

Research Article

Effect of zinc concentration on the growth performance of White leg shrimp, *Litopenaeus vannamei* Boone

Khushbu Sharma*

Department of Zoology and Aquaculture, Chaudhary Charan Singh Haryana Agricultural University, Hisar (Haryana), India

Rachna Gulati

Department of Animal Aquatic Health, Chaudhary Charan Singh Haryana Agricultural University, Hisar (Haryana), India

Karuna Bamel

Department of Zoology and Aquaculture, Chaudhary Charan Singh Haryana Agricultural University, Hisar (Haryana), India

Sushma

Department of Zoology and Aquaculture, Chaudhary Charan Singh Haryana Agricultural University, Hisar (Haryana), India

*Corresponding author. E mail: Khushbu181997@gmail.com

Article Info

<https://doi.org/10.31018/jans.v15i1.3969>

Received: August 23, 2022

Revised: February 12, 2023

Accepted: February 22, 2023

How to Cite

Sharma, K. *et al.* (2023). Effect of zinc concentration on the growth performance of White leg shrimp, *Litopenaeus vannamei* Boone. *Journal of Applied and Natural Science*, 15(1), 289 - 296. <https://doi.org/10.31018/jans.v15i1.3969>

Abstract

Awareness about healthy organic food is increasing, leading to research on contaminants/pollutants and their effect on aquatic fauna and mankind. The effect of zinc sulphate on the growth performance of White leg shrimp, *Litopenaeus vannamei* (Boone) was evaluated under six doses viz; 0.5, 1, 2, 4, 6, and 8 mg/l in *in vitro* study conducted from June to October 2021. The results revealed that higher doses of zinc sulphate showed toxicity against *L. vannamei*. The weight (3.12gm), length (8.95cm), weight gain per shrimp (4.11g), average daily weight gain (0.032), and specific growth rate (1.1g) were significantly (5%) lower at the higher dose (8mg/l) of zinc sulphate than control 11.73g, 16.22cm, 19.75g, 0.156g, and 2.20g respectively. With an increase in zinc sulphate dose from 0 mg/l in control to 8 mg/l, survival of shrimp decreased from 100 % to 7%. The Lethal concentration (LC₅₀) for zinc sulphate treatments was recorded at 0.71 ppm for shrimp. The water quality parameter pH (7.17-8.09), dissolved oxygen (6.13-7.58mg/l), hardness (4000-4772mg/l), and total alkalinity (162-231mg/l) were in an optimum range. The variation in Nitrite- Nitrogen, Nitrate- Nitrogen and Ammonical-Nitrogen (0-0.018, 0-0.4, 0-0.018mg/l) were within a permissible range. Thus, the present study revealed that zinc had a negative potential effect on *L. vannamei* at higher concentrations and may lead to serious economic loss to farmers if ignored.

Keywords: *Litopenaeus vannamei*, Lethal, concentration, Survival, Toxicity, Zinc

INTRODUCTION

Aquaculture an important sector of the world economy, is growing at a rate of more than 10 percent in India. The potential can be seen in aquaculture production increased from 0.75 in 1951-52 to 14.16 million metric tonnes in 2019-20 (FAO, 2018). This increase is due to large coastal areas, technological advancements, increasing demand for fish food as a low-cost high protein diet, and the development of the inland fisheries sector in the states that are away from the coastal region (Naylor *et al.*, 2021). Among Aquaculture, the shrimp culture, due to the standardization of its cultural

practices in India, high growth rate, short culture period, and great export value, has made a significant contribution to the world and Indian economy (Patil *et al.*, 2022). The white leg shrimp *Litopenaeus vannamei* Boone contributes about 80 percent of aquaculture production in India (Khusbhu *et al.*, 2022a). An inappropriate ionic ratio, high ammonia content, low dissolved oxygen, and high heavy metal content limit its production in inland fisheries (Ahmad *et al.*, 2022). Awareness about healthy organic food is increasing, leading to research on contaminants/pollutants and their effect on aquatic fauna and mankind. Several trace metals, including Mn, Fe, Co, Cu, Cr, and Zn, are recognized to

be significant minerals with beneficial effects on fish physiology, nutrition, and metabolism. Although natural processes like rock leaching can release trace quantities of zinc into inland water, human activities such as mining and industrialization can significantly raise zinc levels (Nagajyoti *et al.*, 2010). Among the heavy metals introduced into the environment due to anthropogenic activities and natural resources, zinc is most common, leading to toxicological effects on aquatic organisms (Chatla *et al.*, 2020). Hazardous waste sites may also contain zinc compounds such as zinc sulphate, zinc oxide, zinc chloride, and zinc sulphide. Many zinc salts are extremely water-soluble. The amount of zinc in natural water is determined by the hardness of the water. Its ability to bind to particulate particles varies depending on the physicochemical properties of the aquatic system. It is considered an essential element that acts as a cofactor for various enzymes (Kumar *et al.*, 2022). Even though heavy metals are frequent contaminants, there is comparatively little associated research on them. The toxicity of metals in aquatic organisms is also regulated by environmental conditions like temperature, pH, and salinity. Euryhaline species like *vannamei* shrimp can tolerate zinc over a wide range of concentrations. However, it can become toxic at high concentrations causing severe damage to the hepatopancreas, and intestine (Gao *et al.*, 2012). The decrease in water salinity generally results in the absorption of metals and their toxic effects on marine organisms. *L. vannamei* is a hyper regulator regulating repeated salinity fluctuation by maintaining the internal ionic concentration (Langston, 2018). It was reported that disruption of osmotic concentration and ionic balance occurs after heavy metals like zinc absorption. The present study aimed to evaluate the safe level or critical concentration of Zinc for *L.vannamei* and its effect on the growth of shrimps.

