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Jurnal Ilmiah Ilmu Terapan Universitas Jambi
Vol. 8 No. 1. June 2024, pp. 212-230
DOI. 10.22437/jiituj.v8i1.32094
Research Article

PUMAKKAL FORMULA FOR MAKING SHRIMP POND WASTE FERTILISER

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Article Info
Recieved: Mar 13, 2024
Revised: Apr 28, 2024
Accepted: May 09, 2024
OnlineVersion: May 14, 2024

Abstract
One way to decompose pond waste sediment is by using the liquid nutrient culture media (NB) called Pumakkal as a starter formula. However, bioremediation in shrimp ponds has received less attention, despite being massively promoted by the government. East Lampung has produced 11.6 million m^3 of liquid and 4.077 m^3 of sediment waste in shrimp ponds. They are rich in organic matter, but possibly poison the pond if uncurbed. This study investigated in what manner *Pumakkal* decomposed shrimp pond waste under three parameters: macronutrient and micronutrient contents, and pH. The experiment was conducted using a completely randomised design (CRD) with 15 factorial arrangements. The sample was 65 kg of shrimp pond waste and 65 litres of liquid waste, which were analysed with five treatments: three of liquid waste media (LW), sediment waste (SW), and mixed liquid and sediment waste (MLS). The results showed that the treatment of the CE 15-isolate in MLS significantly improved the fertiliser quality ($p<0.05$). Pumakkal CE worked the finest in MLS, producing the fertiliser with the best macronutrients: Nitrogen (N) 1,3%, Phosphorus (P)2,3%, and Potassium (K) 2,3%; C-organic 23%, C/N ratio 29; micronutrient: Fe:155 ppm, Cu: 51 ppm, Zn: 72 ppm, Mn; 51 ppm, B; 25 ppm, and Mo: 8 ppm, and pH 5-6. The mixture of liquid and pond sediment waste produces the best fertiliser suitable for plant fertiliser users. The study concludes that Pumakkal is effective in decomposing harmful waste sediment to support the bioremediation program.

Keywords: Bioremediation, Nutrient levels, Pumakkal, Shrimp Ponds' Sediment and Wastewater

INTRODUCTION

In recent decades, studies in bioremediation have spread over the globe, including Indonesia. However, this issue should have received more attention despite being massively promoted by the government, particularly shrimp ponds' bioremediation. The current and previous studies are mostly on growth regulators in agriculture (Pérez-Álvarez et al., 2022). Prior studies, such as *Bacillus Probiotics* and Bioremediation (Kamilya & Devi, 2022) have connectivity in that the study in terms of prebiotic bacteria, water quality, and aquaculture. Henceforth, the current study promoted "pumakkal," a trademark (Sutanto, 2021) with essential bioremediation called “Waste Organic Pollutants” (Sutanto, 2016). A report issued various variables such as evaluation of bioremediation with aquaculture sediments (Mandario et al., 2019), as well as on water remediation in shrimp pond (Shinde et al., 2022), and Probiotics and Bioremediation (Kamilya & Devi, 2022). Several variables of bioremediation are studied with attributes of water-exchanged shrimp ponds (Joseph et al., 2021) and active microbiological processes in bioremediation of earthen shrimp ponds (Colette et al., 2023). In short, these studies support the current study by providing scientific contributions to global remediation studies.

In the last five years, total shrimp production in Indonesia has increased by 15.7%. Lampung Province is listed as the largest shrimp-producing area in Indonesia. The national shrimp production reached 348,100 tons, of which 45% was produced from the 2,175-hectare shrimp-producing area in Lampung, producing a volume of 11.6 million m^3 of liquid waste and 4,077 m3 of sediment waste every harvest time (Yuka et al., 2020, 2021). Pond wastewater contains organic matter such as BOD, DO, COD, TSS, phosphate, and high ammonia; 22-57% of the nitrogen that is put into the pond will be wasted with the liquid waste and pollute the pond (Fatimah & Sari, 2018; Marliani, Siagian, 2022); Pond sediment solid waste has a high nutrient content, such as N total 0,67%, P_2O_5 4,78%, K_2O 1%, C-organic 17,87%, pH 6,25, and water content 15,60% (Suwoyo et al., 2017). Meanwhile, the fishery sector in Purworejo Village, Pasir Sakti District, East Lampung Regency is dominated by 80% of the shrimp and fish pond industry. Vaname Shrimp (*Litopenaeus vannamei*) cultivation has a high productivity, resulting in high waste. The amount of sediment waste generated in shrimp ponds with a density of 1,250 fish/m^2 is 21,9 tons, and a density of 1000 fish/m^2 by 20,3 tons (Mulyani & Kartasapoetra, 2002; Novizan, 2002). The problem with shrimp ponds is the accumulation of leftover feed, shrimp excreta, and microorganisms, which reduces water quality and eventually disrupts the life processes of vannamei shrimps. Long-term liquid waste and sediment accumulation will reduce productivity, pond quality, and coastal ecosystems.

Liquid waste and shrimp pond sediments have not been utilised so far. They are high in organic matter content, so potentially be used as a fertiliser through a bioremediation process, in which microbes decompose the organic matter. Bioremediation is an effort to improve the environment by involving the presence of organisms in nature. The process of waste treatment will be more accessible by utilising the activities of microorganisms to break down the substances in the waste materials into simpler ones (Ghosh et al., 2021). Bioremediation requires living agents in the form of plants, animals, and microorganisms, including bacteria and fungi, to to decompose toxic compounds. Consortia *Bacillus sp*. and *Pseudomonas sp*., as well as *Saccharomyces sp., Nitrosomonas sp.,* and *Nitrosobacter sp.*, can reduce the concentration of organic matter in shrimp pond sediments up to 60% (Devaraja et al., 2002). These bacteria were also found in Pineapple Liquid Waste (Sutanto, 2011), indicating that there were 15 isolates of indigenous bacteria with different specifications for degrading organic matter, such as carbohydrates, proteins, starch, and fats. Indigenous bacterial isolates, known as Pumakkal, can decompose organic matter through bioremediation, raise pH, and produce CO_2 and H_2O, which are safe for the aquatic environment (Sutanto, 2011). Organic matters in the shrimp pond contain nutrients and high organic matter, decomposed by Pumakkal bacteria into simpler compounds and become organic fertiliser (Suwoyo et al., 2017). For its effectiveness, it is necessary to assemble five bacterial formulas (consortia). According to their hydrolytic abilities based on groups, quantities, and particular specifications, there are consortia A (CA) consists of 3 isolates, consortia B (CB) 6 isolates, consortia C (CC) 9 isolates, consortia D (CD) 12 isolates, and consortia E (CE) 15 bacterial isolates.

The 15 bacterial isolates used were *Bacillus licheniformis, Bacillus cereus, Bacillus cereus, Bacillus subtilis, Bacillus cereus, Bacillus subtilis, Acinetobacter baumannii, Acinetobacter baumannii, Klebsiella oxitoca, subtilis, Bacillus cereus, Pseudomonas pesudomallei, Actinobacillus iwoffii, Actinobacillus iwoffii,* and *Bacillus* that are firm to degrade protein, starch, and fat. Five formulas of Pumakkal will degrade shrimp pond sediments into fertilisers and be measured by macronutrient and micronutrient parameters. Macronutrient parameters were Nitrogen (N), Phosphorus (P), Potassium (K), C-organic, and C/N. Micronutrient parameters were Fe, Cu, Zn, Mn, B, Mo, and pH 5-6; refer to Indonesian Ministry of Agriculture Regulation Number: 261/KPTS/SR.310/M/4/2019 concerning minimum technical requirements for organic fertilisers, biological fertilisers, and soil amendments (Indonesian Ministry of Agriculture, 2019). The research benefit is to utilise shrimp pond waste (liquid and sediment waste) as organic fertilisers using five Pumakkal bio-remediator formulas and fulfilling the Minister of Agriculture criteria.

