Exploration and remediation ability test of indigenous bacteria from rice field pemalang regency on lead (Pb) contamined soil

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Abstract. The research entitled "Exploration and Isolation of Lead (Pb) Remediator Bacteria from Conventional Rice Farming Land in Pemalang Regency" aims to obtain isolates and determine the morphological characteristics of bacteria that are resistant to Lead (Pb) contamination. The materials and tools used were soil from conventional rice farming land, Nutrient Agar (NA) medium, alcohol 70%, aquades, PbNO3 solution, aluminum foil, cling wrap, heat-resistant plastic, spiritus, physiological solution of 0.85% NaCl, autoclave, test tubes, petri dishes, erlenmeyer flasks, measuring cylinder, beaker glass, laminar air flow (LAF), shaker, magnetic stirrer, micropipette, refrigerator, analytical balance, ose needle, pH meter, vortex, ice box, shovel , tube racks, bunsen, stationery, labels, and other supporting materials and equipment. This research is a sampling research and continued with laboratory tests including bacterial isolation, colony count calculation and macromorphological characterization. The data obtained from morphological observations are presented in the form of descriptions, while quantitative data are presented in the form of numbers and are used as primary data. The results of isolating bacteria with NA medium added with 5 ppm lead, there are five bacterial isolates were selected, namely KMPb O, KT1Pb C, KT2Pb H, KB1Pb H, and KB2Pb K. Based on the TPC (Total Plate Count) test with a range of 30-300 colonies, the number of bacterial colonies ranged from 10,45 x 106 – 28,9 x 106 CFU/ml. The colony morphological characteristics of the five bacterial isolates were dominated by round in shape, smooth texture and flat elevation.

Keywords: remediator, indigenous bacteria, bacterial colonies

1. Introduction

Pemalang Regency is one of the rice producing centers in Central Java. Data from the Central Statistics Agency of Central Java (2022) shows that in 2020 Pemalang Regency was ranked 8th most rice producer in Central Java. Furthermore, data from BPS Central Java (2022) also shows that the rice harvest area in Pemalang Regency in 2019 was 71,086 hectares (Ha) and in 2020 it increased to 73,067 Ha. The productivity of rice crops in 2019 in Pemalang Regency was 57.19 quintals/Ha and in 2020 it decreased to 53.26 quintals/Ha. The decline in rice productivity in Pemalang Regency is not in line with the

increase in rice harvest area. This can happen due to several factors, namely soil fertility, rainfall, fertilizer use, how to grow crops, selection of seedlings, and the presence of plant-disturbing organisms (Ishaq et al., 2016). Excessive use of agricultural inputs such as synthetic chemical fertilizers and pesticides leads to high contamination of heavy metals in the soil (Wisnawa, 2016; Karyadi, 2005) so that it can affect crop productivity. This increasing industrialization is in line with the increasing output of hazardous chemicals to the environment, including waste containing heavy metals (Gerdhart et al., 2008). Suastawan et al., (2016) stated that heavy metals that enter the soil come from the use of chemicals that are directly applied to the soil, accumulation of dust, rain, or sediment, soil erosion, and can also come from waste waste.

Lead (Pb) is a polluting metal often found in agricultural products and environments. Lead comes from nature found in the earth's crust and its excess can cause contamination of air, water, and soil. Pb levels can naturally be found in rocks of about 13 ppm. As a result of human activity, the amount of Pb in the soil can increase until it reaches 1600-2400 ppm (Safitri et al., 2020). Hartini (2011) stated that based on the results of research by the Soil Research Center in 2002, the threshold for heavy metals in the soil allowed was 12.75 ppm.

The presence of heavy metals in agricultural land causes heavy metals to be absorbed into plant tissues through the roots and stomata of leaves, attacks sulfide bonds on cell protein molecules, causes damage to related protein structures, blocks the work of enzymes, and results in metabolic inequalities. (Juhri, 2017). The symptoms of heavy metal poisoning in plants are usually characterized by the presence of chlorosis, necrosis, and late blight earlier (Widowati, 2011). Plants that have been polluted with heavy metals enter the food chain so that when consumed by humans it can cause poisoning, cause cancer, inhibit the activity of enzymes involved in the formation of hemoglobin (Hb), accumulate in the kidneys, liver, nails, fatty tissue, and hair (Juhri, 2017; Hardiani et al., 2011).

