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Marine sponge symbiotic bacterial bioremediation against heavy metal pollutants in tiger prawns: *Penaeus monodon* culture medium

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ABSTRACT

BACKGROUND AND OBJECTIVES: The quality and production volume of the cultivation of tiger prawn *Penaeus monodon* have decreased considerably in the last two decades. However, intensification and extensification efforts, including the application of cultivation technology through pond land recovery, have not produced expected results. Visible symptoms suggest potential issues with the cultivation water possibly originating from exposure to heavy metal pollutants. Therefore, this study aimed to remove heavy metal pollutants by using sponge symbiont bacteria bioremediators to increase the survival rate and quality of tiger prawn production. The achievements of this research are expected to contribute to the scientific development of environmental microbiology, bioremediation, and aquaculture pollution control.

METHODS: The study utilized *Bacillus pumilus* and *Pseudomonas stutzeri* bacteria. The water used for tiger prawn post-larvae cultivation was treated with these bioremediator bacteria. The water had copper and lead ion concentrations that were 20 times greater than the maximum threshold value. The physical and chemical characteristics and parameters, such as dissolved organic matter, nitrite, nitrate, and ammonia contents, of the cultivation water were monitored over a 30-day period. The specific growth rate in terms of weight and body length and the survival rate of the tiger shrimps were measured to evaluate the effect of the bioremediation process on the prawns. The concentrations of copper and lead ions in the cultivation water and within the body of the tiger shrimps were analyzed. The health of the tiger prawns was evaluated by observing signs of tissue damage.

FINDINGS: Among all the treatments, Treatment I with copper ion exposure had the highest average specific growth rate of the tiger prawns in terms of weight and body length, followed by Treatment II with lead ion exposure and Treatment III with a combination of both pollutants (the lowest). The intersection of copper and lead ion concentrations in the tiger prawns and cultivation media occurred in the cultivation period of 15–20 days. The use of *Bacillus pumilus* and *Pseudomonas stutzeri* bacteria as bioremediators effectively remediated the copper and lead pollutants at an average of 99.34 percent and 97.18 percent of the initial concentration, respectively. Despite the bioremediation efforts, the tiger shrimps exhibited symptoms of tissue damage in the head, tail, and shell. These symptoms included necrosis, myopathy, and infiltration, which are indicative of decreased cell function due to the presence of toxic agents.

CONCLUSION: Bioremediation with *Bacillus pumilus* and *Pseudomonas stutzeri* bacteria helped reduce the accumulation of heavy metal pollutants. However, negative effects on the health and growth of tiger prawns were still observed when the prawns were exposed to copper and lead ion concentrations below the allowed threshold value.

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INTRODUCTION

The cultivation of tiger prawn *Penaeus monodon* (TPPM), as one of the leading commodities for supporting the economy of fishing communities, was actively promoted among fish farmers in South Sulawesi between 1985 and 2010 (Mustafa *et al*., 2023). However, over the course of two decades, production volumes gradually decreased, resulting in reduced incomes for farmers (Ribeiro *et al*., 2016). After the initial success of TPPM cultivation, farmers and local governments became complacent with the achievements (Paena *et al*., 2020) as evidenced by the absence of efforts to conduct investigations for improving cultivation technology and maintaining production through land productivity and efficiency (Walker and Winton, 2010). The quality of TPPM production has been decreasing considerably from 2010 to the present. Although the South Sulawesi Provincial Government attempted to restore production volume and quality through intensification and extensification, such effort did not produce positive results (Mustafa *et al*., 2022). Observations and studies performed by related agencies suggested that the decrease is caused by pond land experiencing saturation due to the large-scale exploitation of fertilizers and pesticides in the last two decades (Zhou *et al*., 2012). This condition has deteriorated because of the absence of information on TPPM cultivation technology. Although the two problems have been resolved, the solution provided cannot increase the volume and quality of production (Nguyen *et al*., 2019). Farmers have even complained that TPPM cultivation nowadays is ineffective because the production output is insufficient to cover the costs (Bahiyah *et al*., 2019). This phenomenon has caused some farmers to switch to the cultivation of other species, such as milkfish, seaweed, and other kinds of TPPM (Rajendran *et al*., 2016). Studies on water sources for TPPM cultivation contaminated with toxic heavy metals, polycyclic aromatic hydrocarbon (PAH) compounds, and other potential contaminants, including microplastics, medical and agricultural waste, and residues, are limited (Marzuki *et al*., 2021a). The South Sulawesi Marine Fisheries Service has also raised concerns over potential problems in TPPM cultivation caused by water contamination with heavy metals (Ezemonye *et al*., 2019), PAHs, and other pollutants from agricultural businesses (Syah *et al*., 2014); industrial activities; and the dynamics of community life (Marzuki *et al*., 2020). Previous studies have reported that several types of bacteria have bioremediation capabilities against pollutants, such as heavy metals, PAHs, pesticides, and microplastics (Tavabe *et al*., 2019). These specific bacteria can be isolated from several areas contaminated with heavy metals, including soil, rivers, lakes, and swamps, and from coastal areas and the open sea (Scheuch *et al*., 2020). According to a previous study, bacteria living in lead-contaminated areas (Marzuki, 2020) generally absorb or remediate the metal. In other words, the remediation ability of bacteria is specific to environmental conditions (Medeiros *et al*., 2017). Several related studies have shown that various types of bacteria have a symbiotic relationship with marine biota; for example, sponges exhibit remediation behavior against heavy metal pollutants (Marzuki *et al*., 2023b). Bacteria from sponge marine biota with the capability to remediate the toxic properties of heavy metals include *Acinetobacter calcoaceticus*, which is isolated from *Callyspongia aerizusa*; it can remediate hexavalent chromium (Selvin *et al*., 2009). *Bacillus cohnii*, which is isolated from *Niphates* sp., can remediate pollutants, such as chrome, copper, iron, zinc, cobalt (Marzuki *et al*., 2021b), manganese, and cadmium (Qian *et al*., 2020). *Bacillus licheniformis*, isolated from *Auletta* sp., has the potential to remediate arsenic and mercury pollutants (Shama *et al*., 2010). Other studies have found that bacteria can biodegrade hydrocarbon components, including carcinogenic and mutagenic types of PAHs (Liu *et al*., 2017). The identified types include *Acinetobacter calcoaceticus* isolated from the sponge *Clathria (Thalysias) reinwardtii*. *Bacillus flexus* can degrade saturated and aromatic hydrocarbon components (Scheuch *et al*., 2020).

This study offers a novel bioremediation method to degrade and remove the toxic properties of heavy metals contained in TPPM cultivation media. Group bacteria bioremediators *Bacillus* (Bs) and *Pseudomonas* (Ps) isolated from marine sponges were directly applied by engineering the maintenance media (Yang *et al*., 2006). During the experiment, three different elements were incorporated into a single cultivation medium, and each was subjected to distinct treatments, namely, copper ion (Cu^{2+}) and lead ion (Pb^{2+}) pollutants and the bacteria bioremediators *Bacillus pumilus* and *Pseudomonas stutzeri* (Abbasi *et al*., 2023). The primary objective was to increase the

survival rate (SR) and reduce the exposure to heavy metals without exceeding the maximum allowable limit (Duan *et al*., 2021). The aim of this study was to reduce the toxicity of copper (Rahman, 2024) and lead pollutants by using a bioremediation method that adopts marine sponge symbiont Bs and Ps bacterial bioremediators (Tavabe *et al*., 2029). The hypothesis in this study was that the introduction of sponge symbiont bacteria as bioremediators can effectively reduce the concentrations of heavy metal pollutants (Cu^{2+} and Pb^{2+}) in the cultivation water of TPPM, thereby improving the SR and quality of prawn production without compromising prawn growth (Marzuki *et al*., 2021c). The parameters for determining the success of the method were analyzed based on TPPM growth parameters (body weight and length), SR, specific growth, and health status (tissue damage) in vital parts of the body (brain, tail, and shell; Du *et al*., 2022). The results of this research can be useful in overcoming the problems faced by farmers, especially in South Sulawesi, in relation to the development of environmental management for shrimp farming (Nascimento *et al*., 2017). Handling hazardous and toxic materials (removal of heavy metals) through environmentally friendly bioremediation methods by using Bs and Ps bacteria is the main achievement of this research (Liu *et al*., 2017). Another objective is to analyze the growth and survival of TPPM in the presence of bioremediator bacteria. The success of this study is a new milestone in scientific development, especially in pollutant handling, bioremediation methods, and biotechnology–microbiology of coastal marine environments (Muqsith *et al*., 2019). This study was conducted in Barru and Makassar Regencies, South Sulawesi Province, Indonesia, in 2023.