The current study aims to investigate how *Pumakkal* (with 15 isolates) decomposed shrimp pond waste under three parameters: macronutrient and micronutrient compounds, and pH. The experiment used a completely randomised design (CRD) with a 5 x 3 factorial arrangement. The factorial employs 3 x 5 Factorial Bidirectional Variance Analysis, Variable X1 = Media Variation (X1.1= Liquid Waste; X1.2= Sediment Waste; X1.3= Mixture SPL Waste and Sediment), X2= Formula Pumakal (X2.1 = CO; X2.2= CA; X2.3= CB; X2.4= CC; X2.5= CD; X2.6= CE) and Variable Y= fertiliser quality: macronutrients: Nitrogen (N), Phosphorus (P), Potassium (K); C-organic and C/N ratio; micronutrients: Ferrum (Fe), Cuprum (Cu), Zinc (Zn), Manganese (Mn), Boron (B), and Molybdate (Mo), and Degree of acidity (pH). The study intends to solve the problem of shrimp ponds, which is the accumulation of leftover feed, excreta, and microorganisms that can reduce water quality and eventually disrupt the life processes of Vannamei shrimp. Moreover, long-term liquid waste and sediment accumulation will reduce productivity, pond quality, and coastal ecosystems. Therefore, this research aims to solve the problems as a novelty of the current study.

RESEARCH METHOD

The study employed a quantitative approach under factorial experimental design with five treatments (CO, CA, CB, CC, CD, and CE). The Pumakkal isolate starter formulas were combined with the shrimp pond waste from Pasir Sakti Lampung for the rearing period of 120 days. In details, 65 kg of shrimp pond waste and 65 litres of liquid waste from Pasir Sakti Lampung was used, five formulas of treatment experiments (CO, CA, CB, CC, CD, and CE) were applied, and three treatments of liquid waste media (LW), sediment waste (SW), and mixed liquid and sediment waste (MLS) for in vitro and pilot plan scales. Liquid nutrient culture media (NB) manufactured by Pumakkal were used as the isolate starter formula.

The study involved shrimp pond waste from Pasir Sakti, Lampung. The samples were taken by purposive random sampling. The aim was to produce organic fertiliser by bioremediating the shrimp pond waste.

There were two research stages, in vitro and a pilot plan, according to the procedures of (Sutanto, 2012) and Mekala et al., (2023). The ability of the Pumakkal formula to decompose liquid waste and shrimp pond sediments waste to produce fertiliser is according to the standard of the Indonesian Ministry of Agriculture. *This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the University's Ethics Committee (December 22, 2022/No: 123/II.3.AU/F/UMM/2022).*

Preparation of the Pumakkal starter formula was conducted by using liquid nutrient media/Nutrient Broth (NB) according to the procedure of Abna et al. (2017) and Tantray et al. (2023). There are 5 Pumakkal formulas, each was 750 ml NB. The compositions of the five formulas (a) Consortia A (CA) with 3 isolates, bacterial isolates 2, 3, and 5 with the type of bacteria *Bacillus cereus, Bacillus cereus*, *and Bacillus cereus* to degrade fat, (b) Consortia B (CB) with 6 bacterial isolates, bacterial isolates 4, 5, 6, 7, 12, and 14 with the type of bacteria *Bacillus subtilis, Bacillus cereus, Bacillus subtilis, Acinetobacter, Pseudomonas pesudomallei*, and *Actinobacillus iwoffii* to degrade starch, (c) Consortia C (CC) with 9 bacterial isolates 1, 2, 3, 8, 10, 11, 12, 14, and 15 with the types of bacteria *Bacillus licheniformis, Bacillus cereus, Bacillus cereus, Acinetobacter baumannii, Bacillus subtilis, Bacillus cereus, Pseudomonas pesudomallei, Actinobacillus iwoffii,* and *Bacillus* are firm to degrade protein, (d) Consortia D (CD) with 12 bacterial isolates, bacterial isolates 1, 2, 3, 7, 8, 9, 10, 11, 12, 13, 14, and 15 with the type of bacteria *Bacillus licheniformis, Bacillus cereus, Bacillus cereus, Acinetobacter baummanii, Acinetobacter baummanii, Klebsiella oxitoca, Bacillus subtilis, Bacillus cereus, Pseudomonas pesudomallei, Actinobacillus iwoffii, Actinobacillus iwoffii*, and *Bacillus* are firm to degrade protein and starch, and (e) Consortia E (CE) consisted of 15 bacterial isolates, bacterial isolates 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15, with the type of bacteria *Bacillus licheniformis , Bacillus cereus, Bacillus cereus, Bacillus subtilis*, and *Bacillus* are firm to degrade protein, starch, and fat. The five Pumakkal formulas were used to degrade three media: liquid waste, sediment, and liquid/sediment mixture of shrimp ponds, each volume was 300 ml. Fermentations were conducted for 30 days, and the analyses of the parameters of macronutrients were the contents of Nitrogen (N), Phosphorus (P), Potassium (K), organic carbon (C-organic), C/N ratio, and micronutrients: Fe, Cu, Zn, Mn, B, Mo, and pH. The analysis was conducted at the Chemistry Laboratory of the Universitas

Muhammadiyah Malang, Malang. The data were analysed quantitatively using the ANOVA test and qualitatively to test the quality of fertiliser by comparing them to the Indonesian Ministry of Agriculture Regulation Number: 261/KPTS/SR.310/M/4/2019 (Indonesian Ministry of Agriculture, 2019). Testing the effectiveness of the Pumakkal formula was followed by a pilot plan test, in which a volume of liquid waste, sediment, and a mixture of 1 litre was used. There was also controlled aeration, agitation for 30 days, treatment, and observation according to the steps mentioned previously.

The data analysis was conducted in the Chemistry Laboratory of Universitas Muhammadiyah Malang, Indonesia. The expert laboratory technicians analysed the contents of the organic fertilisers produced from the shrimp pond waste. Data analysis techniques with 3 x 5 Factorial Bidirectional Variance Analysis, Variable X1 = Media Variation (X1.1= Liquid Waste; X1.2= Sediment Waste; X1.3= Mixture SPL Waste and Sediment), X2= Formula Pumakal (X2.1 = CO; X2.2= CA; X2.3= CB; X2.4= CC; X2.5= CD; X2.6= CE) and Variable Y= fertiliser quality: macronutrients: Nitrogen (N), Phosphorus (P), Potassium (K); C-organic and C/N ratio; micronutrients: Ferrum (Fe), Cuprum (Cu), Zinc (Zn), Manganese (Mn), Boron (B), and Molybdate (Mo), and Degree of acidity (pH).

RESULTS AND DISCUSSION

The current study investigates the way Pumakkal (with 15 isolates) decomposed shrimp pond waste under three parameters: macronutrient and micronutrient compounds, and pH. The experiment used a completely randomised design (CRD) with a 5 x 3 factorial arrangement, and the results are recapped in Table 1.

Table 1. Recap of Pumakkal Formula Test Data Analysis on Shrimp Pond Waste to produce Liquid, Solid, and Mixed Fertilisers

