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Heavy Metal Concentration from Shrimp Culture Ponds at Point Calimer Area

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Abstract: Palk strait estuarine water was highly polluted by Cu, Fe, Pb, Zn and Hg. The study was carryout the interactions between heavy metals and microorganisms. The study area paying attention on the role of probiotic bacteria such as *Bacillus cereus, Aeromonas hydrophila, Pseudomonas aeruginosa, Azotobacter vinelandii,* and *Lactobacillus* sp. to remove the metals from polluted nine aquaculture ponds through the way of bioremediation, bioaccumulation and mineralization process. The highest inhibition zone was absorbed in *Pseudomonas aeruginosa* against Hg (3.796 mm) and Cu (0.060 mm), *Aeromonas hydrophila* against Pb (5.526 mm) and *Bacillus cereus* against Zn (4.504 mm) and Fe (1.635 mm). No inhibition zone was produced by *Azotobacter beijerinckii* and control also maintained.

Key words: Heavy metals, palk strait, *Penaeus monodon*

INTRODUCTION

Shrimp farming is the major aquaculture industry in Asian countries contributing 91% of the world production (FAO, 2001). India is rich in natural shrimp resources and nearly 52 species of shrimp are contributed in fishing (Swaminathan, 1980). Of these, 8 shrimp species are economically important and successful culture is practiced for two species viz., *Penaeus monodon* and *P. indicus*. Recent reviews of intensive shrimp aquaculture have emphasised the need for more effective controls on the quality of effluent water discharged into the environment. As in natural environments, good quality water is essential in aquaculture in order to maintain the health, optimal growth and survival of the cultured species, prevent eutrophication and maximize value for the farmer. Heavy metal pollution is one of the major types of pollution in coastal and marine environment. Heavy metals are conventionally defined as elements with metallic properties such as ductility, conductivity, stability as cations, ligand specificity, etc. The study of the interactions between heavy metals and microorganisms has been specially focused on bacterial transformation and conversion of metallic ions by reduction in different polluted environments (Chang *et al*., 1993), the selection of metal-resistant microorganisms from polluted environments (Hiroki, 1994), and the use of resistant microorganisms as indicators of potential toxicity to other forms of life (Doelman *et al*., 1994) as well as on mechanisms, determinants and genetic transfer of microbial metal-resistance (De Rore *et al*., 1994). Many heavy metal ions are "soft" Lewis acids, which mean that their affinity for soft donor atoms such as phosphorus and sulfur are considerably higher than for hard donor atoms such as nitrogen and oxygen. Examples of soft metal ions are Hg^{2+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Au^{3+}, Pt^{4+}, and Ti^{+}. Many soft metal ions are of concern because they are highly toxic and are present in a variety of waste streams that can potentially contaminate the environment if released. Safe, efficient, and cost effective separation and recovery of these toxic metals are of great concern in modern day environmental scenario. In the present study was designed to understand accumulation of the heavy metals in shrimp culture during different culture periods.

MATERIALS AND METHODS

Study area: The shrimp farms selected for the present study is located along the Palk Strait near by Point Calimer, in districts of Thiuruvarur. Nine ponds were selected for the present study and each pond was of 1h area. For convenient the ponds were divided into three groups viz., I, II and III and each group had three ponds. Pond I was treated with high dosage of probiotics, pond II with low dosage and Pond III was kept as control (Plate 1). Further study was conducted C.A.S. in Marine Biology, Annamalai University, Parangipettai.

Sample collection: Healthy *P. monodon* seeds were purchased from a commercial hatchery (CP at Marakkanam, Tamil Nadu). The seeds were stocked at a density of $10/m^2$. Before stocking, the seeds were

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Plate 1: A view of experimental pond along the Palk Strait coast of south east India

acclimatized to the pond environmental conditions. After stocking the survival rate was estimated using survival pens (Happa nets) laid near the outlet of each pond with 100 animals from each pond. Based on the survival rate on the 3rd day, the feed ratio was decided. The shrimps were fed with CP feed (Charoen pokhpond aquaculture India Pvt. Ltd., Chennai). The feeding schedule was based on the feed chart given by the company.