MATERIALS AND METHODS

Materials and equipment

The material used consisted of a bioremediator in the form of two bacteria types that had been characterized for phenotype and genotype. Specifically, the *Bacillus pumilus* strain GLB197 (Bs) isolated from *Niphates* sp. and the *Pseudomonas stutzeri* strain RCH2 (Ps) from *C. (Thalysias) reinwardtii* were used (Orania *et al*., 2018). The experimental setup included TPPM PL 20 days; Pb^{2+} pollutant in lead(II) acetate trihydrate $(CH_3COO)_2Pb.3H_2O$, pro analyst (pa.) preparation, 15 mg/L; Cu^{2+} pollutant in copper(II) sulfate pentahydrate ($CuSO_4.5H_2O$), pa. preparation, 12.5 mg/L, chlorine pa.; triosulfate citrate bile salt sucrose agar media, pa. 70 percent (%) alcohol, formalin pa.; food pellet type 100 MS Prima feed; cultivation media water; and Aquabidest doubly purified water (Abdelrahman *et al*., 2019). Meanwhile, the equipment consisted of a triple-scale platinum resistance temperature detector, digital sense meter for measuring the potential of hydrogen, and dissolved oxygen (DO) meter (Rahman, 2024). Atomic absorption spectroscopy (AAS; variant type AA240FS), a muffle furnace (Thermolyne F6010, Shanghai, China), portable water (quality type 8361, Instrument Corp., Taichung, Taiwan), stainless-steel mesh filters (5 and 0.3 mm), a shaker, a density separator, a vacuum pump, a digital scale, a caliper, a sample bottle, an Eppendorf tube, an electron microscope, an electric aerator, a maintenance box measuring 120 x 75 x 50) cm, a digital camera, a test tube, and a sample bottle were also used (Marzuki *et al*., 2021c).

Procedure

Preparation of materials, such as bacteria suspensions, was conducted by obtaining stock isolates of Bs and Ps bacteria and incubating them for 2 x 24 h. Both isolates were suspended in Aquabidest water, and the number of colonies was counted (Musa *et al*., 2023). The Pb^{2+} solution (1,000 mg/L) was prepared by weighing $(CH_3COO)_2Pb.3H_2O$, dissolving it in 1,000 mL of Aquabidest water, and crushing it until a concentration of 1,000 mg/L was obtained. The same procedure was applied to transform the Cu^{2+} solution of 1,000 mL to a Cu^{2+} solution of 1,000 mg/L (Avnimelech and Ritvo, 2003). TPPM cultivation was performed using a Styrofoam tank measuring (120 x 75 x 50) cm^3 lined with a layer of black plastic and directly filled with 120 L of water from a previously filled tank. Chlorine with a concentration of 20 mg/L was added, and a water pump was installed for aeration and left overnight (Chávez and González. 2013). Treatment was performed through the addition of bacteria suspension with three variations, namely, Box 1 with Bs suspension, Box 2 with Ps bacteria, and Box 3 mixed with Bs+Ps. The colony population of the bioremediator bacteria (Bs with 336,102 cells/mL and Ps with 367,102 cells/mL) was then left for 1 x 24 h (Cortésa *et al*., 2021). Box 1 was subsequently added with ±1,200 L of Cu^{2+} with 1,000 mg/L contaminant

concentration. Box 2 was added with ± 1,200 L of Pb^{2+} with 1,000 mg/L contaminant concentration, and Box 3 was added with 1,200 L each of Cu^{2+} and Pb^{2+} solutions with the same concentration. The engineered cultivation medium was contaminated with two types of pollutants (Cu^{2+} and Pb^{2+}) to reach an average of ±10 mg/L or 20 times higher than the threshold, namely, 0.5 mg/L. L for Cu^{2+} according to SNI 01-3553-2006 and 0.5 mg/L for Pb^{2+} according to SNI 7387:2009 (Bapat and Jaspal, 2016). Treatment I was a combination of three materials, namely, Bs and Ps remediating bacteria and Cu^{2+} contaminant (Cu^{2+}: Bs + Ps). Treatment II consisted of a combination of Bs and Ps together with Pb^{2+} contaminant (Pb^{2+}: *B. pumilus* strain GLB197 + *P. stutzeri* strain RCH2, Bs + Ps; Bibi *et al*., 2016). Treatment III contained Bs and Ps and two types of contaminants, namely, Cu^{2+} and Pb^{2+} (Cu^{2+} + Pb^{2+}: Bs + Ps; Zhou *et al*., 2012). Each treatment box was homogenized for about 5 min by using a water pump for aeration, and all water quality parameters were measured. The concentrations of Cu^{2+} and Pb^{2+} in the cultivation water and TPPM were measured seven times, with each being sampled periodically at maintenance periods of 0, 5, 10, 15, 20, 25, and 30 days (Ezemonye *et al*., 2019). Subsequently, each treatment was left for 1 x 24 h, and, on the following day, the procedure for adding TPPM was applied. TPPM samples that were declared healthy (totaling to 120) were used, and two of them were added to each box to determine the initial average weight and length. The concentration of the heavy metal pollutants was determined before any treatment and regarded as the sample at day 0 (Sarkar *et al*., 2016). The first feeding was conducted three hours after stocking the TPPM seeds and continued every 4 h or 6 times a day for up to 30 days of rearing. Feed portions were given in accordance with the number and age of TPPM, and aeration was performed for 24 h (De Melo *et al*., 2018).

Data presentation and analysis

The data collected through various methods were broadly categorized into two types. The first one involved direct field measurement that included treatment characteristics in the form of temperature in °C, pH, salinity in ‰, oxidation-reduction potential (ORP) in mV, DO percent value, and DO content in mg/L (Niklitschek and Secor, 2009). Data on TPPM growth (weight and length) and mortality were used to calculate the SR value (Du *et al*., 2022). The second type was data obtained after measurements in the laboratory, including bioremediator colonies of Bs and Ps bacteria and histology of TPPM tissue in vital parts, namely, the head, tail, and shell (Sou *et al*., 2017). Measurement of Cu^{2+} and Pb^{2+} concentration was conducted in accordance with the existing sample series (7x each) for each treatment in the cultivation water and TPPM samples by using the Lambert–Beer equation from absorbance data measured by the AAS instrument (Gupta *et al., 2013*). The specific growth, weight, and body length of TPPM were determined using Eqs. 1 and 2 (Musa *et al*., 2023). The average survival was also assessed with Eqs. 1 and 2 (Rahman, 2024).

$$SGR_W (\%) = \frac{\ln Wt - \ln Wo}{t} \times 100\ \%, \quad (1)$$

$$SGR_L (\%) = \frac{\ln Lt - \ln Lo}{t} \times 100\ \%, \quad (2)$$

Where, SGR_W is the specific growth rate of body weight (%/day), SGR_L is specific growth rate of body length (%/day), Wt is the body weight at the end of the treatment (g), Wo is the body weight at the beginning of the treatment (g), Lt is the body length at the end of the treatment (cm), Lo is the initial body length of the TPPM seeds during the treatment (cm), and *t* is the rearing time (days) obtained using Eqs. 3 and 4 (Musa *et al*., 2023).

$$SR (\%) = \frac{PMt}{PMO} \times 100\ \%, \quad (3)$$

$$PM_t = PM_o - PM_1, \quad (4)$$

Where, the SR percentage is the TPPM SR (%), *Penaeus monodon* seeds

(PMo) is the number of TPPM seeds stocked at the start of the treatment, PM_t is the number of TPPM seeds that lived, and PM_1 is the number of deaths during the maintenance period (Mohanty *et al*., 2016).

The bioremediation performance of Bs and Ps bacteria in removing Cu^{2+} and Pb^{2+} pollutants, which can be determined using Eqs. 5 and 6, is an important indicator of the superiority of the bioremediator used in this study (Karimpour *et al*., 2018). The bioremediation performance of Bs and Ps was

determined by calculating the difference in Cu^{2+} and Pb^{2+} concentrations at the beginning of the treatment (Co) in relation to the total concentration of Cu^{2+} and Pb^{2+} pollutants in the maintenance water (C_{CW}) and the concentration of these pollutants adsorbed in the TPPM body (C_{PM}) and given the symbol C1. Eqs. 5 and 6 were used for the calculation (Motaghi and Ziarati, 2016).

$$BR\ (\%) = \frac{C0 - C1}{Co} x100\ \%, \quad (5)$$

$$C_1 = C_{CW} + C_{PM}, \quad (6)$$

Where, BR (%) is the bioremediation performance of Bs and Ps bacteria in %, Co is the initial concentration of each Cu^{2+} and Pb^{2+} pollutant in the cultivation water in mg/L, C_{CW} is the concentration of Cu^{2+} or Pb^{2+} in the cultivation water in mg/L, C_{PM} is the concentration of Cu^{2+} or Pb^{2+} absorbed by TPPM in mg/kg, and W_1 is the sum of the concentrations of Cu^{2+} or Pb^{2+} in the cultivation water and TPPM or $C_{CW} + C_{PM}$ (Musa *et al.*, 2023).