	Pumakkal Formula (CO, CA, CB, CC, CD and CE); Waste Shrimp (Liquid Waste (LW)), Sedimen Waste (SW) and Mix Liquid Sedimen (MLS)																	
	Control (0 Isolate)			Consortia A (3 Isolate)			Consortia B (6 Isolate)			Consortia C (9 Isolate)			Consortia D (12 Isolate)			Consortia E (15 Isolate)		
	LW CO	SW CO	MLS CO	LW CA	SW CA	MLS CA	LW CB	SW CB	MLS CB	LW CC	SW CC	MLS CC	LW CD	SW CD	MLS CD	LW CE	SW CE	MLS CE
Macronutrieat Nitrogen (N) %	0.4a	0.5a	0.9ab	0.6a	0.8b	1ab	1.1a	1.1a	0.9b	1a	1.1a	1.2ab	1a	1.1a	1.1b	1.1a	1.2b	1.3ab
Photophorus (P) %	0.4a	0.7a	0.9ab	0.6a	1.5b	1.3b	0.3a	1.5b	1.3b	0.6a	1.7b	1.5ab	0.3a	1.8b	1.6ab	0.5a	2.3b	2ab
Potassium (K) %	0.2a	0.6b	0.8ab	0.5a	1.6b	1.3ab	0.3a	1.5b	1.4ab	0.5a	1.8b	1.5ab	0.4a	1.9b	1.6ab	0.5a	2.3b	2.1ab
C-Organic %	7a	11b	10ab	12a	16a	14ab	13a	18b	17b	11a	18b	18b	12a	20b	20b	14a	23b	21ab
C/N ratio	-	11a	10b	-	20a	15b	-	17a	17a	-	22a	20b	-	28a	27b	-	29a	26b
Micronutrient Ferum (Fe) ppm	80a	85b	85b	92a	97b	100ab	100a	150b	150b	95a	100b	105ab	96a	98b	99ab	102a	150b	155ab
Cuprum (Cu) ppm	20a	25b	26ab	23a	30b	30b	28a	29	32b	33ab	40a	45b	29ab	40a	40a	32a	41b	61ab
Zink (Zn) ppm	23a	25a	25a	35a	30b	35a	33a	40b	44ab	56a	45b	50ab	60a	55ab	55ab	67a	55b	50ab
Mangan (Mn) ppm	20a	25b	26ab	30a	30a	32ab	45a	50b	50b	47a	58b	60ab	55a	60b	60b	60a	78b	72ab
Boron (B) ppm	4a	5b	5b	9a	9a	10b	10a	11b	12ab	13a	15b	16ab	12a	18b	20ab	14a	20b	25ab
Molibdat (Mo) ppm	1a	3b	3b	1.2a	5b	6ab	1.5a	5b	8ab	2.1a	8b	8b	2.3a	7b	8ab	2.5a	10b	8ab
Degree of acidity (pH)	41	3b	4a	5a	4b	5a	5a	5a	4b	5a	5a	4b	5a	5a	6b	5.5a	5b	6ab

Macronutrient Nitrogen (N)

The percentages of nitrogen (N) after 30 days of fermentation using the Pumakkal formula CO, CA, CB, CC, CD, and CE with liquid media waste (LW), sediment waste (SW), and mixed of liquid and sediment wastes (MLS) of shrimp ponds are presented in Figure 1. ANOVA test results showed a significantly different ($p<0.05$) result. The Pumakkal CE formula with 15 isolates and a mixture of liquid waste and shrimp pond sediment waste showed the highest result of 1,3%. The percentage of nitrogen meets RI Minister of Agriculture Number: 261/KPTS/SR.310/M/4/2019; (N+P+K=2-6%).

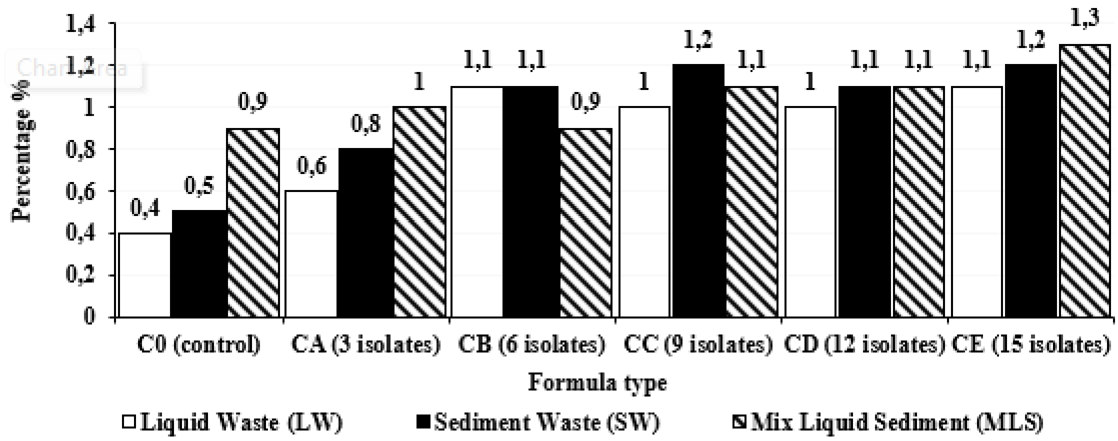

Figure 1. The average percentages of Nitrogen (N) using five Pumakkal formulas (CA, CB, CC, CD, and CE) and three media of liquid waste, sediment waste, and a mixture of shrimp-pond liquid waste and sediment.

Comparison of the ability of the Pumakkal Formula from CO to CE showed an increase in the percentage of nitrogen, where CE and mixed media showed the highest nitrogen content ($p<0.05$), as a result of using the Pumakkal fermenter in protein-rich shrimp pond waste (Hastuti et al., 2011; Paena et al., 2020). This high percentage is due to the decomposition of organic matter by bacteria *Bacillus licheniformis, Bacillus cereus, Acinetobacter baumannii, Bacillus subtilis, Pseudomonas pseudomallei,* and *Bacillus firmus* (Amara et al., 2012) as nitrifying bacteria convert ammonia to nitrate at the end of the fermentation process. In addition, microorganisms contribute to several single-cell proteins obtained during the fermentation. After the decomposition process is completed, nitrogen will be released as one of the components contained in the compost. Due to a breakdown, various types of nutrients, especially N, will be bound in the bodies of microorganisms and will return after their demise (Mulyani & Kartasapoetra, 2002).

Nitrogen formation reaction (Chen et al., 2021; Hastuti, 2011; Novizan, 2002):

Protein Organic Ingredients $\xrightarrow{\text{Aminization Reaction}}$ Amino Acids

Amino Acid $\xrightarrow{\text{Ammonia Ammonification Reaction}}$ NH_3 and Ammonium NH_4^+.

In addition, ammonia nitrification reaction is assisted by bacteria *Nitrosomonas* and *Nitrococcus nitrate*. Aerobic conditions were supplemented by a sufficient abundance of bacteria that help the process of breaking down ammonia into nitrites and nitrates, known as nitrifying bacteria, which will produce an aquatic environment that is conducive and relatively safe from contaminants (Hastuti, 2011). The oxygen layer on the surface of the pond floor is often conveyed to prevent most of the toxic metabolites from getting into the pond water because they are oxidised into non-toxic forms through biological activity when passing through the aerobic layer. Nitrite will be oxidised into nitrate, ferrous to ferric, and hydrogen sulfide (H_2S) to sulfate. In general, the five processes of the nitrogen biogeochemical cycle consist of ammonification, nitrification, nitrogen assimilation, denitrification, and nitrogen fixation. Ammonification is the process of forming ammonia from organic matter. Ammonia can also be directly assimilated into amino acids by diatom groups, cellular algae, and higher plants. Nitrification is an oxidation reaction that forms nitrites or nitrates from ammonia. This process can take place both biologically and chemically.

Phytoplankton, algae, and bacteria conduct nitrogen assimilation by utilising nitrogen to form amino acids in protoplasm. Ammonium and nitrite compounds are essential parts of the nitrogen cycle in nature. Moreover, denitrification is the reduction reaction of nitrate to nitrite, nitric oxide, and nitrogen gas. In contrast, nitrogen fixation is the fixation of nitrogen gas into ammonia and organic nitrogen (Dong et al., 2002). This process occurs in ponds of the coastal area, involving a symbiosis with algae and bacteria (Effendi, 2003; Pramushinta, 2018). In the biogeochemical cycle, there is oxidation and reduction of an inorganic nitrogen compound into another inorganic nitrogen compound. The concentrations of ammonium and nitrite compounds in sediments and waters are also affected by nitrification and denitrification processes. (Syahputra et al., 2011) said that there are three dissimilative nitrate reduction processes in bacteria: denitrification, reduction of nitrate to ammonium, and dissimilative ammonium oxidation (anaerobic ammonia oxidation, anammox). Bacterial denitrification processes use nitrate compounds as the final electron acceptor to obtain energy in low-oxygen or anaerobic conditions (Dodd et al., 1997; Richardson, 2000; Richardson et al., 2001). Nitrite acts as an electron acceptor in the process of becoming nitrogen gas. Metabolism processes form compounds between hydroxyl amines and hydrazine (Hastuti, 2011; Richardson et al., 2001). Nitrogen is an element needed by plants in vegetative growth and protein formation. The lack of nitrogen will cause stunted plants, yellow and fallen leaves, and limited root growth.