Nutrient analysis: Water samples of the ponds were collected in well-cleaned bottles for analysis of nutrients. The estimations were usually made within 24 h of collection. The nutrients like phosphate, nitrate and silicate were estimated following the standard methods described by Strickland and Parsons (1972).

Microbiological analysis: In order to find out the occurrence and distribution of metal resistant bacteria, sediment Samples were collected separately from different parts of the ponds in sterile conical flask and were mixed to make a single sample. This procedure was repeated for every pond and the final samples were brought to the laboratory immediately and were analyzed for microbial counts. It was then transferred to a sterile conical flask (150 ml) containing 99ml of sterile diluents, and serial dilution was performed to get 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} suspension samples. For enumeration of Total Heterotrophic Bacteria (THB), Zobell marine agar medium (Hi-media, Mumbai) was used (Table 5).

Isolation enumeration and identification of microbes: Enumeration of the microbes was done by adopting spread plate method. In this method, sterile media were poured into Petri dishes aseptically and allowed to solidify. One milliliter of serially diluted sample was pipette out into sterile Petri dish. The plates were incubated in an inverted position at 28±2ºC. All the determinations were carried out in triplicates. After the incubation period of 2 to 3 days, the colonies were counted. The microbial load in the given sample was calculated using the following formula and it is expressed as Colony Forming Units (CFU) per gram of the sample.

The dominant groups of microbes that were isolated were subcultured and then was identified by various morphological and biochemical characters given in Bergey' s manual.

Analysis of heavy metals resistant bacteria: Screening of heavy metal was done by qualitative screening assay. This was carried out in petri dishes containing nutrient agar medium and was swabbed with various microbial species separately. Metal salt solutions of Cd Cl_2 100 mM, Co Cl_2 100 mM, Cu SO_4 100 mM and Hg Cl_2 10 mM were prepared. Microbial growth for each bacterium was found separately against various heavy metals by dipping a sterile antibiotic disc in each solution.

Statistical treatment: To know the significance between the various parameters, Analysis of variance (ANOVA) was applied by using the software SPSS version 13.0.

RESULTS

Water quality parameters: The general water quality parameters did not varied significantly between the various ponds. There was no much difference in the average salinity for both treated and control ponds, which actually ranged from 15-40 ppt through out the culture period. The temperature for morning and after noon for both treated and control ponds were taken as average and were 28.5 and 30.0ºC respectively. In general, the average diurnal dissolved oxygen variation was similar (4.5-5.0 mg/L) for both treated and control ponds. Concentration of dissolved oxygen was higher in morning for both treated ponds and in the evening it was higher for control ponds. The pH level was on alkaline side for both treated and control ponds (7.0-8.5).

Nutrients: The nutrient parameters varied significantly between the ponds of various groups (Table 1).

Table 1: Nutrient concentrations of both probiotics treated and control ponds

Nutients	High dosage	Low dosage	Control
NH_4 in ppm	0.19±0.03[a]	0.29±0.04[b]	1.70±0.1[c]
NO_2 in ppm	0.0023±0.0001[a]	0.0035±0.0004[b]	0.043±0.005[c]
NO_3 in ppm	0.0120±0.002[a]	0.0263±0.008[b]	0.41±0.04[c]
PO_4 in ppm	0.0036±0.00004[a]	0.0043±0.00011[b]	0.0057± 0.0006[c]

Values sharing different superscript varies significantly between the groups ($p<0.05$)

Concentration of ammonia was very much lower in the ponds that were treated with probiotics than that of control ponds. Among treated ponds, the ammonium concentrations of the higher dosage ponds were less when compared to lower dosage ponds. Concentrations of nitrite, nitrate and phosphate were higher in control ponds than the other two probiotics treated ponds. Among the treated ponds lower dosage ponds was showing higher amounts of nitrite, nitrate and phosphate. All the nutrients studied for the present study was significantly different ($p<0.001$) between control and probiotics treated ponds.