RESULTS AND DISCUSSION

Physical and chemical changes are likely to occur in TPPM cultivation water media because of several internal and external factors. Internal factors include dirt from TPPM and leftover feed, and external factors comprise excess feed volume, incoming and outgoing water circulation, use of fertilizers and pesticides, accompanying organic materials, lighting, temperature, and potential exposure to heavy metals and hydrocarbon compounds (Girjatowicz and Świątek, 2019). These factors have the potential to influence changes in cultivation water quality parameters, including physical alterations in pH, salinity, oxygen concentration, adequacy of cultivation media, and ORP (Bahiyah *et al.*, 2019). The provision of sponge symbiont bacteria bioremediators interferes with toxic contaminants that can hinder the growth and development of TPPM, thus increasing SR (Paul and Vogl, 2012).

Physical and chemical characteristics of cultivation water

The resistant physicochemical characteristics observed in TPPM cultivation media could be caused by internal and external factors (Engle *et al.*, 2017). The determined chemical parameters of cultivation water quality included levels of dissolved organic matter, nitrite, nitrate, and ammonia (Sun *et al.*, 2019). The use of cultivation media engineered by administering Cu^{2+} and Pb^{2+} contaminants and adding marine sponge symbiont bacterial bioremediators improved the physicochemical quality, thus ensuring the growth and development of TPPM on the basis of rearing time (Ali *et al.*, 2019). The influence of cultivation media engineering is depicted in Tables 1 and 2.

The chemical characteristics of cultivation water and the changes in the dissolved organic matter and levels of nitrite, nitrate, and ammonia contributed to alterations in the chemistry of the media, thereby affecting the growth and development of TPPM (Cavazos and Alonso, 2017). The degree of acidity, temperature (Abdelrahman *et al.*, 2019), salinity, oxygen demand, and ORP of cultivation media were likely to change when the period of cultivation was lengthened (Ribeiro *et al.*, 2016). The fulfillment of the physical quality standards for maintenance water showed that the presence of bioremediators did not cause changes in the physical characteristics of the TPPM maintenance media (Bui *et al.*, 2012). The cultivation media temperature was relatively stable at an average of 28.16 degrees Celsius (°C)–28.68 °C (Table 1) (Duan *et al.*, 2019). The acidity level (pH) tended to increase, but it was in the stable category and ranged within 7.60–7.75 (Du *et al.*, 2022) presumably because of the redox reaction induced by the excess feed and waste from cultivated TPPM. However, this potential was not observed in the oxygen demand, which showed a decreasing trend in terms of concentration and portion contained in the media (Roleda and Hurd, 2019). According to the ORP data, which were all > 100 mV, aeration in all passages was efficient (Table 1) (Tavabe *et al.*, 2019). The chemical characteristics of the cultivation water are presented in Table 2.

The growth and development of TPPM were influenced by several factors, including chemical, physical, cultivation, contamination, disease, and other effects (Tables 1 and 2). The data presented in Tables 3 and 4 and Fig. 1 show the growth trend of TPPM during 30 days of cultivation (Du *et al.*, 2022). Dissolved organic matter decreased with increasing cultivation time. The result implies that the innate organic material content was consumed as nutrition by TPPM, but this could cause problems

Table 1: Characteristics of water quality medium for cultivating TPPM

Treatment	Measurable parameters	Measurement and maintenance period (days)							Average	Standard quality	Sources
		0	5	10	15	20	25	30			
(I) Cu^{2+}; (Bs+Ps)	Temp. (^{0}C)	28.90	28.20	29.10	28.70	28.17	28.87	28.91	28.69	26 – 33	Abdelrahman *et al*., 2019
	pH	8.06	8.03	7.59	7.57	7.50	7.45	7.31	7.64	7.8 – 8.3	Millard *et al*., 2021
	Salinity (‰)	36.22	35.72	39.50	36.14	39.44	39.22	39.61	37.98	0.5–45.0	Mohanty *et al*., 2016
	OD (%)	73.60	79.20	79.40	75.60	61.67	44.45	63.21	68.16	> 60.00	Qian *et al*., 2020
	OD (mg/L)	4.51	5.01	4.72	4.30	3.01	2.77	3.26	3.94	> 3.0	Qian *et al*., 2020
	ORP (mV)	66.20	70.70	71.40	63.20	58.10	50.01	71.90	64.50	≤ 100.0	Kanjal *et al*., 2023
(II) Pb^{2+}; (Bs+Ps)	Temp. (^{0}C)	28.80	28.20	28.00	29.30	28.21	28.07	28.10	28.38	26 – 33	Abdelrahman *et al*., 2019
	pH	8.38	8.08	7.78	7.30	7.23	7.21	7.19	7.60	7.8 – 8.3	Millard *et al*., 2021
	Salinity (‰)	36.27	35.64	39.53	36.52	39.16	39.16	39.33	37.94	0.5–45.0	Mohanty *et al*., 2016
	OD (%)	62.60	89.10	57.30	76.30	60.10	60.52	65.10	67.29	> 60.00	Qian *et al*., 2020
	OD (mg/L)	3.89	5.01	3.59	5.52	3.72	3.71	4.01	4.21	> 3.0	Qian *et al*., 2020
	ORP (mV)	54.20	78.10	56.40	79.90	58.52	58.05	59.60	63.54	≤ 100.0	Kanjal *et al*., 2023
(III) (Cu^{2+}+Pb^{2+}) (Bs+Ps)	Temp. (^{0}C)	28.2	29.10	27.50	28.90	27.79	27.65	28.01	28.16	26 – 33	Abdelrahman *et al*., 2019
	pH	8.50	7.89	7.79	7.69	7.54	7.45	7.36	7.75	7.8 – 8.3	Millard *et al*., 2021
	Salinity (‰)	36.51	39.50	40.04	39.26	39.09	39.91	39.44	39.11	0.5–45.0	Mohanty *et al*., 2016
	OD (%)	70.00	79.40	42.20	61.20	47.60	44.74	6.7	58.12	> 60.00	Qian *et al*., 2020
	OD (mg/L)	4.32	4.72	2.54	3.79	2.99	2.89	3.01	3.47	> 3.0	Qian *et al*., 2020
	ORP (mV)	46.20	71.40	53.60	58.10	50.52	52.40	54.40	55.23	≤ 100.0	Kanjal *et al*., 2023

Table 2: Chemical characteristics of the cultivation water for TPPM

Treat-ment	Quality of cult. water (mg/L)	Measurement period (days)							Average (mg/L)	Standard quality (mg/L)	Sources
		0	5	10	15	20	25	30			
(I) Cu^{2+}; (Bs+Ps)	TOM	60.50	52.69	52.21	52,27	33.43	29.67	23.34	43.4443	≤ 90	Musa *et al*., 2021
	NO_2-N	0.0052	0.0034	0.0027	0,0019	1.0810	0.1765	0.2138	0.2121	≤ 0.5	Juliyanto *et al*. 2021
	NO_3-N	0.2352	0.2425	0.4654	0,6654	2.3461	1.1896	0.1678	0.7589	0.4 – 0.8	Kamilia *et al*., 2021
	NH_3-N	0.2531	0.2480	0.2457	0,2422	0.4047	1.3421	2.0363	0.6817	< 0.80	Chen *et al*., 2016
(II) Pb^{2+}; (Bs+Ps)	DOM	62.51	59.36	57.23	55,81	43.84	34.87	24.05	48.2386	≤ 90	Musa *et al*., 2021
	NO_2-N	0.0054	0.0022	0.0018	0,0011	1.2408	0.9876	0.4648	0.3862	≤ 0.5	Juliyanto *et al*. 2021
	NO_3-N	0.2356	0.1906	0.1867	0,1754	2.0244	0.1586	0.1537	0.4464	0.4 – 0.8	Kamilia *et al*., 2021
	NH_3-N	0.2542	0.2494	0.2573	0,2803	0.2190	0.2679	1.4409	0.4241	< 0.80	Chen *et al*., 2016
(III) (Cu^{2+} + Pb^{2+}); (Bs+Ps)	DOM	60.50	50.39	50.22	50,05	10.14	21.98	23.37	38.0929	≤ 90	Musa *et al*., 2021
	NO_2-N	0.0054	0.0033	0.0030	0,0026	1.1858	0.7872	0.5235	0.3587	≤ 0.5	Juliyanto *et al*. 2021
	NO_3-N	0.2344	0.1357	0.1976	0,2434	2.5747	1.9874	0.1984	0.7959	0.4 – 0.8	Kamilia *et al*., 2021
	NH_3-N	0.2545	0.2294	0.2347	0,2575	0.2028	0.5681	1.6526	0.4857	< 0.80	Chen *et al*., 2016

in TPPM's health status (Nkuba *et al*., 2021). The nitrite and nitrate contents increased with increasing maintenance time, but no fixed pattern was observed (Furtado *et al*., 2014). The ammonia content also increased with increasing cultivation time, but the increase was inconsistent or exhibited fluctuations (Velthof *et al*., 2012) across all treatments (Table 2).