Total Nitrogen (TN) consists of Total Ammonia Nitrogen (TAN), Nitrite (NO_2), and Nitrate (NO_3). Nitrogen is generally divided into inorganic (NH_3, NH_4, NO_2, and NO_3) and organic (proteins, amino acids, and urea). Nitrogen sources in WWTPs are generally uneaten feed and shrimp excreta, which are transformed by phytoplankton and microorganisms during assimilation, fixation, nitrification, ammonification, and denitrification processes. Enzymes that work to break down proteins are Proteases, such as poly-peptidases, oligo-peptidases, and di-peptidases. An enzyme breaks down proteins into simpler peptides or amino acids. The amino acids then undergo a transamination, deamination, decarboxylation, or dehydrogenation to be simpler substances (Yuniati et al., 2015). The percentage of nitrogen from the fermentation of the CE Pumakkal formula (with 15 isolates) in the mixed media of liquid waste and sediment produces organic fertiliser suitable for plant fertiliser in agriculture.

Macro Nutrient Phosphorus (P).

The percentages of phosphorus (P) after 30 days of fermentation using the Pumakkal formula CO, CA, CB, CC, CD, and CE with liquid media waste (LW), sediment waste (SW), and mixed liquid waste and sediment waste (MLS) are presented in Figure 2. ANOVA test results were significantly different ($p<0.05$). The Pumakkal CE formula with 15 isolates and sediment media and a mixture of shrimp ponds had the highest percentages of phosphorus, 2,3% and 2% respectively. The percentage of phosphorus meets the requirement stated in the regulation in Indonesian Ministry of Agriculture Number: 261/KPTS/SR.310/M/4/2019; (N+P+K=2-6%).

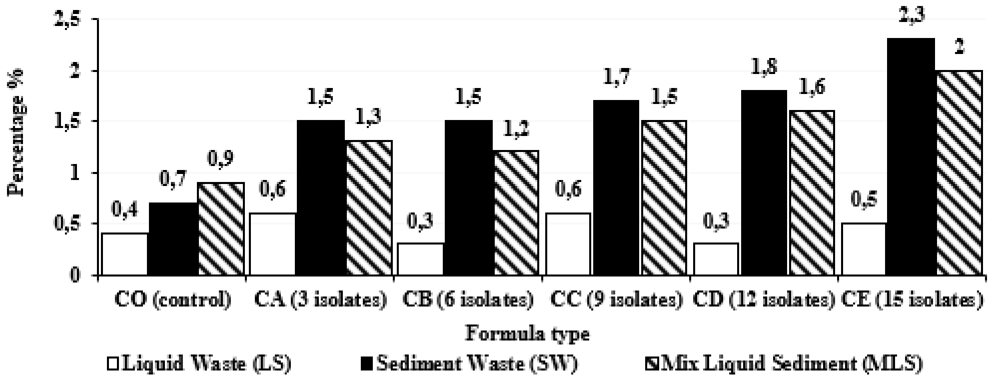

Figure 2. The average percentages of phosphorus (P) using five Pumakkal formulas (CA, CB, CC, CD, and CE) and three media of liquid waste (LW), sediment waste (SW), and a mixture of liquid waste and sediment waste (MLS) of shrimp ponds.

Comparison of the ability of the Pumakkal Formula from CO to CE showed an increase in phosphorus percentages, where the highest of it was CE in mixed media ($p<0,05$). This result is due to the decomposition of organic matter, exceptionally high protein, by bacteria *Bacillus licheniformis, Bacillus cereus, Acinetobacter baumanii, Bacillus subtilis, Pseudomonas pseudomallei,* and *Bacillus firmus. Bacillus, Pseudomonas, Aerobacter,* and *Xanthomonas* are bacteria that play a role in the breakdown of phosphorus and dissolve phosphate so that plants can utilise it. Important microorganisms involved in S oxidation are a group of bacteria belonging to the genera of *Acidithiobacillus, Thiobacillus,* and heterotrophic bacteria, including *Cytobacillus firmus, Enterobacter cloacae, Enterobacter ludwigii, Klebsiella oxytoca*, *Phytobacter diazotrophicus*, and *Pseudomonas stutzeri* (Bünemann et al., 2018; Shinde et al., 2022; Williams & Kelly, 2013). The shrimp farming wastewater contains total organic matter (BOT), total suspended solids (TSS), total N, and PO_4 as the sources of bacterial nutrition, and potential for organic matter in large shrimp pond waste (Fahrur et al., 2016). The retentions of N and P in vannamei shrimp culture are 22,27% and 9,79%, respectively, so that nutrients are released into the aquatic environment, where each pond reaches 77,73% Nitrogen and 90.21% Phosphorus. Phosphorus input in vannamei shrimp ponds is 58,3%, consisting of 7,73% feed, 4.05% fertiliser, probiotic media <1%, whilst the source of phosphorus in the pond is caused by 51% feed input, 26% erosion, and water flow by 10% (Funge-Smith & Briggs, 1998; Hendarajat et al., 2007). This happens because phosphorus is insoluble, forms a complex with calcium under aerobic conditions, and precipitates in sediments so that algae are unable to utilise it (Sudrajat & Bintoro, 2016). Orthophosphate can also be utilised directly by phytoplankton, while polyphosphate undergoes hydrolysis to form orthophosphate, which is influenced by pH and temperature. The polyphosphate changes into orthophosphate occur rapidly at high temperatures and low pH. The change of polyphosphate to orthophosphate in wastewater-containing bacteria occurs faster than in clean water. The process of decomposing shrimp pond waste using the Pumakkal formula is due to the performance of bacteria to produce appropriate enzymes. The enzyme that works to decompose fat is lipase, which can hydrolyse long-chain triglycerides into diglycerides, monoglycerides, glycerol, and fatty acids. Microorganisms producing lipase enzymes are widely used in waste treatment to decompose fats into harmless compounds, as the lipase enzymes produced are able to decompose fat (Arfiati, Pratiwi, et al., 2021). The high percentage of phosphorus in the sediment and mixed waste of shrimp-ponds came from fat and protein, and it meets the criteria of the Ministry of Agriculture. Thus, fermented fertiliser using Pumakkal is suitable for agricultural use since plants use phosphorus to accelerate root growth, flower formation, fruit ripening, and grain production (Takahashi & Katoh, 2022).

Macro Nutrient Kalium (K).

The percentages of Potassium (K) after 30 days of fermentation using the Pumakkal formula CO, CA, CB, CC, CD, and CE with liquid media (LW), sediment waste (SW), and mixed (MLS) are presented in Figure 3. Pumakkal CE formula with 15 isolates in sediment waste and a mixture of liquid waste and sediment waste media for shrimp ponds produced the highest percentages of kalium at 2,3% and 2% ($p>0,05$) respectively. The percentage of potassium meets the levels stated in the regulation of the Indonesian Ministry of Agriculture Number: 261/KPTS/SR.310/M/4/2019; (N+P+K=2-6%).

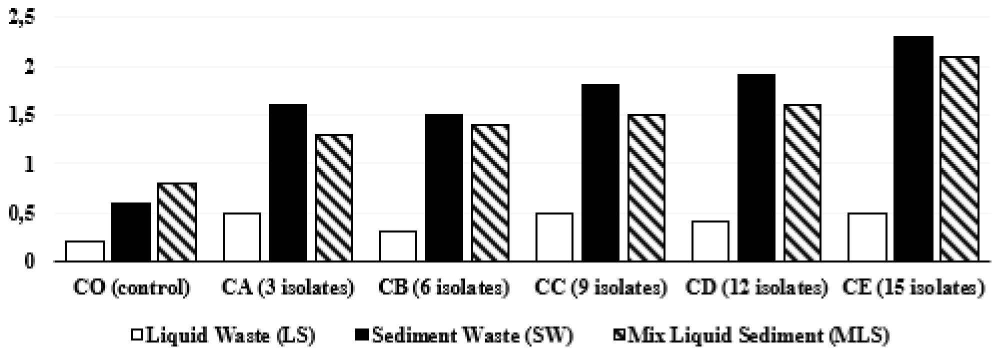

Figure 3. The average percentages of Potassium (K) using five Pumakkal formulas (CA, CB, CC, CD, and CE) in three media of liquid waste (LW), sediment waste (SW), and a mixture of liquid waste and sediment waste (MLS) of shrimp-ponds.