One of the efforts that can be made to reduce Pb contamination on agricultural land is through bioremediation. Bioremediation is the process of using microorganisms that have been selected to be grown on certain pollutants as an effort to reduce the levels of these pollutants (Priadie, 2012). The bioremediation mechanism is by changing the properties of the originally active metal to inactive which is indicated by a decrease in Pb metal in the interchangeable phase along with the increase in Pb metal in the residual phase due to microbial activity (Hardiani et al., 2011).

The majority of farmers in Pemalang Regency still apply the conventional rice cultivation system so that it has the potential to bring Pb heavy metal contamination to agricultural land. If this continues, it is feared that it will aggravate the condition of lead pollution in the land and agricultural products of Pemalang Regency. This study aims to obtain isolates and determine the morphological characteristics of bacteria that are resistant to lead contamination (Pb). The results of this study are expected to be used as a reference in the use of bacteria from the root of rice plants in Pemalang Regency as a remediator of lead-polluted agricultural land (Pb).

2. Materials and methods

1.1. Research Location

This research was conducted in Mandiraja Village, Moga District, Pemalang Regency and the Agroecology Laboratory of the Faculty of Agriculture, Jenderal Soedirman University, Banyumas Regency, Central Java.

1.2. Materials and tools

The materials used are soil from conventional rice planting fields (5 samples from different locations), Nutrient Agar (NA) medium, 70% alcohol, aquaades, PbNO3 solution, aluminum foil, cling wrap, heat-resistant plastic, spiritus, 0.85% NaCl physiological solution, and other supporting materials. The tools used are autoclaves, test tubes, petri dishes, erlenmeyer flasks, measuring cups, beaker glass, laminar air flow (LAF), shakers, magnetic stirrs, micropipettes, refrigerators, analytical scales, ose needles, vortex, ice boxes, shovels, bunsen, and other supporting equipment.

1.3. Trial Design

This research is a sampling study and continued with laboratory tests including bacterial isolation, calculation of the number of colonies and macromorphological characterization.

1.4. How it works

1.4.1. Soil Sampling

Sampling is carried out in a composite way, namely combining soil samples from 5 different points with a depth of 15 cm. Soil sampling is carried out using a shovel pre-sterilized with 70% alcohol. The soil sample is put into a plastic that has been labeled with the name of the location and then stored in an ice box.

1.4.2. Preparation of tools and material

The tool is sterilized using a 10% solution of bleach (bayclin) for 10 minutes and then drained and sprayed using 70% alcohol. After that it is sterilized with an autoclave for 15 minutes with a temperature of 121℃ and a pressure of 1 atm. NA media is made with 4g of NA added with 190 ml of aquaades and then homogenized with a magnetic stirrer. The mixed media is then boiled and sterilized with an autoclave for 20 minutes with a temperature of 121℃ and then 5 ppm lead heavy metal is added.

1.4.3. Bacterial Isolation

Isolation was carried out with 10 grams of soil introduced into the erlenmeyer and a 90 ml physiological solution of NaCl was added and then homogenized with a 120 rpm shaker for 60 minutes (Saragih et al., 2015). Homogeneous samples are diluted to a dilution rate of 10-6 for later dissolution to be carried out on dilution of levels 10-5 and 10-6 by the pour plate method using NA media to which a 5 ppm lead solution has been added. The results of the captivity were incubated 24-48 hours at room temperature, then bacterial morphology and calculated the number of bacterial colonies in CFU / mL using the Total Plate Count (TPC) method (Pratiwi et al., 2012).

1.4.4. Calculation of the number of colonies

The calculation of the number of colonies is carried out by the TPC (Total Plate Count) method. According to Sukmawati (2018), the range of the number of bacterial colonies analyzed is between 30-300 colonies.

1.4.5. Characterization of bacterial isolates

This macromorphological characterization is carried out by direct observation of bacterial colonies growing on petri dishes. Macromorphological observations include: size, shape, texture, color, edge, pigmentation and elevation (Kandi, 2015).

1.4.6. Bacterial purification

Bacterial isolates were then purified by the quadran streak method using ose on NA media. Purification of these isolates is aimed at obtaining a pure culture. In the purification of these isolates, it is carried out twice so that completely pure isolates are obtained.

1.4.7. Data analysis

Data analysis is carried out descriptively qualitatively on data that have been collected from morphological observations. The quantitative data obtained are presented in the form of numbers and are used as primary data.

3. Result and discussion

The initial stage in bacterial isolation is soil sampling. The soil samples in this study were taken from conventional rice plantations in five locations of Mandiraja Village, Moga District, Pemalang Regency precisely in Dukuh Kembang, Dukuh Krajan Timur I, Dukuh Krajan Timur II, Dukuh Krajan Barat I, and Dukuh Krajan Barat II. Soil sampling is carried out by means of composites from several points of the same depth and then combined (Irfan, 2014).