MATERIALS AND METHODS

The experiment was carried out in the College of Fisheries Science, Chaudhary Charan Singh Haryana Agricultural University Hisar, Haryana, India, for one culture season (June- to October 2021). *L. vannamei* juveniles were obtained from a commercial shrimp farm, Hisar, and transported to the laboratory in live condition. After three days of acclimatization, *L. vannamei* juveniles were transferred to experimental aquaria containing 100 liters of saline water (20ppt) in each at a stocking density of 5 juveniles /aquarium. Individual aquariums were equipped with a filter, submerged air diffuser, and thermostat to maintain an adequate temperature (27 ± 0.5 °C).

The stock solution of zinc sulphate was prepared from which six different doses; 0.5, 1, 2, 4, 6, and 8mg/l were made by dilution method. Each dose was evaluated

under triplicate conditions and compared with the control in which no zinc sulphate was added. Before introducing Zinc sulphate solution in an individual aquarium, zinc concentration was estimated by Atomic Absorption Spectroscopy (AAS) (Sprague, 1970). Shrimps were fed with commercial feed (crude protein 36%) four times daily according to the 5 percent body weight. Uneaten food and fecal matter were removed daily and only the water lost by siphoning was exchanged daily.

Effect of zinc on shrimp growth

Observations of the changes in weight and length of shrimps were recorded weekly for four months. At the end of the study period, average daily weight gain, weight gain per shrimp, specific growth rate (SGR), survival, and mortality rate were assessed using the below-mentioned standard formula:

$$\text{Average daily gain (ADG, g/shrimp/day)} = (\text{Fw} - \text{Iw}) / n \quad (\text{Eq. 1})$$

Where, n is the duration period; Iw is the initial mean weight of shrimp in gram, and Fw is the final mean weight of the shrimp in gram.

$$\text{Weight gain (WG, g/shrimp)} = (\text{Fw} - \text{Iw}) \quad (\text{Eq. 2})$$

$$\text{Specific growth rate (SGR)} = 100 \times \ln \text{Fw} - \ln \text{Iw} / \text{days} \quad (\text{Eq. 3})$$

Where;

In is the natural logarithm

$$\text{Survival rate (\%)} = \text{Fw} / \text{Iw} \times 100 \quad (\text{Eq. 4})$$

Water quality parameters

Water samples were collected from the aquarium (20-30cm below the surface of the water to estimate quality parameters). Dissolved oxygen (DO), temperature, and pH were measured twice a day (10:00 am and 5:00 pm), whereas ammonia nitrogen, nitrite nitrogen and nitrate nitrogen were measured twice a week using the colorimetric method. The salinity, total hardness, and total alkalinity were measured weekly. The salinity was measured by using the microprocessor CONDS-TDS-SAL meter (LT-51). The total hardness and total alkalinity were measured by using the Standard titration method (APHA, 1995).

Zinc determination

The concentration of zinc was determined in the water of each replicate of six dosages and control. Water samples were collected weekly from each aquarium and analyzed in atomic absorption spectrophotometer (AAS) following standard methodology. The data were recorded on a weekly basis for each aquarium.

RESULTS

Morphological and behavioural changes

Shrimp exposed to higher doses (2, 4, 6 and 8 mg/l) of zinc sulphate developed black spots on their body, tel-



Fig. 1. Morphology of *Litopenaeus vannamei* A) before and B) after zinc sulphate treatments

son, and on carapace region. Slow and sluggish movement of the pleopods and less feeding of the shrimps exposed were recorded during these experiments (Fig. 1 B).

Growth performance of shrimp

The results on the growth performance of shrimp revealed that higher doses of zinc sulphate showed toxicity against *L. vannamei*. The number of live shrimps decreased in various treatments, leading to a significant decrease in shrimp weight (CD=0.11; $p=0.05$) (Table 1). The weight of shrimps was significantly higher (11.73 g) in control (0 mg/l) and it decreased to 8.47, 7.88, 5.30, 3.83, 3.37, 3.12g with an increase in zinc sulphate dose from 0.5mg/l to 8 mg/l. Statistical analysis revealed a significant effect of the observation period. The weight of shrimps significantly increased to 11.16g on the 119th day as compared to the initial 1.40 g due to the number of live shrimps in control (CD=0.11; $p=0.05$). The ANOVA revealed a significant interaction between zinc sulphate treatment and the observation dates (CD=0.28; $p=0.05$) (Table 1). This showed that higher doses of zinc sulphate were more potent in reducing the live shrimps and weight at each observation date than lower doses.

Similar results were obtained for changes in the length of shrimps due to zinc sulphate treatments. Due to the mortality of shrimps, significantly lower lengths of shrimps were recorded at 0.5mg/l (13.33cm), 1mg/l (12.21cm), 2mg/l (11.32cm), 4mg/l (10.47cm), 6mg/l (9.61cm) and 8mg/l (8.95cm) concentrations as compared with control (16.2cm) (CD=0.40; $p=0.05$) (Table 2). Irrespective of dose, the length of shrimps increased significantly at each observation period due to the number of live shrimps in control (CD=0.25; $p=0.05$). The length of shrimps did not differ significantly from each other on the 0th and 7th day. Interaction between the treatment and observation periods was found significant (CD=0.25; $p=0.05$), which showed that at the end of the study period (119th day), lower shrimp length was