The increase of potassium levels was due to the decomposition process carried out by decomposer microorganisms originating from Pumakkal. Those microorganisms increase several nutrients, especially nitrogen, phosphorus, and potassium. These nutrients can return through the weathering of the living things remaining when the microorganisms die (Sundari et al., 2014b). The decomposition of organic matter consists of primary and secondary decomposition. The primary decomposer is the mesofauna, which decomposes organic matter, such as Colembolla and Acarina, which crumble organic matter/litter into smaller sizes. Earthworms eat the remains of the crumbs, which are then excreted as faeces after going through digestion in their bodies. Secondary decomposers are microorganisms that decompose organic matter, such as *Trichoderma reesei, T. harzianum, T. koningii, Phanerochaeta crysosporium, Cellulomonas, Pseudomonas, Thermospora, Aspergillus niger, A. terreus, Penicillium,* and *Streptomyces*. The existence of soil fauna activity enables microorganisms to utilise organic matter so that the mineralisation process runs faster and provides better nutrients for plants (Fan et al., 2022). Potassium in the compost mostly comes from organic matter.

Organic materials can increase the cation exchange capacity. This is related to the negative charges originating from the –COOH and OH groups, which dissociate into COO^- and H^+ and $O^- + H^+$. This negative charge causes humus to potentially adsorb cations such as Ca, Mg, and K, which are bound to moderate strength, to easily exchange or undergo a cation exchange process (Syafruddin et al., 2012). The macronutrient Kalium (K) catalyses proteins, cell division, and carbohydrates and activates enzymes (Kaya et al., 2014). A potassium deficiency will decrease photosynthesis effectiveness while increasing plant respiration. The functions of micronutrients include influencing oxidation and reduction processes, regulating acid levels, acting as a catalyst (stimulant), influencing osmotic value, assisting growth, and affecting the absorption of other nutrients (Sudarmi & Wartini, 2018). The high potassium content is due to the element Kalium (K), a catalyst for microbes or microorganisms to speed up fermentation. In addition, bioactivators in the manufacture of liquid fertiliser also affect the high potassium levels in the produced fertiliser. If the fermentation process is sustained and is accompanied by suitable supporting raw materials, the potassium content will increase. Potassium in the compound of Potassium Dioxide (K_2O) in the substrate material, used by microorganisms as a catalyst, thus affects the presence of bacteria and their activities in the fermentation process (Andriawan et al., 2022). Kalium is bound and stored in the cells by bacteria and fungi. Potassium will be available if it is re-degraded (Rahmawati et al., 2021). The highest percentage of potassium was found in the formula Pumakkal CE 15-isolate in pond sediment media at the level of 2,3%, which meets the criteria of the Minister of Agriculture as a feasible organic fertiliser.

Percentage of organic C.

The percentages of organic C after 30 days of fermentation using the formula Pumakkal CO, CA, CB, CC, CD, and CE with liquid waste (LW), sediment waste (SW), and mixed liquid waste and sediment waste (MLS) are presented in Figure 4. Pumakkal CE formula with 15 isolates in sediment waste and a mixture of shrimp ponds produces the highest percentage of organic C at the levels of 23% and 21% ($p< 0,05$), respectively. These numbers meet the criteria of the Indonesian Ministry of Agriculture Number: 261/KPTS/SR.310/M/4/2019 (minimum 10% liquid and 15% solid fertiliser).

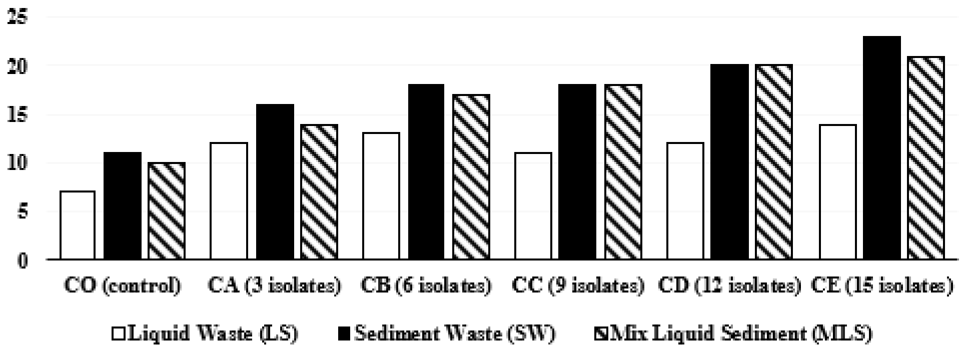

Figure 4. The average percentages of organic C using five Pumakkal formulas (CA, CB, CC, CD, and CE) and three media of liquid waste (LW), sediment waste (SW), and mixed liquid and sediment waste (MLS) of shrimp ponds.

The high levels of organic C are due to the use of Pumakkal as a starter for decomposer microorganisms, among others, *Bacillus cereus* and *Bacillus subtilis,* which can break down organic compounds such as carbohydrates and proteins into simpler compounds during the fermentation process so that plants can utilise. These microorganisms use carbon to decompose organic matter during fermentation (Sutanto, 2017; Yulipriyanto, 2010). The reaction process is as follows:

$$\text{Organic ingredients} + O_2 \xrightarrow{\text{Aerobic Microbes}} H_2O + CO_2 + \text{Nutrient} + \text{humus} + E$$

It is followed by an anaerobic process that takes place gradually. In the first stage, several facultative bacteria will decompose organic matter into fatty acids. Secondly, the other microbial groups will convert fatty acids into ammonia, methane, carbon dioxide, and hydrogen. The heat generated in the anaerobic process is lower than the aerobic one. The following is a reaction that occurs under anaerobic conditions:

$$\text{Organic ingredients} \xrightarrow{\text{Anaerobic Microbes}} CH_4 + \text{Nutrients} + \text{Humus}$$

The total organic C in liquid organic fertiliser is influenced by the method of materials decomposition, the quality of the organic matter, and the activity of microorganisms involved in the process. Carbon is a source of energy used by microorganisms to fix nitrogen. The organic C content decreased under the treatment of the Pumakkal CC and CD liquid waste formulas because carbon was consumed by microbes as food and an energy source in the decomposition process of organic matter. Microbes obtain/take energy to decompose organic matter from calories produced in biochemical reactions. Since the conversion of carbohydrates to CO_2 and H_2O occurs continuously, the carbon content in organic fertilisers decreases gradually. Then, the organic C levels increased in the middle or at the end of the fermentation process, most likely due to lower activity of microorganisms, or their demise (Pramushinta, 2018). The organic C content is an essential factor in determining the quality of soil minerals. The higher the total organic C content, the better the soil mineral quality. Soil organic matter is important in improving soil physical properties, increasing soil biological activity, and increasing plant nutrient availability. Organic matter is a vital ingredient in creating soil fertility in terms of soil physics, chemistry, and biology. The factors affecting the organic matter decomposition can be divided into three groups: 1) the nature of the plant type, age, and chemical composition; 2) soil, including aeration, temperature, humidity, acidity, and fertility; and 3) climatic factors, especially humidity and temperature. The threshold of organic C fertiliser is 10% liquid fertiliser and 15% solid fertiliser, so the active carbon content contained in all treatments in this study meets the required standards and is suitable for organic fertilisers.

Ratio of Carbon and Nitrogen (C/N).