Specific growth rate of TPPM

The specific growth rate of TPPM is a crucial measure of successful cultivation, and development and growth, specifically the increase in weight and body length, are essential for economic viability (Niklitschek and Secor, 2009). In this study, the increase in the weight and body length of TPPM fluctuated when the rearing time was increased (Saiya and Katoppo, 2015). As determined by the linear equations for the three treatments, the body weight and length of TPPM showed a positive trend in direct proportion to increased rearing time (Sarkar *et al*., 2016). The correlation value was close to 1, suggesting that the increase in rearing time positively influenced the weight and body length of TPPM (Tables 3 and 4). Media engineering through the provision of bioremediator materials of Bs and Ps bacteria isolated from marine sponges reduced the toxic properties of Cu^{2+}, Pb^{2+}, and the mixture of both. This intervention was reflected in the unstable, fluctuating increase in TPPM weight across all treatments (Ma *et al*., 2021).

TPPM continued to show growth and development during the 30 days of maintenance. Physical observations and measurements of body weight and length were conducted every 5 days. The results

Table 3: Development of the body weight of TPPM on the basis of rearing time

Treatment	Development of TPPM weight (g) based on maintenance period (days)									Equality linear	Correlation (R^2)
	0	5	10	15	20	25	30	Amount (g)	Average (g)		
(I)	0.160	0.230	0.390	0.550	0.490	0.530	1.220	3.570	0.510	y = 0.0277x + 0.0943	0.7449
(II)	0.120	0.440	0.360	0.390	0.450	0.620	0.790	3.170	0.453	y = 0,0176x + 0.1893	0.8151
(III)	0.180	0.220	0.380	0.410	0.740	0.690	0.870	3.490	0.499	y = 0.0241x + 0.1375	0.9348

Table 4: Increase in the body length of TPPM on the basis of rearing time

Treatment	Development of body length (cm) of TPPM based on maintenance period (days)									Equality linear	Correlation (R^2)
	0	5	10	15	20	25	30	Amount (cm)	Average (cm)		
(I)	2.60	3.00	3.98	4.30	4.54	4.23	5.55	28.20	4.03	y = 0,0848x + 2.7568	0.8688
(II)	2.20	3.11	3.41	3.70	4.43	4.60	4.50	25.95	3.71	y = 0.0779x + 2.5393	0.9096
(III)	2.50	2.34	2.65	3.82	4.65	4.67	4.80	25.43	3.63	y = 0,0969x + 2.1800	0.8841

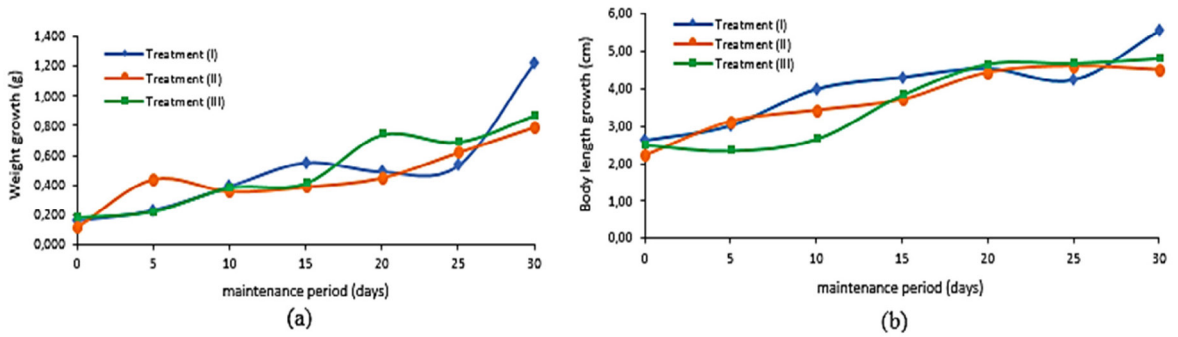


Fig. 1: Development and growth of TPPM in relation to the maintenance period: (a) development of the weight of TPPM and (b) increase in the body length of TPPM.

showed an increase in the average body weight of TPPM in the three treatments; the average body weight reached 0.49 g or 220.26% of the average initial weight (Musa *et al*., 2023). The average increase in body length also increased considerably and reached 3.79 mm or 55.97% of the initial average. This finding implies that TPPM was not physically affected by the presence of Cu^{2+} and Pb^{2+} pollutants (Cortésa *et al*., 2021). The R^2 value showed that the increase in the body length of TPPM was the most stable in Treatment III, followed by Treatments II and I (Du *et al*., 2022).

The data in Table 5 show that the average specific growth of TPPM in terms of weight and body length was the highest in Treatment I containing Cu^{2+}, followed by Treatment II composed of Pb^{2+} and Treatment III engineered with a combination of both pollutants (the lowest). This finding suggests that TPPM was more tolerant to Cu^{2+} than to Pb^{2+} (Rahman, 2024).

The toxicity of Pb^{2+} was stronger than that of Cu^{2+}, and in general, the average specific growth rate, specifically the weight (6.10%/day) and length (2.36%/day) of the body, was high presumably because of the bioremediator that remediated the two types of pollutants and reduced the pressure

Table 5: Average specific body growth and body length of TPPM prawns during the rearing period

Treatment	W_0 (g)	Wt (g)	Lo (cm)	Lt (cm)	t (days)	SGR_W (%.day^{-1})	SGR_L (%.day^{-1})
(I)	0.160	1.220	2.60	5.55	30	6.7717	2.5277
(II)	0.120	0.790	2.20	4.50	30	6.2820	2.3853
(III)	0.180	0.870	2.50	4.80	30	5.2517	2.1743

Table 6: Concentration of heavy metal contaminants in the water for cultivating TPPM in relation to the maintenance period

Treatment	Concentration of heavy metals (mg/L) based on maintenance period (days)							Average (mg/L)	Equality Linear	Correlation (R^2)
	0	5	10	15	20	25	30			
Cu^{2+}-CW/ (I)	9.433	0.169	0.123	0.106	0.084	0.076	0.067	1.437	y = -0.0038x + 0.171	0.9045
Pb^{2+}-CW/ (II)	11.408	0.828	0.724	0.644	0.517	0.276	0.253	2.093	y = -0.0248x + 0.9748	0.9629
Cu^{2+}-CW/ (III)	9.432	0.143	0.098	0.084	0.071	0.065	0.057	1.421	y = -0.0031x + 0.1401	0.8527
Pb^{2+}-CW/ (III)	11.443	0.677	0.655	0.540	0.437	0.402	0.391	2.078	y = -0.0131x + 0.7466	0.9304

of toxicity on TPPM growth (Musa *et al*., 2023). This relationship presumably plays a role in helping Bs and Ps remediator bacteria stabilize the quality of cultivation water. The unstable increase in weight was also accompanied with fluctuations in body length (Figs. 1a and 1b). Table 6 and Figs. 2 and 3 show the effects of Bs and Ps bacteria bioremediators on removing heavy metal pollutants to minimize the spread of toxic properties, resulting in the enhanced growth and average survival of TPPM (Paul and Vogl, 2012). Lead, copper, and other heavy metals do not decompose in the body and have accumulative abilities. As their concentrations increase, their toxicity puts increasing pressure on the body during prolonged exposure (Heidarieh *et al*., 2013).

The richness of the various rearing media increased with increasing exposure time (Lebel *et al*., 2010). The administration of Bs and Ps bacterial bioremediators also reduced the level of exposure to Cu^{2+} and Pb^{2+}. The growth and development of TPPM in terms of weight and body length increased, indicating that the TPPM in the engineered media was not resistant to the presence of Bs and Ps remediator bacteria (Kasan *et al*., 2019).

Trends in the bacteria bioremediation of copper and lead ion pollutants

A bacteria bioremediator can also reduce the concentration of heavy metal contaminants in cultivation media (Liu *et al*., 2017a). The trends of pollutant exposure in TPPM and cultivation media in relation to the length of rearing time are presented in Table 6.