observed at higher doses than at lower doses. The effect of Zinc sulphate treatments on the survival of *vannamei* is illustrated in Fig. 2. Zinc sulphate treatments resulted in less survival of shrimps. It was 53, 40, 40, 20, 7 and 7 percent in 0.5, 1, 2, 4, 6 and 8 mg/l treatments as compared to 100 percent survival in control. Likewise, minimum weight gain per shrimp (4.11g) (Fig. 3) and average daily weight gain (0.032) (Fig 4), specific growth rate (1.09gm) (Fig 5) were recorded at a higher dose of 8 mg/l of zinc sulphate as compared to weight gain per shrimp, average daily weight gain, and specific growth rate (19.75g, 0.156g, and 2.20g) in control where no extra dose was given to shrimps, lesser gain in weight was recorded in 0.5 mg/l (13.74g), 1 mg/l (13.21g), 2mg/l (7.54g), 4mg/l (5.35g) and 6mg/l (4.41g), respectively. The average daily weight gain was (0.156g) in control which decreased to 0.032 at 8mg/l doses, respectively. A similar trend was witnessed in the specific growth rate of *L. vannamei* (Fig. 5). It ranged between 2.2g at the control and 1.1g at 0.08 mg/l showing a lower growth rate at higher doses of Zinc sulphate treatment.

Determination of concentration-mortality response (LC₅₀)

The LC₅₀ values (concentration at which 50 percent mortality occurred in *L. vannamei*) along with regression statistics for Zinc sulphate were calculated using the standard probit analysis method and are presented in Table 3. The LC₅₀ value was 0.71 mg/l. The value of slope was 1.36. This showed that further increases in concentrations would lead to mortality in shrimp. The intercept value was 5.18.

An analysis through graphical representation showed the changes in Nitrite-Nitrogen (mg/l) (Fig. 7), Nitrate-Nitrogen (mg/l) (Fig. 8) and Ammonical-Nitrogen (mg/l) (Fig. 9) in culture water. The variations in these parameters (0-0.018, 0-0.4, 0-0.018 mg/l, respectively) were within the permissible limits (0-0.05, 0-0.5 and 0-0.05)

Table 1. Effect of zinc sulphate (ZnSO₄) on the weight of shrimp, *Litopenaeus vannamei*

| Days of observation | Shrimp weight (g) in different treatment of ZnSO ₄ | | | | | | | Mean |
|---------------------|---|------------|------------|-----------|-----------|-----------|-----------|-------|
| | Control | 0.5mg/l | 1mg/l | 2mg/l | 4mg/l | 6mg/l | 8mg/l | |
| 0 | 1.28 (15) | 1.44(15) | 1.34(15) | 1.56 (15) | 1.38(15) | 1.44 (15) | 1.39 (15) | 1.40 |
| 7 | 1.73 (15) | 2.58(15) | 4.03(15) | 2.26(15) | 1.49(15) | 1.53 (15) | 1.78 (12) | 2.20a |
| 14 | 3.17 (15) | 3.07(15) | 2.46(15) | 2.15(15) | 1.78(15) | 1.66 (15) | 1.30 (9) | 2.23a |
| 21 | 4.50 (15) | 3.54 (15) | 3.73 (15) | 3.06 (15) | 1.92(14) | 1.52(13) | 1.28 (8) | 2.79 |
| 28 | 6.25 (15) | 4.55 (14) | 3.98 (14) | 2.94 (14) | 2.33(14) | 2.22(13) | 1.70 (8) | 3.42 |
| 35 | 7.85 (15) | 5.93 (14) | 5.00 (12) | 3.07 (14) | 2.36 (14) | 2.11(10) | 1.99 (8) | 4.05 |
| 42 | 8.63(15) | 6.53 (14) | 5.44 (12) | 3.52 (14) | 2.71 (14) | 2.18 (10) | 2.00 (7) | 4.43 |
| 49 | 9.30 (15) | 7.42 (14) | 6.62 (11) | 4.62 (12) | 3.55 (10) | 2.67 (6) | 2.30 (7) | 5.21 |
| 56 | 10.78 (15) | 8.11 (14) | 6.88 (11) | 5.02(12) | 3.93 (10) | 2.95 (6) | 2.81 (5) | 5.78 |
| 63 | 12.03 (15) | 8.36 (14) | 7.26 (11) | 5.43(11) | 4.15 (10) | 3.61 (5) | 3.20 (5) | 6.29 |
| 70 | 13.77 (15) | 10.42 (14) | 8.79 (11) | 5.67(11) | 4.38 (10) | 3.92(2) | 3.75 (5) | 7.24 |
| 77 | 15.23 (15) | 10.38 (12) | 9.66 (10) | 6.56 (9) | 4.71 (8) | 4.14(2) | 4.00 (5) | 7.81 |
| 84 | 17.01 (15) | 11.55 (12) | 10.78 (10) | 7.16 (8) | 4.85(8) | 4.59(2) | 4.02 (4) | 8.56 |
| 91 | 18.48(15) | 12.46 (10) | 11.82 (10) | 7.86 (8) | 5.16(5) | 4.61(2) | 4.23 (4) | 9.23 |
| 98 | 19.53 (15) | 13.20 (8) | 12.56 (10) | 7.97(6) | 5.34(5) | 4.86(2) | 4.62 (1) | 9.73 |
| 105 | 20.01 (15) | 13.57 (8) | 13.02 (9) | 8.38 (6) | 5.72(4) | 5.21(1) | 5.09 (1) | 10.14 |
| 112 | 20.50 (15) | 14.12 (8) | 13.81(6) | 9.04 (6) | 6.40(3) | 5.54(1) | 5.15 (1) | 10.65 |
| 119 | 21.03 (15) | 15.18 (8) | 14.69 (6) | 9.11(6) | 6.74(3) | 5.85(1) | 5.49 (1) | 11.16 |
| Mean | 11.73 | 8.47 | 7.88 | 5.30 | 3.83 | 3.37 | 3.12 | |