C/N test results using the Pumakkal formula CO, CA, CB, CC, CD, and CE in sediment waste (SW) and mixed liquid and sediment wastes (MLS) are presented in Figure 5. Among other treatments, the Pumakkal CE formula with the 15 best isolates in sediment media, and CD 9 isolates in mixed media of shrimp-ponds gave the highest C/N ratio, at the levels of 29% and 27% ($p>0.05$) respectively. This Carbon and Nitrogen ratio meets the RI Minister of Agriculture Number 261/KPTS/SR.310/M/4/2019 (minimum 25%).

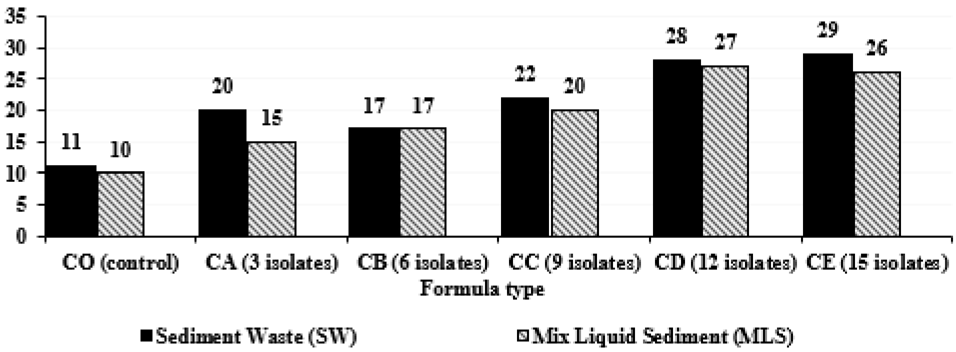

Figure 5. Average organic C/N using five Pumakkal formulas (CA, CB, CC, CD, and CE), two sedimentary waste media (SW), and a mixture of liquid waste and sediment waste (MLS) for shrimp ponds.

Composting (aerobic process) is the decomposition of organic waste/sediment waste into compost by microorganisms. Composting is influenced by C/N ratio, which should be around 20-40, microorganisms, moisture content (50-55%), temperature (30-55°C), pH (5,5-8), aeration, and particle size. The C/N ratio of 25-35 is required (Purnomo et al., 2017) to compost sheep faeces with a low C/N ratio. Additional media is needed to increase the C/N ratio of the compost so that the composting process runs well and produces good compost. The indicators of compost quality include the Nitrogen (N) content, Phosphorus (P_20_5), and Potassium (K_2O). The compost quality standards are based on SNI 19-7030-2004, which should contain a minimum of 0,40% Nitrogen (N), 0,1% Phosphorus (P_20_5), and 0,20% Potassium. As a biological process, optimal composting needs to consider influential environmental factors that affect the rate of composting, including material size, C/N ratio, humidity and aeration, temperature, degree of acidity, and the microorganisms involved. The smaller size of the raw material used resulted in a better and faster composting process. However, the minute raw material will cause the decrease of air voids, so that the pile becomes more compressed than it should be, and the supply of oxygen to the pile will decrease, too. This condition will cause the deficient work of microorganisms in the waste compost.

The C/N ratio is the most important factor in the composting process, since the process depends on the activities of microorganisms that require carbon as an energy source and cell building, and nitrogen for cell formation. The value of the C/N ratio depends on the type of waste. A good composting process will produce an ideal C/N ratio of 20–40, with 30 as the best one. The C/N ratio has an inverse relationship to microorganism activity. If the C/N ratio is high, the activity of the microorganism will decrease. In addition, several life cycles of microorganisms are needed to complete the degradation of the compost material. Thus, the composting time will be longer, and the quality of compost produced will be lower. If the C/N ratio is too low (less than 30), excess nitrogen (N), which is not used by microorganisms, cannot be assimilated and will be lost through volatilisation as ammonia or denitrification (Purnomo et al., 2017). Decomposing shrimp ponds involve Pumakkal microbes, and they use carbon and nitrogen to grow, so the C/N ratio is the ratio of carbon mass to nitrogen in a substance. Fresh organic matter has a higher C/N ratio than the C/N ratio after the composting process. This means that composting is an effort to reduce the C/N ratio of organic matter to a lower C/N ratio that can be absorbed by plants, which is at least 25.

The C/N ratio contained in liquid fertiliser also indicates the liquid fertiliser's maturity level. Hence, from this study's results, it is revealed that liquid organic fertiliser is immature since it has not been decomposed completely. When the C/N ratio is too high, where the carbon amount is greater than the nitrogen, the metabolism becomes inadequate. It means there is carbon in the substrate that is not fully converted, so maximum methane yields will not be achieved. On the other hand, a surplus of nitrogen can lead to the formation of excessive amounts of ammonia (NH_3), which, even in low concentrations, will inhibit bacterial growth (Pramushinta, 2018). In addition, the low content of the C/N ratio of liquid organic fertiliser is also caused by the content and activity of microorganisms. A longer fermentation process results in a lower C/N ratio, since the C content in the raw material used has been greatly reduced, utilised by microorganisms as food or energy source. Along with that, the nitrogen content increased due to the decomposition process of liquid fertiliser ingredients by microorganisms. The process produced ammonia and nitrogen, so the C/N ratio decreased (Trivana & Pradhana, 2017).

The C/N ratio of organic matter is the most important factor in producing liquid fertiliser, as microorganisms need carbon to provide energy (Yudi et al., 2023) and nitrogen to maintain and form their body cells (Yudi et al., 2023). A high C/N ratio will cause the fermentation process to run slowly due to the low nitrogen content. Conversely, if the C/N ratio is too low, it will cause ammonia to form, causing nitrogen to be lost in the air (Yudi et al., 2023). Hence, the C/N ratio can be used as an indicator of the fermentation process. The fermented fertiliser can be used if the total ratio between carbon and nitrogen is between 20% to 30%. The difference in C and N contents will determine the continuity process of the liquid fertiliser fermentation, and affect the quality of the liquid fertiliser produced (Pancapalaga, 2011). The anaerobic bacterial organic waste decomposition also produces CO_2, NH_4, NO_3, SO_4, and $H_2PO_4^{2-}$.

There is a close relationship between the ratio of carbon and nitrogen to the decomposition rate of organic matter. Plasma cell formation requires 1 part for every 10 parts of Carbon (Moriarty, 1997). A good range of C/N ratio for aquaculture is 10 to 15 (Tucker & Hargreaves, 2004). Nitrogen fluctuations in ponds are influenced by the bulk content of undecomposed organic matter in ponds. Some nutrients settle at the bottom of the pond and form organic sediments. Those that dissolve in water will be converted into inorganic nutrients used by seaweed and plankton, and some of the N evaporates into the air. Meanwhile, an estimated 60-80% of the total carbon content will be released as CO_2 under aerobic conditions. The types of organic matter will affect its quality and quantity. Organic materials with a low C-N ratio (<25) will cause the decomposition process to speed up. In contrast, organic matter with a high C-N ratio (> 25) can cause immobilisation, humus formation, organic matter accumulation, and sulfur content augmentation. Moreover, the increasing levels of lignin and polyphenols will inhibit the decomposition process of organic matter (Kismi et al., 2014). The ratio of carbon and nitrogen while Pumakkal CD and CE fermenters were utilised in producing fertilisers were 27 and 29, which are suitable for agriculture, according to the requirements of the Ministry of Agriculture.

Shrimp Pond Waste Micronutrients.

Percentages of Fe, Cu, Zn, Mn, B, and Mo were calculated using the formula Pumakkal CO, CA, CB, CC, CD, and CE in liquid media (LW). Figures 6, 7, and 8 show Pumakkal CE formula with 15 isolates in shrimp pond mixed media as the best in micronutrients yielded ($p>0.05$) with the contents of Fe:155 (standard 90-900), Cu: 51 (standard 25-500), Zn: 72 (standard: 25-5000, Mn; 51 (standard 25-5000, B; 25 (standard: 12-250), and Mo: 8 (standard: 2-10) (p.0.05) comply with the regulation.

