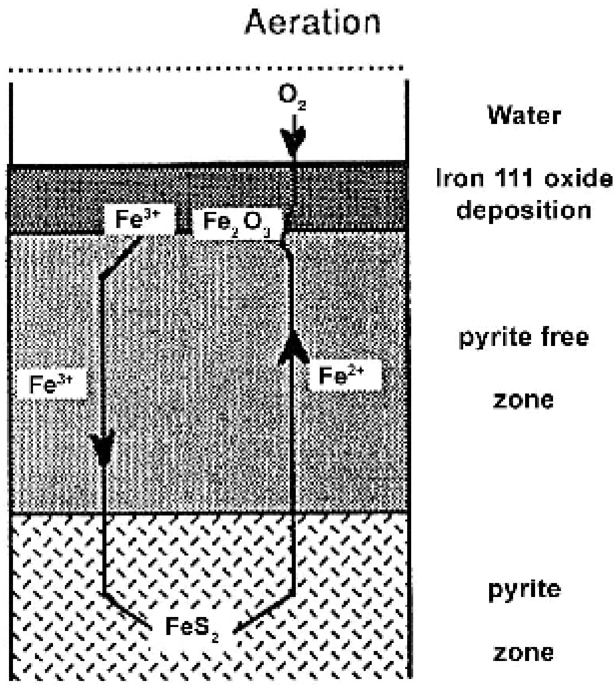

Figure 4. Model of pyrite oxidation in shrimp culture ponds constructed using acid sulphate soils.

Soluble Mn^{2+} is presumed to be the most stable form of manganese in the acid sulphate soil environment under the Eh-pH conditions observed in the present study (Ponnamperuma et al. 1967; Collins and Buol 1970a and 1970b). But in situations where iron hydroxide precipitation occurs, manganese also has a very strong tendency to co- precipitate with iron (Jenne, 1968; Collins and Buol 1970 b) as follows:

$$Fe(OH)_3 \text{ (colloidal)} + Mn^{2+} + 2H^+ \longrightarrow MnO_2 \text{ (colloidal)} + H_2O + 2Fe^{2+}$$

The highly significant correlation between gill manganese and iron obtained in the present study ($r = 0.981$, $p < 0.001$) may indicate that coprecipitation of manganese with iron did occur.

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