Figures in parentheses are the number of live shrimps in treatment; Values denoted by similar letter do not differ significantly with each other; CD (p=0.05) for Date of observation =0.11; SE=0.04; CD (p=0.05) for Zinc sulphate treatments =0.07; SE= 0.02; CD (p=0.05) for Date of observation × Zinc sulphate treatments = 0.28; SE= 0.10

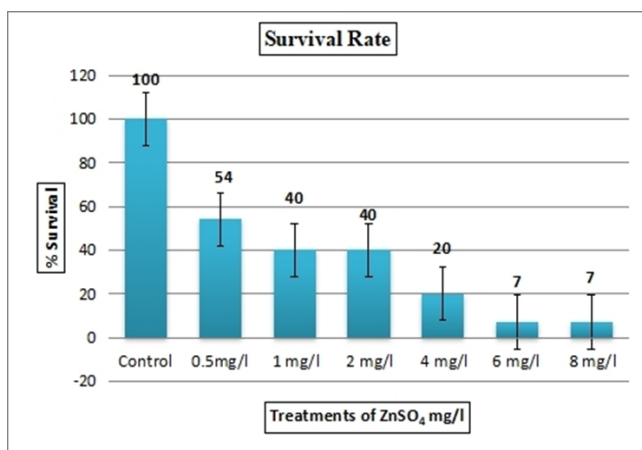


Fig. 2. Effect of zinc sulphate treatments on survival of *Litopenaeus vannamei*

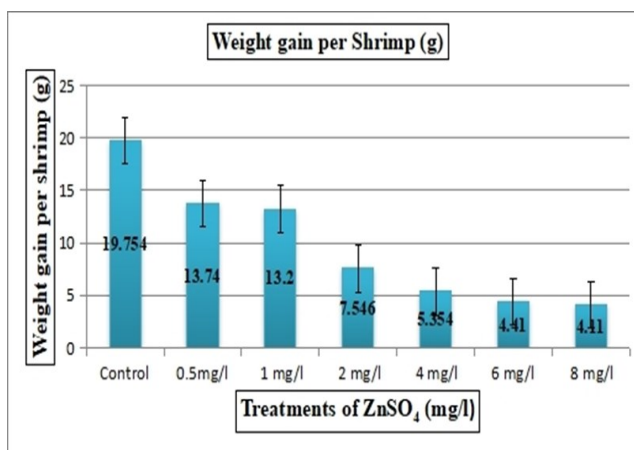


Fig. 3. Effect of zinc sulphate treatments on weight gain of *Litopenaeus vannamei*

Table 3. Effect of zinc sulphate (ZnSO₄) on the length of shrimp, *Litopenaeus vannamei*

| Days of Observation | Shrimp length (cm) in zinc sulphate treatments (mg/l) | | | | | | | Mean A |
|---------------------|---|------------|------------|------------|------------|-----------|-----------|--------------------|
| | Control | 0.5mg/l | 1 mg/l | 2mg/l | 4mg/l | 6mg/l | 8mg/l | |
| 0 | 5.89 (15) | 5.56 (15) | 5.42 (15) | 4.88 (15) | 4.81 (15) | 4.64 (15) | 4.70 (15) | 5.130 ^a |
| 7 | 6.82 (15) | 5.77 (15) | 5.61 (15) | 5.43 (15) | 4.97 (15) | 4.83 (15) | 4.79(12) | 5.463 ^a |
| 14 | 8.83 (15) | 6.77 (15) | 5.78 (15) | 5.66 (15) | 5.22 (15) | 5.09 (15) | 4.95 (9) | 6.043 |
| 21 | 10.76 (15) | 7.77 (15) | 6.02 (15) | 5.94 (15) | 5.62 (14) | 5.29 (13) | 5.16 (8) | 6.651 |
| 28 | 12.53 (15) | 8.71 (14) | 6.77 (14) | 6.31 (14) | 5.89 (14) | 5.56 (13) | 5.42 (8) | 7.31 |
| 35 | 14.53 (15) | 9.71 (14) | 7.77 (12) | 6.77 (14) | 6.30 (14) | 5.78 (10) | 5.66 (8) | 8.08 |
| 42 | 14.74 (15) | 10.71 (14) | 8.70 (12) | 7.77 (14) | 6.73 (14) | 5.80 (10) | 5.78 (7) | 8.62 |
| 49 | 15.89 (15) | 11.71 (14) | 10.70 (11) | 8.70 (12) | 7.77 (10) | 6.77 (6) | 5.89 (7) | 9.63 |
| 56 | 16.56 (15) | 12.71 (14) | 11.71 (11) | 10.72 (12) | 8.72 (10) | 7.77 (6) | 6.77 (5) | 10.70 |
| 63 | 18.16 (15) | 13.71 (14) | 12.70 (11) | 11.70 (11) | 10.72 (10) | 8.84 (5) | 7.84 (5) | 11.95 |
| 70 | 19.53 (15) | 14.57 (14) | 13.70 (11) | 12.71 (11) | 11.71 (10) | 10.71 (2) | 8.84 (5) | 13.10 |
| 77 | 19.83(15) | 15.14 (12) | 14.56 (10) | 13.69 (9) | 12.77 (8) | 11.71 (2) | 10.70 (5) | 14.05 |
| 84 | 20.02 (15) | 16.86 (12) | 15.14 (10) | 14.58 (8) | 13.70 (8) | 12.72 (2) | 11.70 (4) | 15.11 |
| 91 | 21.13 (15) | 17.18 (10) | 16.86 (10) | 15.14 (8) | 14.57 (5) | 13.69 (2) | 12.70 (4) | 15.74 |
| 98 | 21.25(15) | 19.83 (8) | 17.18 (10) | 16.86 (6) | 15.14 (5) | 14.57 (2) | 13.70 (1) | 16.91 |
| 105 | 21.27 (15) | 20.27 (8) | 19.83 (9) | 17.18 (6) | 16.86 (4) | 15.14 (1) | 14.57 (1) | 17.84 |
| 112 | 21.97 (15) | 21.27 (8) | 20.02 (6) | 19.80 (6) | 17.18 (3) | 16.86 (1) | 15.14 (1) | 18.89 |
| 119 | 22.43 (15) | 21.97 (8) | 21.26 (6) | 20.02 (6) | 19.83 (3) | 17.18 (1) | 16.86 (1) | 19.93 |
| Mean | 16.22 | 13.33 | 12.21 | 11.32 | 10.47 | 9.61 | 8.95 | |