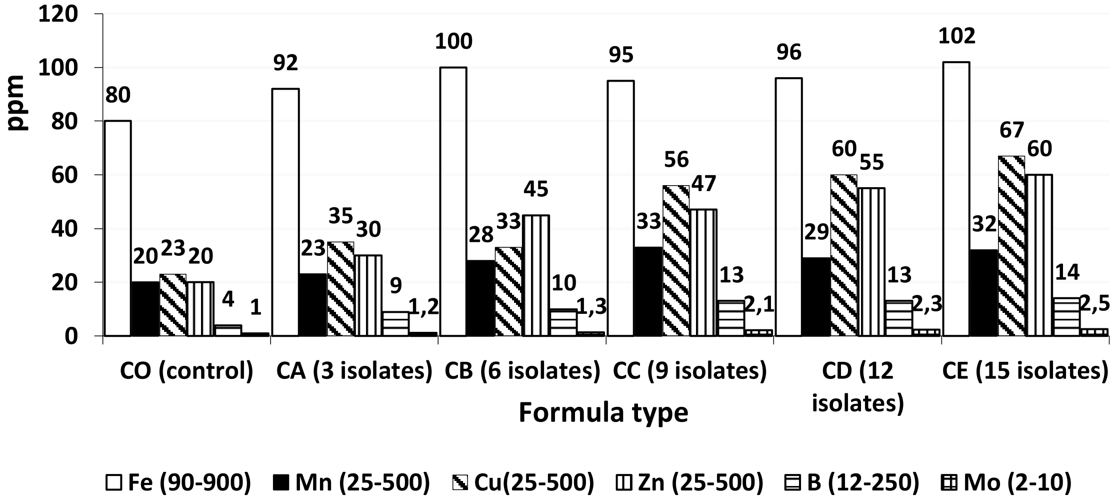

Figure 6. Fe, Cu, Zn, Mn, B, and Mo using five Pumakkal formulas (CA, CB, CC, CD, and CE) for shrimp pond wastewater.

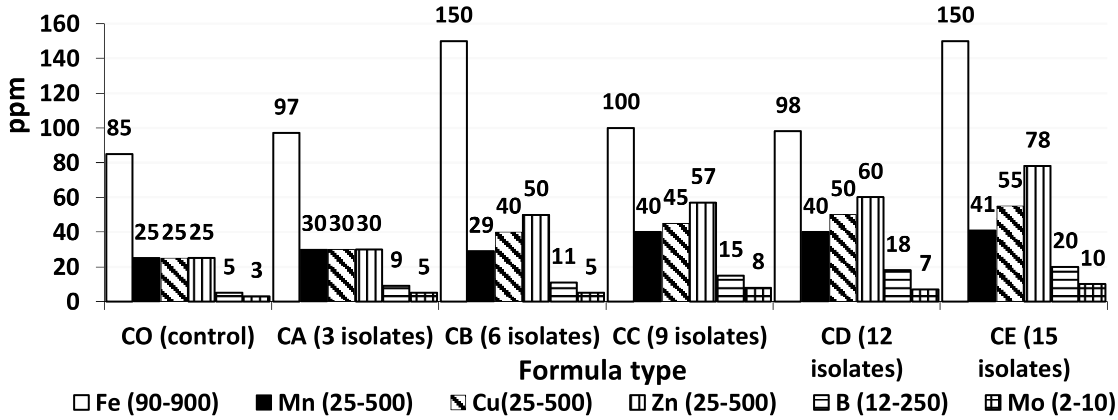

Figure 7. Fe, Cu, Zn, Mn, B, and Mo using five Pumakkal formulas (CA, CB, CC, CD, and CE) for shrimp pond liquid sediment media.

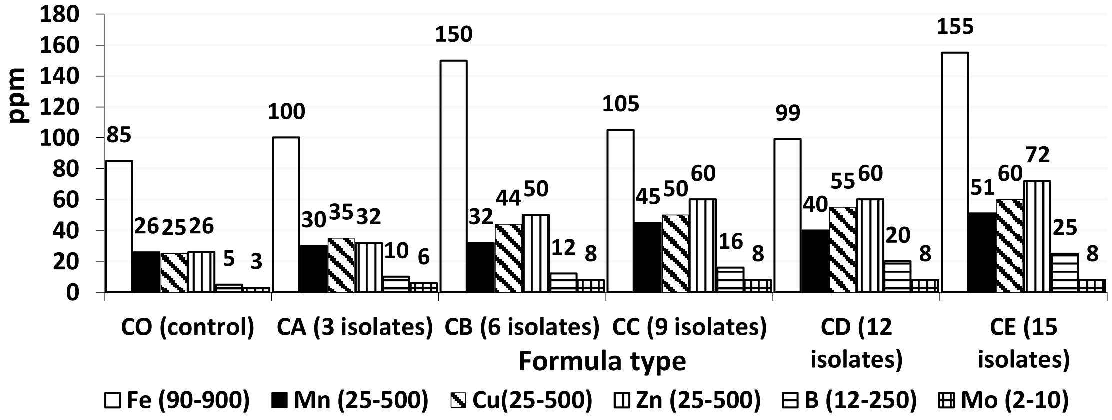

Figure 8. Fe, Cu, Zn, Mn, B, and Mo using five Pumakkal formulas (CA, CB, CC, CD, and CE) for mixed media for shrimp ponds.

Micronutrients are obtained from the decomposition of shrimp pond waste by Pumakkal microbes: bacteria *Bacillus licheniformis, Bacillus cereus, Acinetobacter baumanii, Bacillus subtilis, Pseudomonas pseudomallei,* and *Bacillus firmus.* Meanwhile, other bacteria, including *Bacillus, Pseudomonas, Aerobacter,* and *Xanthomonas,* play a role in the revamp of phosphorus. Genera like *Pseudomonas sp*. and *Bacillus sp.* have the greatest ability to dissolve insoluble phosphate and change it into a soluble form in the soil (Tangguda et al., 2015). Bacteria species with this ability are *P. striata, P. rathonis, B. polymyxa*, and *B. Megaterium.* Saputra et al., (2023) found that *Bacillus sp*. can reduce lipid levels by as much as 25% due to membrane-bound oxygenase enzymes. The enzymes are produced by bacteria to increase direct contact between the oil and bacteria so that bacteria can utilise the oil as a carbon source. **J-**types of bacteria from the *Bacillus* genus, which are capable of degrading lipids, are *B. polymixa, B. licheniformis, B. stearothermophilus, B. brevis,* and *B. coagulans.* In addition, there are also *Alcaligenes sp.* and *Coryne bacterium sp*. that function as bioremediation agents (Tangguda & Prasetia, 2019). These microorganisms produce enzymes, which can change the structure of toxic pollutants to be less complex so that they become non-toxic and non-dangerous compounds (Priadie, 2012). Nitrification consists of two reactions, nitritation and nitration. The first one is changing the ammonia into nitrite, which is carried out by the bacteria Nitrosomonas sp., and the second one is changing the nitrite into nitrate, carried out by the bacteria *Nitrobacter sp*. (Tangguda & Prasetia, 2019).

Nitrifying bacteria in shrimp pond solid waste illustrate that nitrification can occur properly. The Pumakkal CE formula with the 15 isolates in the mixed media of shrimp-ponds produced the best micronutrients as follow: Fe=155 (90-900 standard), Cu= 51 (25-500 standard), Zn= 72 (25-5000 standard, Mn= 51 (25-5000 standard), B= 25 (standard: 12-250), and Mo= 8 (standard: 2-10) comply with Indonesian Ministry of Agriculture decree Number: 261/KPTS/SR.310/M/4/2019. Comparing the capabilities of the Pumakkal Formula from CO to CE, there was an increase in micronutrients, the type of sedimentary media and mixtures were significantly different ($p>0.05$), and the sediment media contained high micronutrients.

Degree of Acidity (pH).