Cu^{2+} and Pb^{2+} exposure on TPPM cultivation media in all the treatments exhibited a decreasing trend on days 0–5 and sloped on days 10–30. This result reveals the bioremediation of the toxic properties of the pollutants by Bs and Ps bacteria (Bellebcir *et al*., 2023). The result was supported by the regression equation data, which showed a positive trend and an R^2 value close to 1. However, further analysis is needed to determine if the reduction in Cu^{2+} and Pb^{2+} pollutant concentrations on cultivation media is caused by sponge symbiont bacteria bioremediators (Table 6 and Figs. 2a and 2b; Esteves *et al*., 2016).

A decreasing concentration of Cu^{2+} and Pb^{2+} pollutants was observed in the TPPM cultivation water engineered by administering heavy metal contaminants and a bioremediator (Figs. 3a and 3b). The decrease in Pb^{2+} concentration in Treatment I was sharper than that in Treatment II (Table 6 and Fig. 3b; Roleda and Hurd, 2019). This disparity could have been caused by the potential for competition among bacteria cells to obtain nutrients, hindering optimal cell division. Consequently, the population of both types of bacteria decreased, leading to reduced bioremediation performance against heavy metal Cu^{2+} and Pb^{2+} concentrations (Armus *et al*., 2020). A considerable reduction in pollutant levels occurred in the cultivation media. The initial

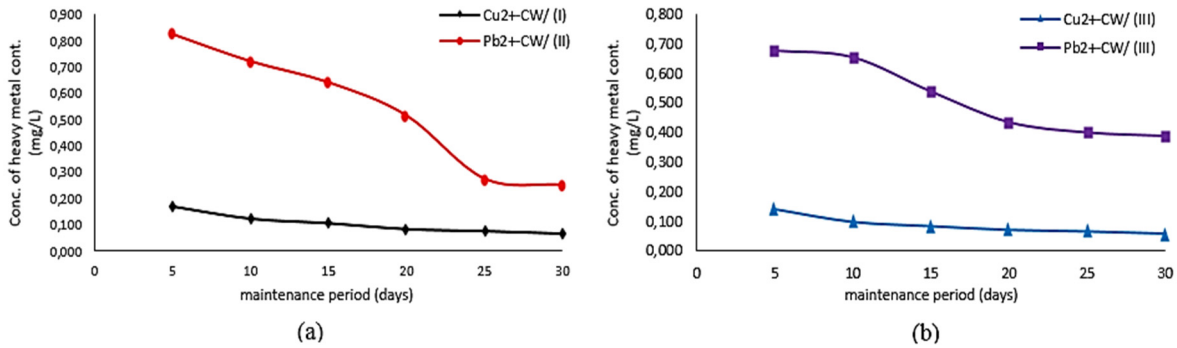


Fig. 2: Concentration of heavy metal contaminants in TPPM cultivation water. (a) Cu^{2+} vs. Pb^{2+} concentrations for Treatments I and II and (b) Cu^{2+} vs. Pb^{2+} concentrations for Treatment III.

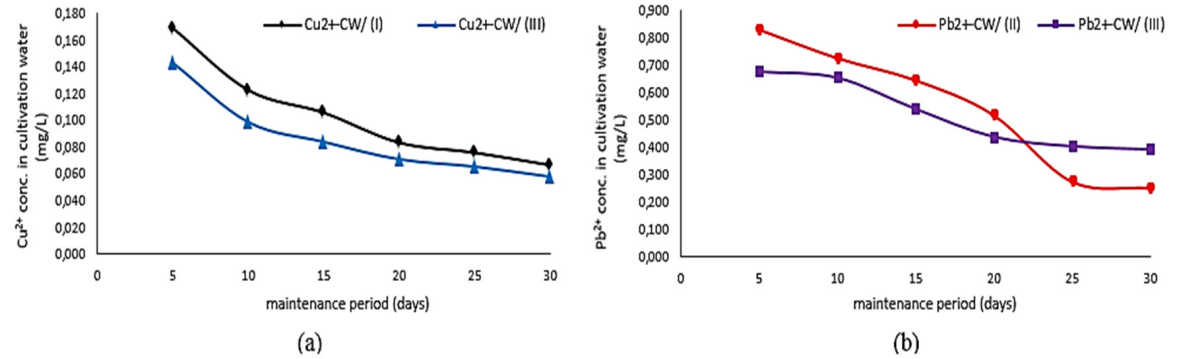


Fig. 3: Comparison of the concentrations of heavy metal contaminants in the cultivation water of TPPM: (a) Cu^{2+} for Treatments I and III and (b) Pb^{2+} for Treatments II and III.

concentration of the two pollutants (i.e., ±10 mg/L) decreased to ±0.1557 mg/L for Cu^{2+} and 0.7526 mg/L for Pb^{2+} on day 5 of rearing (Marzuki *et al*., 2023b). However, the concentration of both pollutants continued to decrease, and the decline was gradual until the maintenance period reached 30 days. The concentration in this range was between 1.4213 and 1.4367 mg/L, with an average decrease of 84.85% for the Cu^{2+} pollutant. Similarly, the concentration range of 2.0779–2.0928 mg/L was reduced by 81.75% for Pb^{2+} (Table 6; Al-Busaidi *et al*., 2011). The pathway for reducing the concentration of heavy metal pollutants in cultivation water was assumed to be distributed in two parts; some accumulated in the body of TPPM, and the others underwent remediation by the marine sponge symbiont bacteria Bs and Ps (Heidarieh *et al*., 2013).

The level of heavy metal contamination in TPPM treated with a sponge symbiont bacteria bioremediator through the addition of Cu^{2+} and Pb^{2+} pollutants to engineered cultivation media with several treatment variations is presented in Table 7.

The concentration of the two pollutants in the body of TPPM was in the range of 1.6036–2.2442 mg/L equivalent to 12.02% for Cu^{2+} and 3.5632–5.0182 mg/L equivalent to 22.97% for Pb^{2+} (Table 7). The difference in the concentration of pollutants between the body and the cultivation media relative to the initial concentration was attributed to the performance of the Bs and Ps bioremediators in detoxifying the toxic properties of Cu^{2+} and Pb^{2+} (Lorenzon *et al*., 2001). The Cu^{2+} and Pb^{2+} concentrations in TPPM increased with increasing cultivation time primarily because the two types of heavy metals were not decomposed but accumulated in the body (Karbassi *et al*., 2016). If cultivated TPPM is exposed for a long period, even though the Cu^{2+} and Pb^{2+} levels in the cultivation media do not exceed the threshold of 0.5 mg/L set by SNI 01-3553-2006, the concentration in the body can exceed the required threshold value of 0.8–1.2

Table 7: Concentration of heavy metal contaminants in TPPM engineered by administering a marine sponge symbiont bacterial bioremediatory

Treatment	Concentration of heavy metals (mg/kg) in TPPM based on the maintenance period (days)							Average (mg/kg)	Equality linear	Correlation (R^2)
	0	5	10	15	20	25	30			
Cu^{2+}-PM/ (I)	0.154	0.272	0.311	0.311	0.331	0.385	0.481	0.321	y = 0.0088x + 0.1891	0.8929
Pb^{2+}-PM/ (II)	0.219	0.221	0.459	0.481	0.577	0.692	0.915	0.509	y = 0,0225x + 0,1715	0.9482
Cu^{2+}-PM/ (III)	0.099	0.141	0.141	0.187	0.264	0.319	0.452	0.229	y = 0,0110x + 0,0641	0.9081
Pb^{2+}-PM/ (III)	0.317	0.382	0.410	0.789	0.912	0.982	1.227	0.717	y = 0,0317x + 0,2416	0.9482

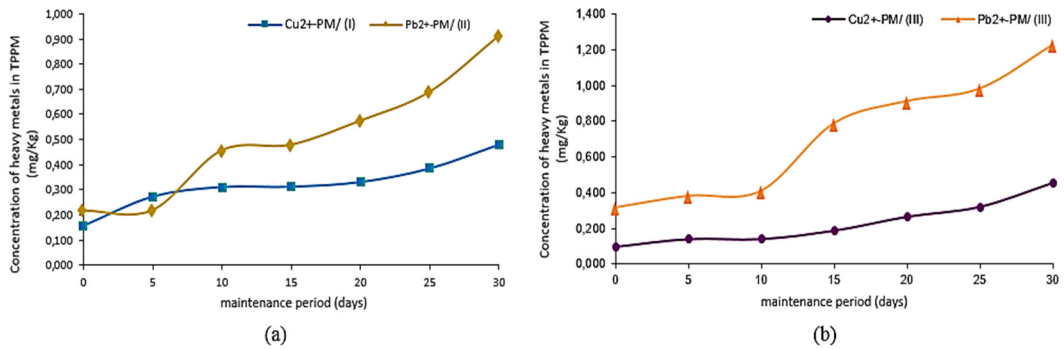


Fig. 4: Concentration of heavy metal contaminants in TPPM cultivation media. (a) One heavy metal (Cu^{2+} or Pb^{2+}) in Treatment I/II and (b) mixed Cu^{2+} and Pb^{2+} in Treatment III.

mg/kg for Cu^{2+} (BPOM No. 03725/B/SK/VII/2009) and <10 mg/kg for Pb^{2+} (PBPOM No. 32/2019). On the average, the Cu^{2+} concentration in the TPPM body ranged within 0.2291–0.3206 mg/kg with a rearing period of 30 days for the two different treatments in the current study (Table 7; Nascimento *et al*., 2017). A similar result was obtained after exposure to the heavy metal Pb^{2+}, the concentration of which ranged within 0.5090–0.7169 mg/kg in the cultivated TPPM but was still lower than the required threshold (Table 6).