Values denoted by similar letter do not differ significantly with each other CD (p=0.05) for date of observation =0.40; SE= 0.14 CD (p=0.05) for zinc sulfate treatments =0.25; SE=0.09; CD (p=0.05) for date of observation × zinc sulfate treatments =1.08; SE=0.39

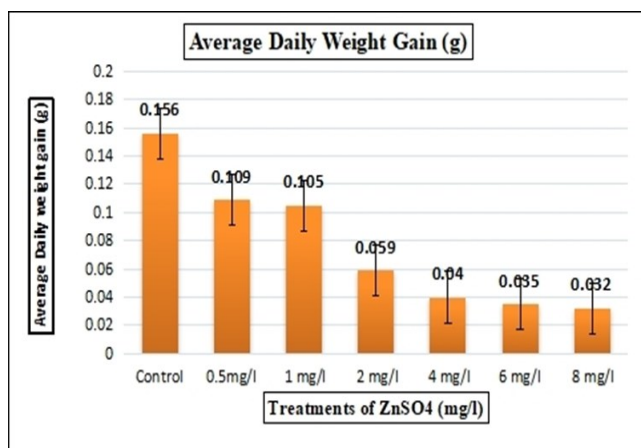


Fig. 4. Effect of zinc sulphate treatments on average daily weight gain of *Litopenaeus vannamei*

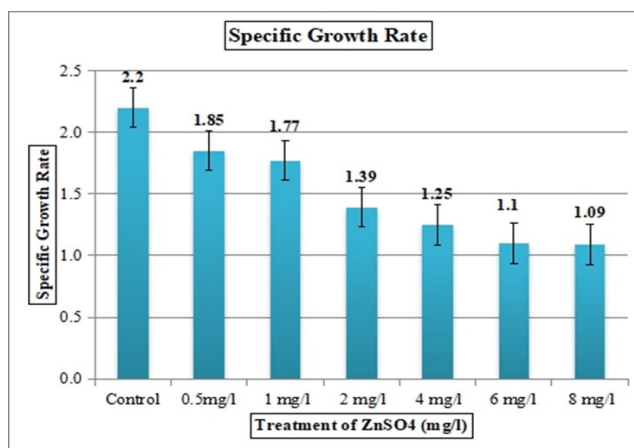


Fig. 5. Effect of Zinc sulphate treatments on specific growth rate of *Litopenaeus vannamei*

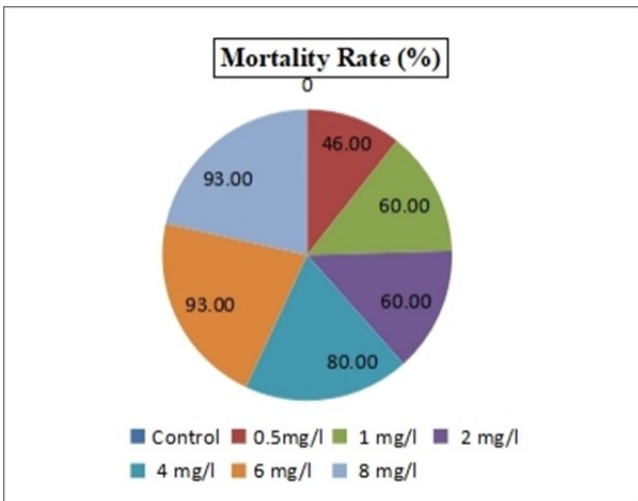


Fig 6. Mortality rate (%) due to zinc sulphate treatments in *Litopenaeus vannamei*

(APHA, 1995; FAO, 2018).

DISCUSSION

During the present study, at 0.5, 1, 2, 4, 6, and 8mg/l zinc sulphate treatments- 46, 60, 60, 80, 93, and 93 percent mortality in *L. vannamei* was recorded (Fig. 6). The findings of this study also illustrated a significant (5%) decrease in the survival rate of *L. vannamei* when treated with higher doses of zinc sulphate. Similar results were recorded in a previous study (Chen *et al.*, 2020), at high concentrations in which zinc showed toxic effects. The most obvious sign of toxicity recorded in the present study was the reduction in growth performance, similar to the study conducted by Chen *et al.*

(2020). The hepatopancreas of *vannamei* is the main target site of zinc accumulation. Excessive zinc impaired the hepatopancreas and also reduce body length, weight gain, specific growth rate, survival rate, food intake, nutritional status, and health status of shrimp (Chen *et al.*, 2020; Pérez and Hoang, 2017; Wu and Chen, 2005). Bambang *et al.* (1995) revealed that osmotic control is a key mechanism for environmental adaptation in marine species. Osmotic Concentration (OC) which is sensitive to zinc toxicity may be utilized as a criterion of aquatic environmental disturbance (Khushbu *et al.*, 2022b). Changes in hemolymph osmolarity in *L. vannamei* exposed to higher zinc concentration led to stunted growth and mortality (Wu and Chen, 2004). A study by Sejati *et al.* (2022) recorded that when shrimp (*L. vannamei*) were treated with high concentrations of zinc sulphate, it gets accumulated in their body due to low excretion rate. Silva *et al.* (2016) recorded that the shrimp (*L. vannamei*) usually died at concentrations more than 1.80 mg/l, but in concentrations between 0.88 and 1.80 mg/l mortality was further delayed. When *L. vannamei* were exposed to high doses of zinc, it caused cytological and histochemical damage to the gill epithelium (Wu and Chen, 2004). These gill alterations caused oxygen consumption inhibition, which would explain the mortalities observed in the present study (Barbieri, 2009; Soegianto *et al.*, 1999). According to Viswanathan and Manisseri (1993), the LC₅₀ for zinc to *Penaeus indicus* was 1.67 ppm. The LC₅₀ in *Metapenaeus sp.* was 1.7 ppm (Sivadasan *et al.*, 1986), which was greater than the value found in the present study (0.71ppm).