Growth and survival: At the end of culture period the survival rate of treatment ponds with higher dosage was 94.3% and lower dosage was 90.1%. Whereas the survival rate of control pond was only 73.5% (Fig. 1). Maximum growth was observed in the ponds treated with different dosages of probiotics during each sampling interval and by the end of the experiment. Average weight gained for the shrimps that were supplied with probiotics was approximately 20% greater than that of the control (Fig. 2). The growth was not only showed significant difference between control and treatment ponds but also between low and high dosage ponds.

Heavy metal concentration in the pond sediment: Various heavy metals analyzed in the present study varied significantly between final and initial days in all the ponds ($p<0.05$). However, the control pond showed increase in the heavy metal concentration in the final day compared with the initial day (Table 2).

Identification of the bacteria: The microbes present in the sediment of the treated pond were identified. These are *Bacillus cereus*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Azotobacter vinelandii* and *Lactobacillus* sp., The characteristics of the microbes used for identification are given in Table 3-5.

Screening of heavy metal resistance: Qualitative screening assay done for screening of heavy metal resistance revealed that all the microbial species analyzed

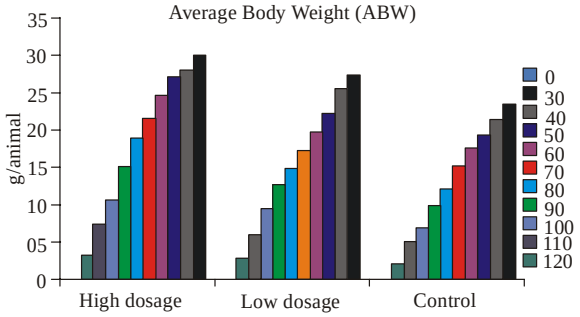

Fig. 1: Survival rate of *P. monodon* treated with different dosages of probiotics and control ponds

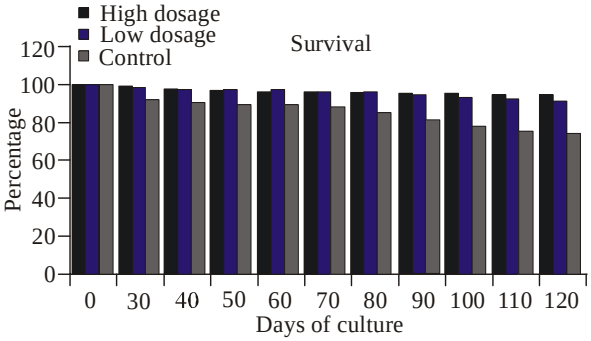

Fig. 2: Average body weight of *P. monodon* treated with different dosages of probiotics and control ponds

in the present study exhibit the heavy metal resistance property. However the there was some zones for the *Bacillus cereus* against Zn, *Aeromonas hydrophila* against Pb, *Pseudomonas aeruginosa* against Hg, *Azotobacter beijerinckii and Lactobacillus sp.* exhibited any zones. Hence these species are considered as best source of metal resistant bacteria (Table 6).

DISCUSSION

The quality of water during the culture period will deteriorate mainly due to the accumulation of metabolic wastes of living organisms, decomposition of unutilized feed and decay of biotic materials. However, addition of some commercial preparations as probiotics is reported to effectively deal with these substances and that way helpful in maintaining water quality parameters thereby improving growth rate, weight gain and survival rate with an attractive FCR in farmed organisms (Sissons, 1989). If

Table 2: Heavy metal concentrations of both probiotics treated and control ponds

	Control		Low dosage		High dosage	
Heavy metals	Initial	Final	Initial	Final	Initial	Final
Cu	0.054[a]	0.058[a]	0.057[a]	0.037[b]	0.061[a]	0.033[b]
Fe	1.635[a]	1.745[a]	1.621[a]	1.616	1.629[a]	1.521[a]
Pb	0.027[a]	0.029[b]	0.022[a]	0.017[a]	0.023[a]	0.003[b]
Zn	3.904[a]	5.526[a]	3.795[a]	3.437[a]	3.77[a]	3.123[a]
Hg	0.095[a]	0.171[b]	0.092[a]	0.029[b]	0.088[a]	0.045[b]

Values sharing different superscript varies significantly between the groups ($p<0.05$)