The comparison of the exposure levels of Cu^{2+} and Pb^{2+} in the TPPM body for the same treatment showed that Pb^{2+} exposure was stronger than Cu^{2+} exposure. This finding suggests that the TPPM body's resistance to Pb^{2+} was lower than its resistance to Cu^{2+} (Figs. 4a and 4b; Zhen *et al*., 2022). Low exposure to both pollutants in TPPM cultivated for 30 days revealed the role and contribution of the bioremediator that remediated the toxic properties (Zhou *et al*., 2012). Theoretically, exposure to heavy metal Cu^{2+} should be much higher than the results of the current experiments performed for an interaction period of 30 days (Marzuki, 2020b). The cultivation medium was engineered by adding the Cu^{2+} pollutant to two treatments at concentrations of 9.4335 and 9.4317 mg/L or almost 20 times higher than the threshold, namely, 0.5 mg/L (Ali *et al*., 2019). Similarly, Pb^{2+} was added to two treatments at initial concentrations of 11.4077 and 11.4425 mg/L or more than 20 times higher than the threshold, namely, 0.5 mg/L (Table 6 and Figs. 4a and 4b). These findings show that the sponge symbiont Bs and Ps bioremediator materials added to each treatment played a remarkable role (Bibi *et al*., 2016).

The two bioremediators helped remediate the toxic properties of the Cu^{2+} and Pb^{2+} pollutants and reduce the amount adsorbed through nutrition and circulation (Ghosh *et al*., 2023). A comparison of exposure levels to the heavy metal Cu^{2+} in TPPM was conducted for two different treatments. In Treatment I, the cultivation medium was engineered by adding

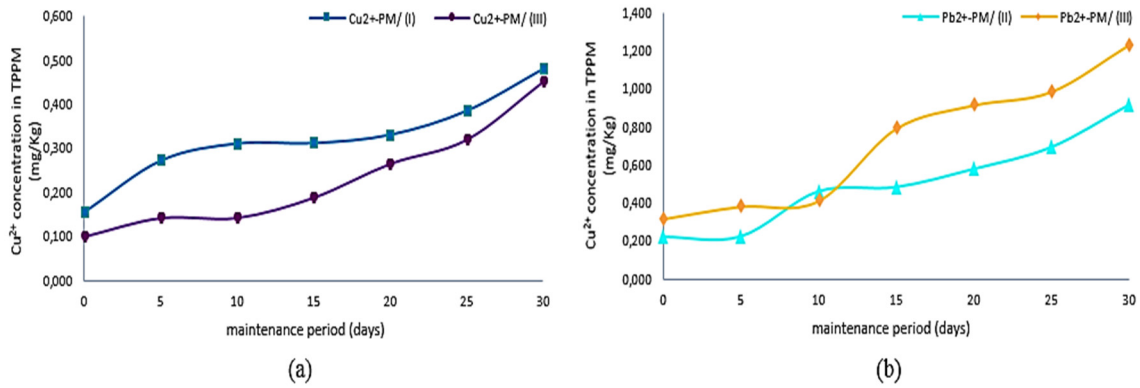


Fig. 5: Comparison of the adsorption concentrations of Cu^{2+} and Pb^{2+} pollutants in TPPM. (a) Cu^{2+} in Treatment I vs. Treatment III and (b) Pb^{2+} in Treatment II vs. III.

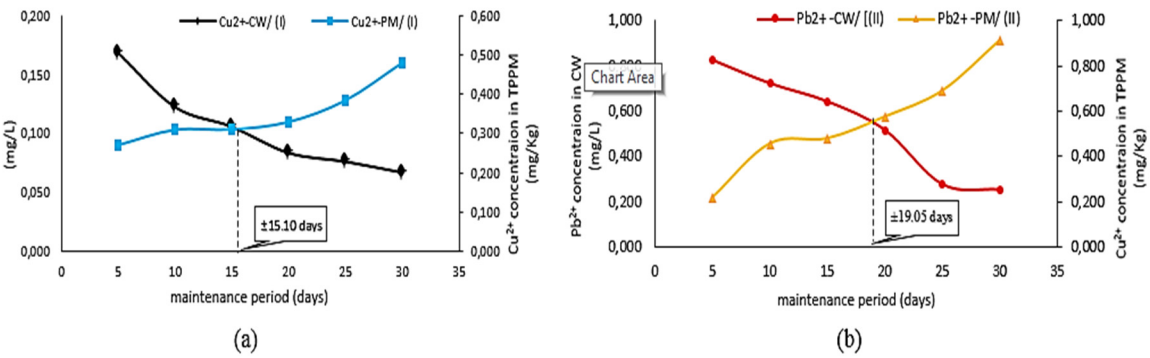


Fig. 6: Comparison of heavy metal pollutant concentrations in the cultivation water and PM prawns. (a) Cu^{2+} concentration in Treatment I and (b) Pb^{2+} concentration in Treatment II.

Cu^{2+} to bioremediators Bs and Ps. Meanwhile, in Treatment II, the bioremediator number and type were the same, but two heavy metal pollutants were provided (Fig. 5a). The results of the two experiments confirmed Cu^{2+} exposure. The values of Treatment I were higher than those of Treatment II (Duan *et al.*, 2019). Similar steps were implemented for a comparative analysis of the exposure to the heavy metal Pb^{2+}. The results fluctuated and overlapped in the first 10 days of contact. In the 30-day rearing period, exposure to Pb^{2+} in Treatment II was more dominant than that in Treatment I (Fig. 5b; Marzuki *et al.*, 2023a).

The other engineered cultivation media involved the provision of two bioremediators and heavy metal. The analysis results showed that the level of exposure in TPPM and the concentration in the cultivation water had an inverse relationship. The concentration of Cu^{2+} and Pb^{2+} in TPPM increased with increasing cultivation time, and the concentration in the cultivation water decreased with increasing exposure time (Figs. 6a and 6b; Velthof *et al.*, 2012). Fig. 7 shows a comparison of the contamination rates of Cu^{2+} and Pb^{2+} in TPPM and the cultivation water.

In this treatment, the two intersection points for Cu^{2+} concentration were visible in the cultivation water and TPPM with a contact period of 15–20 days. Meanwhile, the Pb^{2+} pollutants were observed during the 20-day maintenance period (Figs. 6a and 6b). In the subsequent treatment, two types of bioremediators (Bs and Ps) and two types of heavy metals were provided. The intersection of Cu^{2+} and Pb^{2+} concentration was visible in TPPM and the cultivation media and occurred in the cultivation period of 15–20 days (Figs. 7a and 7b; Marzuki *et al.*, 2021b). For Treatment I, the equilibrium point for Cu^{2+} concentration in the cultivation water versus

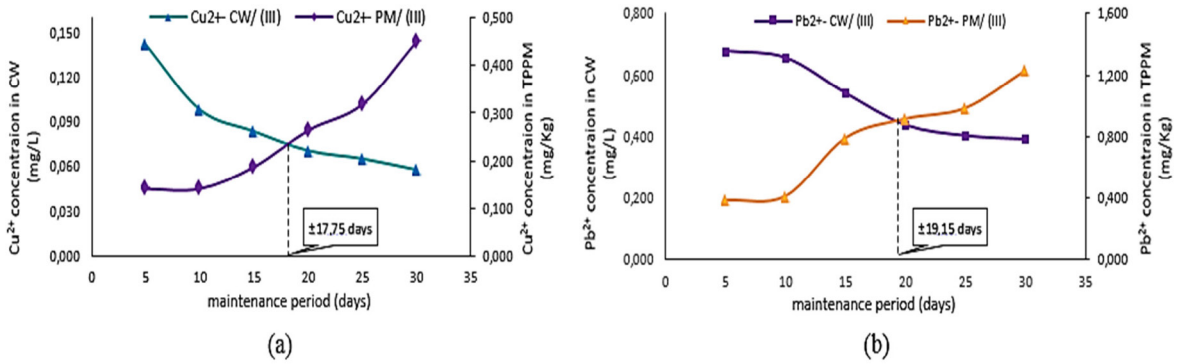


Fig. 7: Comparison of heavy metal pollutant concentrations in the cultivation water and PM prawns. (a) Cu^{2+} concentration in Treatment III and (b) Pb^{2+} concentration in Treatment III.