Table 3. LC₅₀ of *Litopenaeus vannamei* for zinc sulphate

| <i>L. vannamei</i> | Zinc sulphate treatments | | | | | |
|--------------------|--------------------------|-------|-----------|----------------------|----------------|----|
| | n | Slope | Intercept | LC ₅₀ (%) | χ ² | Df |
| | 15 | 1.36 | 5.18 | 0.71 | 12.59 | 6 |

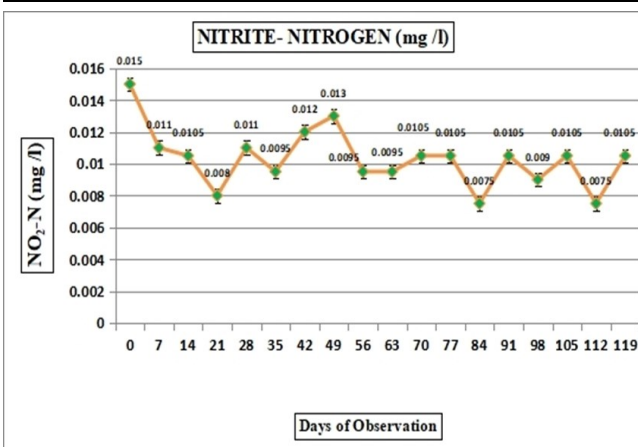


Fig. 7. Changes in Nitrite- Nitrogen (mg/l) during experiment

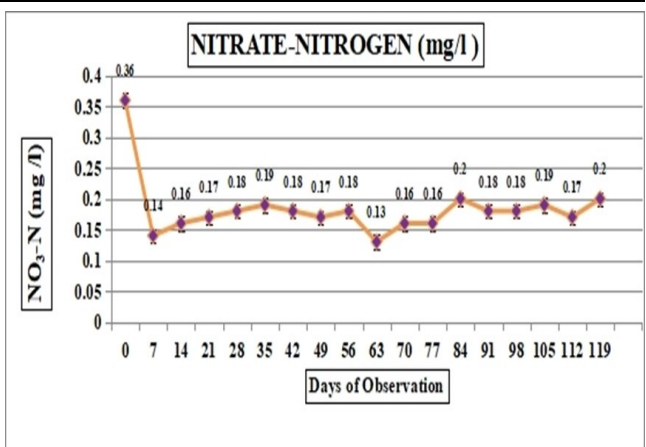


Fig. 8. Changes in Nitrate- Nitrogen (mg/l) during experiment

Table 4. Physico-chemical parameters of water during the experiment

| Days of observation | pH | | Dissolved Oxygen (mg/l) | | Hardness (mg/l) | Alkalinity (mg/l) |
|---------------------|-------------------------------|-------------------------------|-------------------------|-----------------------|-------------------------|---------------------------|
| | Morning | Evening | Morning | Evening | | |
| 0 | 7.62 ^{e,f,g,h,l,j} | 7.41 ^{e,f,g,h,l,j,k} | 6.48 | 6.13 | 4319.28 ^f | 193.07 ⁱ |
| 7 | 7.49 ^{b,c,d} | 7.21 ^{a,b} | 7.09 ^{a,b} | 6.73 ^a | 4078.57 ^{b,c} | 162.42 |
| 14 | 7.49 ^{a,b,c} | 7.27 ^{b,c} | 7.19 ^c | 6.84 ^{b,c} | 4211.42 ^e | 186.50 ^{g,h} |
| 21 | 7.53 ^{b,c,d,e} | 7.33 ^{c,d,e} | 7.04 ^a | 6.80 ^{a,b} | 4000.00 ^a | 179.14 ^{c,d,e} |
| 28 | 7.46 ^{a,b} | 7.25 ^{a,b,c} | 7.20 ^c | 6.90 ^{c,d} | 4008.57 ^a | 168.64 ^a |
| 35 | 7.38 ^a | 7.17 ^a | 7.41 ^{e,f} | 7.00 ^e | 4100.71 ^{b,c} | 174.78 ^{b,c} |
| 42 | 7.49 ^{b,c} | 7.27 ^{b,c} | 7.49 ^g | 7.19 ^{h,i} | 4159.28 ^d | 182.28 ^{d,e,f,g} |
| 49 | 7.46 ^{a,b} | 7.30 ^{b,c,d} | 7.58 | 7.34 ^l | 4,192.14 ^{d,e} | 177.57 ^{c,d} |
| 56 | 7.61 ^{e,f,g,h,i} | 7.36 ^{c,d,e,f} | 7.55 ^h | 7.31 ^l | 4280.71 | 196.43 ^{i,j,k} |
| 63 | 7.64 ^{e,f,g,h,l,j,k} | 7.42 ^{e,f,g,h,l,j} | 7.51 ^g | 7.27 ^{i,k} | 4335.00 ^f | 172.57 ^{a,b} |
| 70 | 8.09 | 7.88 | 7.37 ^{d,e} | 7.04 ^{e,f,g} | 4,406.43 ^g | 211.29 |
| 77 | 7.93 | 7.65 | 6.69 | 6.33 ^l | 4458.21 ^{h,i} | 186.14 ^{g,h} |
| 84 | 7.57 ^{c,d,e,f,g} | 7.36 ^{c,d,e,f} | 7.24 | 6.99 ^{d,e} | 4399.29 ^g | 193.86 ^{i,j} |
| 91 | 7.53 ^{b,c,d,e} | 7.28 ^b | 7.54 | 7.19 ^{h,j} | 4458.57 ^{h,i} | 196.43 ^{i,j,k} |
| 98 | 7.55 ^{b,c,d,e,f} | 7.37 ^{c,d,e,f,g,h} | 7.54 | 7.21 ^{h,k} | 4565.71 ^j | 181.00 ^{d,e,f} |
| 105 | 7.63 ^{e,f,g,h,l,j} | 7.38 ^{d,e,f,h,i} | 7.46 ^{f,g} | 7.21 ^{h,j,k} | 4587.14 ^j | 206.07 |
| 112 | 7.59 ^{c,d,e,f,g,h} | 7.37 ^{c,d,e,f,g} | 7.37 ^d | 7.12 ^{g,h} | 4644.29 | 195.57 ^{i,j,k} |
| 119 | 7.70 ^{i,j,k} | 7.44 ^{f,g,h,l,j} | 7.10 ^{a,b} | 6.79 ^{a,b} | 4720.00 | 231.14 |
| CD | 0.11 | 0.10 | 0.66 | 0.59 | 34.20 | 4.82 |
| SE | 0.39 | 0.36 | 0.33 | 0.29 | 12.27 | 1.73 |