The results of the degree of acidity (pH) test using the Pumakkal formula CO, CA, CB, CC, CD, and CE in liquid media (LW), sediment (SW), and mixed (MLS) in Figure 9 show that CE (15 isolates) in the mixed media (MLS) had the significantly ($p>$ 0,05) highest pH, which were around 5-6, met the standard of Minister of Agriculture 4-9.

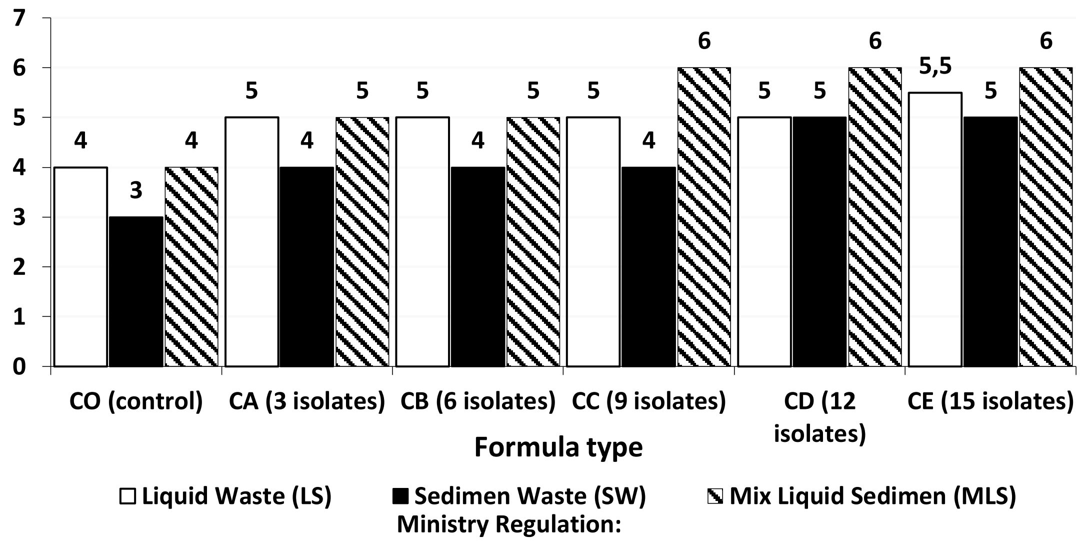

Figure 9. Ranges of pH using five Pumakkal formulas (CA, CB, CC, CD, and CE) and three media of liquid waste (LW), sediment waste (SW), and a mixture of liquid waste and sediment (MLS) for shrimp ponds.

The pH change to neutral was caused by an acid-base reaction that formed between Pumakkal bio-activators during the fermentation process, with the following reaction:

$$H^+ + OH^- \longrightarrow H_2O.$$

Pumakkal bio-activators contain bacteria *Acinetobacter baumanni* and *Pseudomonas pseudomallei,* which can decompose organic acids in waste (Sutanto, 2017). There is a significant influence of pH on fertilisers in determining the plants' uptake of nutrient ions. Generally, plants will easily absorb nutrients at a pH of 6-7 because most of the nutrients will dissolve in water at that pH condition. Fertiliser application may cause acidic conditions in the soil, where aluminium (Al) will be found, bind phosphorus so that plants cannot absorb it, and finally poison the plants.

In contrast, in alkaline conditions, elements such as Na (Sodium) and Mo (Molybdenum) can poison the plants. The microorganisms, such as fungi and bacteria, which decompose organic matter, will develop well at a pH of 5.5 – 7 (Sundari et al., 2014a). After harvesting and drying, the ponds will be filled up with shrimp droppings, leftover shrimp feed, and decaying shrimp as debris on the floor of the pond. The residue will dry and can be used for shrimp biosolids. These biosolids are considered waste and are usually disposed of in landfills. These biosolids or shrimp pond residues are valuable sources of N, P, K, and other useful plant nutrients. The highest content in these biosolids is nitrogen (Dufault et al., 2013). The environmental impact of shrimp pond activities is closely related to managing wastewater and settling pond sludge. The pond sludge has the potential to be reused as organic fertiliser in the presence of high levels of nutrients and organic matter (Rahaman et al., 2013). Shrimp pond solid residue alone cannot be used as a complete fertiliser and must be applied with commercial fertilisers instead. Pond solid residue has a very high amount of organic matter, and soil minerals with low organic matter, which are expected to increase fertility, and then contribute to successive crops planted (Dufault et al., 2013). Pumakkal, as bio-activators, can decompose shrimp pond sediments into compost that meets the required criteria of C, N, C/N, P. Ca, K, and pH content.

The suitable pH level ranges from 6-7, and the optimum pH level for decomposing bacteria ranges from 5,5-7,5. In the hydrolysis and acidogenesis phases, the optimum number is at pH 5,5-6,5 and in the methanogenesis phase is at pH 6,5-8,2 (Sari et al., 2016). The activity of acidic bacteria increases pH due to NH^{4+} binding to OH^- to form NH_4OH, which is essential for plants (Sutanto, 2017). The enzymes that decompose carbohydrates include starch-breaking enzymes such as amylase, invertase, lactase, and cellulase. There are also pectin-breaking enzymes, including polygalacturonase and methyl pectin esterase (Arfiati, Lailiyah, et al., 2021). The amount of feed given daily affects the amount of sediment that settles and triggers a decrease in the sediment's pH due to the decomposition process. At the beginning of the process, for all treatments, the pH value decreased and then increased moderately.

The pH value drops at the beginning of decomposing organic matter due to the activity of bacteria producing organic acids such as lactic, acetic, and pyruvic acid. The formation of lactic acid results from the decomposition of organic matter by the bacteria *Lactobacillus sp.* The emergence of other microorganisms from the decomposed material causes its pH to rise again after a few days (Rhys et al., 2016). The pH value, which increases again, is possibly caused by the biological activity of microorganisms in breaking down organic nitrogen (Fitria et al., 2008). Through enzymes, microorganisms utilise organic pollutants, converting them into simple compounds to help produce energy and nutrients (Abatenh et al., 2017; Singh et al., 2014). Bacteria will decompose complex organic compounds into simpler ones with enzymes. Organic compounds will be oxidised to CO_2, H_2O and new biomass. The Pumakkal bioremediation formula of CE (15 isolates) can decompose the shrimp-pond wastewater, sediment, and pond mixtures wastes into fertilisers, which meet the requirements of the Minister of Agriculture in terms of macronutrients, micronutrients, and pH in so that they are suitable for use as plant fertilisers.

CONCLUSION

Five types of consortia pumakkal Application (CA, CB, CC, CD dan CE) were used, and the Pumakkal CE formula (15 isolates) in the mixture of shrimp pond sediments produced the highest macronutrients: Nitrogen (N) 1,3%, Phosphorus 2,3%, and Potassium 2,3%; C-organic 23%, C/N ratio 29; and micronutrients, such as Fe:155 (90-900 standard), Cu: 51 (25-500 standard), Zn: 72 (25-5000 standard), Mn; 51 (25-5000 standard), B; 25 (standard: 12-250), and Mo: 8 (standard: 2-10) and the pH value of 5-6 ($p>0,05$). These results meet the Indonesian Ministry of Agriculture standard Number: 261/KPTS/SR.310/M/4/2019 and are suitable for agriculture.

ACKNOWLEDGMENTS

Thank you to the research funders of the Ministry of Research and Technology and Higher Education and the Chemistry Laboratory of Muhammadiyah Malang University, who assisted in the research. This work was supported by Kemenristek Dikti; Grant numbers [DRPM Kemenristek Dikti Nomor. 1230/SP2J/LT/LL2/2021, April 16, 2021] and [additional grant]). Authors 1, 2, 3, and 4 have received research support from Universitas Muhammadiyah Metro.

AUTHOR CONTRIBUTIONS

All authors have contributed to this article. The first and the third author are responsible for the whole manuscript. The fourth and fifth authors are responsible for reviewing the method and flows of discussion. In contrast, the second and sixth authors are responsible for proofreading, crosschecking (in-text citation and list of references), and reference improvement.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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