Table 8: SR and relationship between TPPM mortality rate and treatment

Treatment	ΣPM_O	ΣP_{M1}	ΣP_{Mt}	SR (%)
(I)	120	48	72	60.00
(II)	120	47	73	60.83
(III)	120	26	94	78.33

TPPM was reached on day 15.10 of the rearing period, and the equilibrium point for Pb^{2+} concentration was reached on day 19.05 (Figs. 6a and 6b). The equilibrium points for Cu^{2+} and Pb^{2+} concentrations in Treatment II were achieved on days 17.75 and 19.15, respectively (Figs. 7a and 7b). Figs. 6 and 7 show that adsorption of Cu^{2+}/Pb^{2+} pollutants still occurred in TPPM and led to an increase in the concentration even after passing the equilibrium point (Liu *et al.*, 2017a). This phenomenon was attributed to the nature of the two pollutants, which can accumulate in objects as the interaction time increases. Meanwhile, the concentration tended to decrease after the equilibrium point (Ezemonye *et al.*, 2019). The effect of the heavy metal pollutants on TPPM growth was reflected in the growth and SR values, including the microscopic analysis and tissue damage specifically in the head, tail, and shell (Table 8).

In general, the SR of TPPM was high when the growth parameters, including the conditions required for survival, were met. However, this result does not guarantee that TPPM with high viability can be considered healthy and free from contamination by dangerous toxic, carcinogenic, and mutagenic materials, such as heavy metals, PAHs, and microplastics (Walker and Winton, 2010). Exposure to heavy metal pollutants in TPPM cannot be physically observed, so SR is not a measure of safety and suitability for consumption. According to the results, SR reached 60.83% in Treatment II, but it was only 60% in Treatment I (Table 8; Beardsley *et al.*, 2011). Among all the treatments, Treatment III produced the highest SR value of 78.33% (Table 8; Qian *et al.*, 2020). This finding offers several perceptions, including the idea that the difference in SR values among the three treatments reflects the influence or role of the Bs and Ps bioremediators in remediating the toxic properties of heavy metals (Suo *et al.*, 2017). The SR of TPPM increased in all the treatments and reached an average of 66.33%. This value is higher than the average SR (i.e., 54.67%) of similarly cultivated TPPM without the addition of Bs and Ps bioremediators (Du *et al.*, 2022). These findings show that the presence of remediating bacteria can increase SR (Tavabe *et al.*, 2019). Another assumption is that TPPM exhibits different immunity to each heavy metal contaminant. The bioremediation performance of Bs and Ps bacteria against Cu^{2+} and Pb^{2+} pollutants is presented in Table 9.

Bs and Ps bacteria's bioremediation performance against Cu^{2+} is better than their bioremediation performance against Pb^{2+} (Marzuki *et al.*, 2021a). In the current study, the value for Cu^{2+} reached 94.19% in Treatment I and 94.60 % in Treatment III. Meanwhile, the value for the Pb^{2+} pollutant was low, namely,

Table 9: Bioremediation performance of Bs and Ps bacteria against Cu^{2+} and Pb^{2+} pollutants in TPPM cultivation media after 30 days of cultivation

Treatment	Co (mg/L)	C_{CW} (mg/L)	C_{PM} (mg/kg	$C_1 = (C_{CW} + C_{PM})$ (mg/L)	Percent BR (%)
Cu^{2+}; (I)	9.4335	0.0668	0.4809	0.5477	94.19410
Pb^{2+}; (II)	11.4077	0.2529	0.9152	1.1681	89.76042
Cu^{2+}; (III)	9.4317	0.0575	0.4517	0.5092	94.60119
Pb^{2+}; (III)	11.4425	0.3908	1.2272	1.6180	85.85973

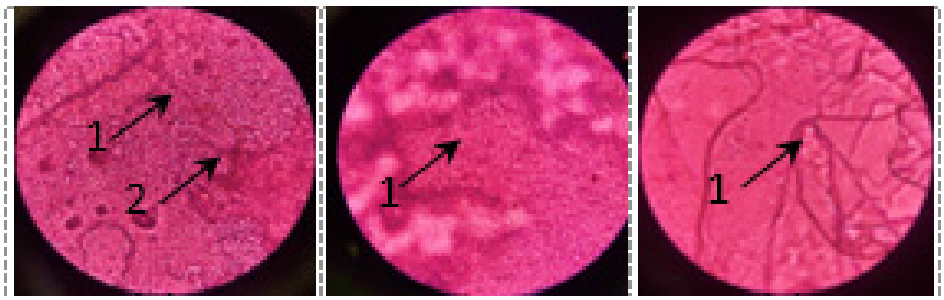


Fig. 8: Damage to cell tissue in the head of TPPM in (a) Treatment I, (b) Treatment II, and (c) Treatment III.

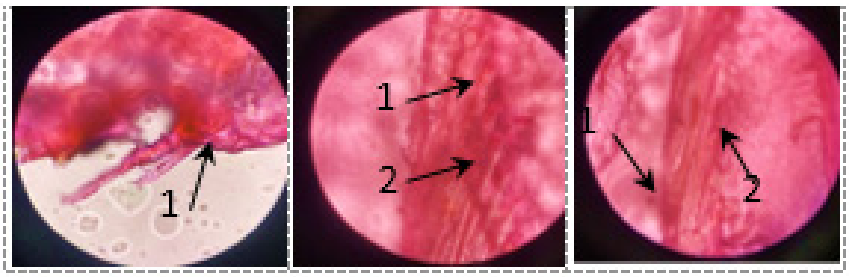
89.76% in Treatment II and 85.86% in Treatment III, as shown in Table 9 (Armus *et al*., 2020). Pb^{2+} was assumed to have stronger toxicity than Cu^{2+}, making it difficult for bioremediator bacteria to initiate cell division and increase their population (Marzuki *et al*., 2023a). The bioremediation performance of Bs and Ps bacteria in remediating the toxic properties of Cu^{2+} averaged 94.40% in Treatments I and III (Qian *et al*., 2020). A similar technique was applied to examine the role of these bacteria in remediating the toxic properties of Pb^{2+}, and an average of 87.81% was obtained for Treatments II and III (Zhou *et al*., 2012).

SR and the influence of $Cu^{2+}$and Pb^{2+}pollutants on the health status of TPPM

The immunity of TPPM to Pb^{2+} pollutants is reportedly stronger than that of Cu^{2+} (Lebel *et al*., 2010). The tissue damage in several important parts of TPPM in the three treatments was analyzed in this study, and the head produced different results (Fig. 8). This condition is in line with the previous assumption that the immunity of TPPM to different heavy metal pollutants varies (Du *et al*., 2022). Analysis of the damage to head tissue showed symptoms of necrosis and infiltration (*Sites 1 and 2* in Fig. 8a, respectively), myoma or muscle abnormalities (*Site 1* in Fig. 8b), and infiltration (*Site 1* in Fig. 8c) (Walker and Winton, 2010).

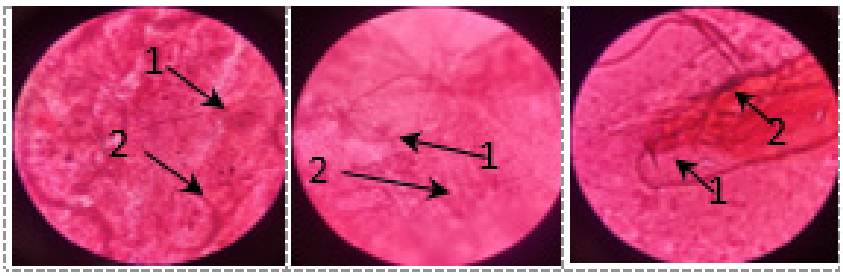
These symptoms were caused by exposure to the Cu^{2+} and Pb^{2+} pollutants (Marzuki *et al*., 2023b). Damage to cell tissue was also observed in the tail, which exhibited symptoms of necrosis (*Site 1* in Fig. 9a) and myopathy and infiltration (*Sites 1 and 2* in Figs. 9b and Fig. 9c) (Ali *et al*., 2019). Furthermore, symptoms of tissue damage, specifically necrosis and infiltration in *Sites 1* and *2* (Figs. 10a and 10b), necrosis in *Site 1* in Fig. 10c, and infiltration in *Site 2* in Fig. 10c were observed (Beardsley *et al*., 2011). All cell disorders or damage in the head, tail, and shell (Figs. 8–10) were caused by exposure to heavy metal pollutants Cu^{2+} and Pb^{2+} (Karimpour *et al*., 2018).