Values denoted by similar letter do not differ significantly with each other

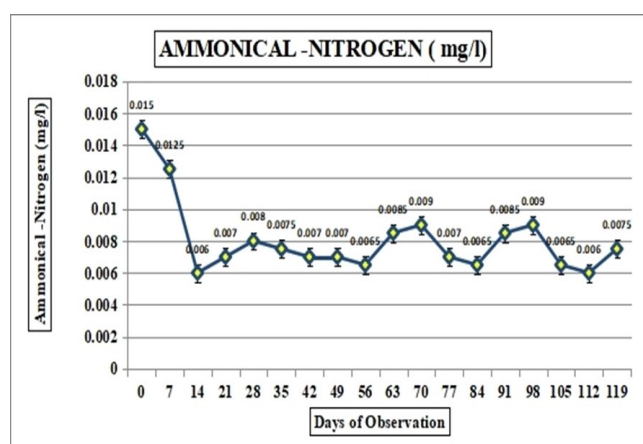


Fig. 9. Changes in Ammonical-Nitrogen (mg/l) during experiment

Conclusion

This study revealed that higher doses of zinc sulphate were toxic to *L. vannamei*. Average daily weight gain (0.032), and specific growth rate (1.1g) was significantly (5%) lower at the higher dose (8mg/l) of zinc sulphate than the control ones. With an increase in zinc sulphate dose from 0 mg/l in control to 8 mg/l, survival of *L. vannamei* decreased from 100 % to 7%. The LC₅₀ for zinc sulphate treatments was recorded at 0.71 ppm.

To increase the sustainability of the aquaculture sector and provide safe shrimp for human consumption, the competent authorities at the local, state, and national levels should conduct frequent monitoring of the shrimp and the surrounding environment. Thus the present study concluded that zinc had a negative potential effect at higher concentrations and may lead to serious economic loss to farmers.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

This study was supported by the Council of Scientific and Industrial Research (CSIR, India) under sanction number (09/303(0310)/2019-EMR-I).

Ethical approval

This study did not require any ethical approval.

REFERENCES

- Ahmad, A. L., Chin, J. Y., Harun, M. H. Z. M. & Low, S. C. (2022). Environmental impacts and imperative technologies towards sustainable treatment of aquaculture