Table 3: Morphological, culture and biochemical characteristics of bacteria

Organism	*A h*	*B c*	*P a*
Gram Stain	-Rod	+Rod	-R od
Lactose	±	-	-
Dextrose	+	A	-
Sucrose	±	A	-
H_2S Production	+	-	-
NO_3 reduction	+	+	+
Indole production	+	-	-
MR Reaction	±	-	-
VP Reaction	+	±	-
Citrate Use	-	-	+
Urease Activity	-	-	-
Catalase Activity	+	+	+
Oxidase Activity	+	-	+
Gelatin liquefaction	+	+, fast	+
Starch Hydrolysis	+	+	-
Lipid Hydrolysis	-	±	+

(A = Acid production, AG = Acid and Gas production, ± = variable) *A h*: *Aeromonas hydrophila*; *B c*: *Bacillus cereus*; *P a*: *Pseudomonas aeruginosa*

Table 4: Morphological, culture and biochemical characteristics of *Lactobacillus* sp.

Color	White, cream color
Gram staining	+, Rod
Carbohydrate utilization*:*	
Galactose	+
D glucose	+
D fructose	+
Maltose	+
Lactose	+
Sucrose	+
Starch hydrolysis	+
H_2S	-
Catalase activity	-
Gelatin liquefaction	-
Indole	-

Table 5: Composition of Zobell marine agar medium

Composition	Amount (g)
Peptone	5.0
Yeast extract	1.0
K_2HPO_4	0.5
$FeS0_4$	Trace
Agar	15
50% seawater	1000 mL
pH	7.2

Table 6: Morphological, culture and biochemical characteristics of *Azotobacter vinelandii*

Organism	*Azotobacter vinelandii*
Gram Stain	-, Large ovoid rods,
Motility	+
Pigment	Yellow
Carbohydrate utilization:	
Starch	-
Mannitol	-
Rhamnose	-
Nitrate utilization	+
Catalase	+

the salinity is low the shell will be weak and prone to diseases. The optimum salinity was important for normal growth. Muthu (1980) and Karthikeyan (1994) recommended a salinity range of 10-35 ppt was ideal for *P. monodon* culture. The optimum range of pH 6.8 to 8.7 was maintained for maximum growth and production of penaid species (Ramanathan *et al*., 2005). Ramakrishna (2000) was recommended pH of 7.5 to 8.5 for *P. monodon* culture. Ravi *et al*. (1998) described the benefits of probiotics in maintaining water quality and enhancing growth rate in Indian White Prawn, *P. indicus*. There are many approaches for the use of bacteria to remove heavy metals from the environment, bioaccumulation and bioabsorption, oxidation and reduction, methylation and demethylation, and ligand degradation by bacteria (Bolton and Gorby, 1995). Lower costs and higher efficiency at low metal concentrations make biotechnological processes very attractive in comparison with physicochemical methods for heavy metal removal (Gadd, 1992). There are several studies on the heavy metal accumulation of different microbial species. Okino *et al*. (2002) studied the effect of initial cell concentration of removal of $HgCl_2$ and concluded that the removal rate increased with increasing initial cell concentrations. The resting cells of Pseudomonas aeruginosa have been reported to take up upto 110 mg Pb g-1 dry cell mass whereas the inactivated cells could absorb 70 mg Pb g-1 dry cell (Chang *et al*., 1993). However a few obvious examples are the remediation of hazardous or radioactive wastes, remediation of contaminated groundwater, and recovery of precious and or toxic metals from industrial processing solutions. A variety of well-known techniques are necessary for the chemists or engineers for these tasks, including solvent extraction, ion-exchange chromatography, and precipitation of heavy metal concentration.

CONCLUSION

The principal pollutant transformation processes, microbial degradation or biotransformation may be the most efficient way for removal of chemical pollutants and their toxicity from the environments. Microorganisms have a high surface area-volume ratio because of their small size and therefore provide themselves with a large contact area that can interact with matter in the surrounding environment. This revealed all the tested microbes exhibited the heavy metal resistance power. Hence it is very well clear that the probiotic microorganisms are capable of removing the heavy metal present in the aquaculture ponds.

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