According to these results, TPPM was more resistant to Cu^{2+} than to Pb^{2+}. This resistance suppressed the growth rate, resulting in a reduced SR value (Du *et al*., 2022). Bioremediators Bs and Ps contributed positively to reducing the accumulation of heavy metal pollutants and ensured that TPPM was appropriately exposed to Cu^{2+} and Pb^{2+} pollutants below the allowed threshold value (Zhen *et al*., 2022). The analysis of the cell tissue in several vital parts of TPPM showed symptoms of tissue damage or problems with decreased cell function attributed to the presence of toxic agents in the head, tail, and shell (Karimpour *et al*., 2018). These symptoms included necrosis, myopathy, and infiltration (Kasan *et al*., 2019).



(a) (b) (c)

Fig. 9: Damage to cell tissue in the tail part of TPPM in (a) Treatment I, (b) Treatment II, and (c) Treatment III.



(a) (b) (c)

Fig. 10: Damage to cell tissue in the shell part of TPPM in (a) Treatment I, (b) Treatment II, and (c) Treatment III.

The analysis of tissue damage to the head, tail, and shell of TPPM revealed the strong effects of the heavy metal pollutants on TPPM's health after being exposed to a range of concentrations between 0.2291 and 0.3206 mg/Kg for Cu^{2+} and 0.5090 and 0.7169 mg/Kg for Pb^{2+} in the 30-day maintenance period (Table 6). These concentrations are considerably lower than the maximum allowable limit but can still cause health problems (Muqsith *et al.*, 2019). These results are alarming because exposure to heavy metals in low concentrations can exert substantial negative effects on the health status of TPPM, and these effects are characterized by symptoms of necrosis, infiltration, and myopathy or cell tissue damage in several parts of the body (Sarkar *et al.*, 2016), specifically the head, tail, and shell (Figs. 8–10; Ghosh *et al.*, 2023). Bioremediators, such as bacteria with biosorption capabilities against the toxic properties of heavy metals, can be used to address this problem (Abbasi *et al.*, 2016). In this study, sponge isolates of Bs isolated from *Niphates sp*. and Ps from *Clathria (Thalysias) reinwardtii* were used to remediate contaminants. Bacterial bioremediators should be given early before the spread of TPPM seeds (Orania *et al.*, 2018).

CONCLUSION

In conclusion, the bioremediation process considerably reduced the concentrations of Cu^{2+} and Pb^{2+} pollutants in the cultivation water and thus contributed to a safe environment for TPPM growth and development. The presence of the bioremediator bacteria did not negatively affect the physical growth of TPPM, as evidenced by the increase in the average body weight and length over the 30-day maintenance period. Despite the positive effects on growth, symptoms of tissue damage were still observed in TPPM, indicating that even subthreshold levels of heavy metal exposure can exert detrimental effects on the health of marine organisms. Another critical finding of the study is that subthreshold levels of heavy metal exposure could still cause tissue damage in TPPM, underscoring the importance of stringent monitoring and management of pollutant levels. The bioremediation performance of Bs and Ps bacteria against Cu pollutants (±94.40%) is relatively higher than for Pb pollutants (±87.81%) possibly because the toxicity level of Pb is higher than that

of Cu, thus affecting the bioremediation performance of both bacteria. The positive response or immunity of TPPM to heavy metal pollutants Cu^{2+} and Pb^{2+} differs, so TPPM can survive in environments contaminated with heavy metals. Three physical parameters in this study showed that TPPM can live and adapt to a cultivation environment contaminated with Cu and Pb pollutants. First, the average SR of TPPM was high in the cultivation medium that was not engineered with remediating bacteria. Second, the body weight and length of TPMM increased. Last, the daily specific growth, body weight, and body length of TPPM were above the average values for conventionally cultivated shrimp. Hence, TPPM can survive with positive SR in environments exposed to Cu^{2+} and Pb^{2+} pollutants with low concentrations. Exposure of TPPM to Cu^{2+} and Pb^{2+} even at low concentrations (below the maximum allowable threshold value) can exert negative effects on TPPM and damage cells and tissues, as characterized by symptoms of neurosis, myopathy, and infiltration in the head, tail, and shell of cultivated TPPM. Moreover, the physical and chemical characteristics of the culture media engineered with the addition of Bs and Ps bioremediator bacteria were relatively similar to those of the cultivation water without any engineering, which means that TPPM can adapt to remediator bacteria. These results show that the presence of Bs and Ps bioremediator bacteria does not cause resistance and does not have a negative effect on the growth of TPPM, indicating that TPPM can adapt to bioremediator bacteria.

AUTHOR CONTRIBUTIONS

I. Marzuki guided the second to fifth authors in the data analysis during study implementation; data analysis of bacterial bioremediation performance, SR, TPPM growth, and tissue damage; data interpretation; and manuscript preparation. I. Pratama contributed to the analysis of heavy metal pollutant concentrations in the cultivation water and TPPM and helped prepare the article's writing material. R. Asaf, contributed to the water quality analysis and played a role in writing the article. A. Athirah contributed to the engineering, physical, and chemical analyses of the cultivation media. K. Nisaa contributed to the analysis of the disease and other health problems of TPPM and helped write the article. N. Nurbaya participated in the interpretation of TPPM disease. M. Muslimin contributed to the processing and interpretation of data on the relationship between pollutants and TPPM growth. N. Nurhidayah contributed to data processing and determining the level of bacterial bioremediation. S. Suwardi participated in the interpretation of TPPM SR and preparation of the article for publication. A. Sahrijanna contributed to the calculations and analysis of TPPM growth and development. K. Kamaruddin analyzed the relationship between Bs and Ps bioremediators on the level of pollutant remediation.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, ethical issues, informed consent, plagiarism, infringement, falsification and/or falsification of data, multiple publication and/or submission, and redundancy have been fully considered by all authors involved in the study and writing of the manuscript.

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ABBREVIATIONS

<	Greater than
>	Smaller than
%	Percent
$(CH_3COO)_2Pb.3H_2O$	Lead (II) acetate trihydrate
‰	Per milli
^0C	Degrees Celsius
AAS	Atomic absorption spectrophotometry
BPOM	Food and Drug Regulatory Agency
BR	Bioremediation
Bs	*Bacillus pumilus* strain GLB197
Bs + Ps	*Bacillus pumilus* strain GLB197 + *Pseudomonas stutzeri* RCH2
C_1	Final concentration
C_{CW}	Concentration in cultivation water
cells/mL	Cells per milliliter
cm	Centimeter
cm^3	Cubic centimeter
C_o	Initial concentration
C_{PM}	Concentration in prawn PM
Cu^{2+}	Copper ion with a valence of 2
$CuSO_4.5H_2O$	Copper (II) sulfate pentahydrate
CW	Cultivation water
DO	Dissolved oxygen
DOM	Dissolved organic matter
Eq.	Equation
g	Grams
L_o	Initial length
L_t	Final length
mg/kg	Milligrams per kilogram
mg/L	Milligrams per liter
mL	Milliliter
mm	Millimeter
mV	Millivolt
NH_3-*N*	Nitrogen bound in ammonia
NO_2-*N*	Nitrogen bound in nitrite
NO_3-*N*	Nitrogen bound in nitrate
OD	Oxygen demand
ORP	Oxidation reduction potential
pa	Pro analytics
PAHs	Polycyclic aromatic hydrocarbons
Pb^{2+}	Lead ion with a valence of 2
PF	Food pellets
pH	Potential of hydrogen
PL	Post-larvae
PM	*Penaeus monodon*
PM_o	*Penaeus monodon* seeds
PM_1	Dead *Penaeus monodon*
PM_t	Living *Penaeus monodon*
Ps	*Pseudomonas stutzeri* RCH2
R^2	Correlation
SNI	Indonesian National Standards
SGR_w	Specific growth rate of weight
SGR_L	Specific growth rate of body length
sp	Species
SR	Survival rate
t	Times (day)
TCBSA	Triosulfate citrate bile salt sucrose agar
RTD	Resistance temperature detector
TPPM	Tiger prawns (*Penaeus monodon)*
W_o	Initial body weight
W_t	Final body weight

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