- wastewater: A review. *Journal of Water Process Engineering*, 46, 102553. <https://doi.org/10.1016/j.jwpe.2021.102553>
2. APHA (1995). Standard methods for the examination of water and wastewater, 19th edn. NewYork, USA. <http://www.sciencedirect.com/reference/36447>
 3. Bambang, Y., Thuet, P., Charmantier-Daures, M., Trilles, J. P. & Charmantier, G. (1995). Effect of copper on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* Bate (Crustacea, Decapoda). *Aquatic Toxicology*, 33(2), 125-139. [https://doi.org/10.1016/0166-445X\(95\)00011-R](https://doi.org/10.1016/0166-445X(95)00011-R)
 4. Barbieri, E. (2009). Effects of zinc and cadmium on oxygen consumption and ammonium excretion in pink shrimp (*Farfantepenaeus paulensis*, Pérez-Farfante, 1967, Crustacea). *Ecotoxicology*, 18(3), 312-318. <https://link.springer.com/article/10.1007/s10646-008-0285-y>
 5. Chatla, D., Padmavathi, P. & Srinu, G. (2020). Wastewater treatment techniques for sustainable aquaculture. *Waste management as economic industry towards circular economy*, 159-166. https://link.springer.com/chapter/10.1007/978-981-15-1620-7_17
 6. Chen, C., Xu, C., Qian, D., Yu, Q., Huang, M., Zhou, L., Qind., G. J., Chenc., L. & Lia, E. (2020). Growth and health status of Pacific white shrimp, *Litopenaeus vannamei*, exposed to chronic water born cobalt. *Fish & Shellfish Immunology*, 100, 137-145. <https://doi.org/10.1016/j.fsi.2020.03.011>
 7. FAO (2018). The State of World Fisheries and Aquaculture 2018, FAO, Fisheries and Aquaculture Department, Rome, Italy. <https://www.fao.org/3/I9553EN/i9553en.pdf>
 8. Gao, W., Tan, B., Mai, K., Chi, S., Liu, H., Dong, X. & Yang, Q. (2012). Profiling of differentially expressed genes in hepatopancreas of white shrimp (*Litopenaeus vannamei*) exposed to long-term low salinity stress. *Aquaculture*, 364, 186-191. <https://doi.org/10.1016/j.aquaculture.2012.08.024>
 9. Khushbu, Gulati, R., Sushma & Bamel, K. (2022a). Microsporidian Enterocytozoon hepatopenaei (EHP) in Shrimp and Its Detection Methods. *Bulletin of Pure and Applied Sciences-Zoology*, 41A (1), 179-187. DOI:10.5958/2320-3188.2022.00022.5
 10. Khushbu, Gulati, R., Sushma & Sharma, P. (2022b). Shrimp culture (*Litopenaeus vannamei*) and its management. *Agricultural Science: Research and Review*, 7, 62-76. https://www.researchgate.net/publication/362292019_Shrimp_culture_Litopenaeus_vannamei_an_d_its_management
 11. Kumar, N., Banerjee, C., Chang, J. S. & Shukla, P. (2022). Valorization of wastewater through microalgae as a prospect for generation of biofuel and high-value products. *Journal of Cleaner Production*, 132114. <https://doi.org/10.1016/j.jclepro.2022.132114>
 12. Langston, W. J. (2018). Toxic effects of metals and the incidence of metal pollution in marine ecosystems. *Heavy metals in the marine environment*, 101-120. <https://www.taylorfrancis.com/chapters/edit/10.1201/9781351073158-7/toxic-effects-metals-incidence-metal-pollution-marine-ecosystems-langston>
 13. Nagajyoti, P. C., Lee, K. D. & Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environmental Chemistry Letters*, 8(3), 199-216. <https://doi.org/10.1007/s10311-010-0297-8>
 14. Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., ... & Troell, M. (2021). A 20-year retrospective review of global aquaculture. *Nature*, 591(7851), 551-563. <https://doi.org/10.1038/s41586-021-03736-4>
 15. Patil, P. K., Mishra, S. S., Pradhan, P. K., Manna, S. K., Abraham, J. T., Solanki, H. G., ... & Jena, J. (2022). Usage pattern of chemicals, biologicals and veterinary medicinal products in Indian aquaculture. *Reviews in Aquaculture*. <https://doi.org/10.1111/raq.12688>
 16. Pérez, E. & Hoang, T. C. (2017). Chronic toxicity of binary metal mixtures of cadmium and zinc to *Daphnia magna*. *Environmental toxicology and chemistry*, 36(10), 2739-2749. <https://doi.org/10.1002/etc.3830>
 17. Sejati, R. A. W., Hanum, G. R. & Pramushinta, I. A. K. (2022). Test of Lead (Pb) and Zinc (Zn) on Tiger Shrimp (*Penaeus monodon*) at Kalanganyar Market, Sidoarjo with Atomic Absorption Spectrophotometer (AAS). *Medicra (Journal of Medical Laboratory Science/Technology)*, 5(1), 56-61. <https://doi.org/10.21070/medicra.v5i1.1624>
 18. Silva, E., Viana, Z. C. V., Onofre, C. R. E., Korn, M. G. A. & Santos, V. L. C. S. (2016). Distribution of trace elements in tissues of shrimp species *Litopenaeus vannamei* (Boone, 1931) from Bahia, Brazil. *Brazilian Journal of Biology*, 76, 194-204. <https://doi.org/10.1590/1519-6984.17114>
 19. Sivadasan, C. R., Nambisan, P. N. K. & Damodaran, R. (1986). Toxicity of mercury, copper and zinc to the prawn *Metapenaeus dobsoni* (MIER). *Current Science*, 337-340. <https://www.jstor.org/stable/24089404>
 20. Soegianto, A., Charmantier-Daures, M., Trilles, J. P. & Charmantier, G. (1999). Impact of copper on the structure of gills and epipodites of the shrimp *Penaeus japonicus* (Decapoda). *Journal of Crustacean Biology*, 19(2), 209-223 <https://doi.org/10.1163/193724099X00015>
 21. Sprague, J. B. (1970). Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. *Water Research*, 4(1), 3-32 [https://doi.org/10.1016/0043-1354\(70\)90018-7](https://doi.org/10.1016/0043-1354(70)90018-7)
 22. U.S. Environmental Protection Agency (EPA). (2017). *Water Quality Standards Handbook: Chapter 3: Water Quality Criteria*. EPA-823-B-17-001. EPA Office of Water, Office of Science and Technology, Washington, DC. Accessed November 2018. <https://www.epa.gov/sites/production/files/2014-10/documents/handbook-chapter3.pdf>
 23. Viswanathan, S. & Manisseri, M. K. (1993). Histopathological studies of zinc toxicity in *P. indicus*. Milne Edwards. MSc Dissertation. Cochin University of Science and Technology. India. <http://eprints.cmfri.org.in/id/eprint/11136>
 24. Wu, J. P., & Chen, H. C. (2004). Effects of cadmium and zinc on oxygen consumption, ammonium excretion, and osmoregulation of white shrimp (*Litopenaeus vannamei*). *Chemosphere*, 57(11), 1591-1598. <https://doi.org/10.1016/j.chemosphere.2004.07.033>
 25. Wu, J. P. & Chen, H. C. (2005). Metallothionein induction and heavy metal accumulation in white shrimp *Litopenaeus vannamei* exposed to cadmium and zinc. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 140 (3-4), 383-394. <https://doi.org/10.1016/j.cca.2005.03.006>