Soil sampling was carried out by the X cross method at five points at each location. The soil taken is a surface horizon layer with a depth of between 15-20 cm. Nurjana's research (2001), revealed that the number of soil bacteria populations at a depth of 0-20 cm is higher than the depth of 100-110 cm. . The high number of bacterial populations at ground level or rhizosphere is thought to be because the soil surface has growing conditions that are suitable for bacterial growth. According to Purwaningsih (2005), the high population of bacteria on the soil surface is caused by the plant root system which allows the availability of substrate and food supply so that plant root metabolites will increase nutrients in the soil which affects the soil bacteria population.

Isolation is the activity of taking microbes from their original habitat and then bred on an artificial medium with the aim of separating one type of microbe from other types of microbes derived from a mixture of various microbes (Sabbathini et al., 2017). Bacterial isolation activities are also accompanied by purification to obtain bacterial isolates consisting of only one type of species (Sabbathini et al., 2017). Isolation of indigenous bacteria in rice fields was carried out using NA medium enriched with Pb with a concentration of 5 ppm. The bacteria that grow in this isolation are then characterized by their macromorphology and the number of colonies is calculated.

Bacteria growing on the medium will appear in the form of colonies. A colony is a collection of microorganisms that all come from a single stem cell so that they are genetically similar. Generally, each type of bacteria will form a different colony morphology (Fahruddin et al., 2019). Bacterial isolates from exploration are presented in Table 1 and Table 2.

Table 1. Bacterial isolates from exploration

Sample	U	Colour	Shape	Size	Edge	Elevation	Texture	Pigmentation	Ammount	Code
KMPb	1	Yellow	Round	Point	Soft	Flat	Soft	Yellow	8	A
		Broken white	Round	Small	Soft	Flat	Soft	White	60	B
		Transparent white	Round	Point	Soft	Flat	Soft	Transparent	230	C
	2	Milk White	Round	Point	Soft	Flat	Soft	Transparent	81	D
		Red	Round	Point	Soft	Flat	Rough	Transparent	4	E
		Transparent	Round	Point	Soft	Raised	Soft	Transparent	72	F
		White	Round	Small	Soft	Raised	Soft	White	6	G
		Yellow	Irregular	Big	Corrug ated	Flat	Soft	Yellow	1	H
KT1Pb	1	Broken white	Round	Small	Soft	Flat	Soft	Yellow	22	A
		Transparent white	Round	Big	Soft	Flat	Soft	Transparent	5	B
		White	Round	Small	Soft	Flat	Soft	White	136	C
		White	Irregular	Big	Wavy	Convex	Rough	Transparent	1	D
	2	White	Round	Small	Soft	Flat	Soft	White	43	E
		White	Irregular	Big	Wavy	Flat	Rough	White	1	F
		Yellow	Round	Point	Soft	Flat	Soft	Yellow	1	G
KT2Pb	1	Yellow	Round	Point	Soft	Flat	Soft	Yellow	4	A
		White	Round	Small	Soft	Flat	Soft	White	50	B
		Greenish White	Round	Small	Soft	Flat	Soft	Transparent	225	C
	2	Transparent White	Irregular	Big	Wavy	Flat	Soft	Transparent	82	D
		White	Round	Point	Soft	Flat	Soft	White	217	E
KB1Pb	1	Transparent White	Round	Small	Soft	Flat	Soft	Transparent	33	A
		White	Round	Medi um	Soft	Flat	Soft	Transparent	5	B
		Red	Round	Point	Soft	Flat	Rough	Transparent	1	C
		White	Round	Small	Soft	Flat	Soft	Transparent	44	D
	2	White	Round	Point	Soft	Flat	Soft	White	19	E
		Transparent White	Round	Small	Soft	Flat	Soft	Transparent	18	F
		Transparent White	Round	Big	Soft	Flat	Soft	Transparent	1	G
		Yellow	Round	Point	Soft	Flat	Soft	Yellow	3	H
		Greenish White	Round	Point	Soft	Flat	Soft	Transparent	146	I

Sample	U	Colour	Shape	Size	Edge	Elevation	Texture	Pigmentation	Ammount	Code
KB2Pb	1	Yellow	Round	Point	Soft	Flat	Soft	Transparent	2	A
		White	Round	Big	Soft	Flat	Soft	White	1	B
		White	Irregular	Big	Wavy	Flat	Rough	Transparent	1	C
		Yellowish White	Round	Big	Soft	Flat	Soft	Transparent	7	D
		White	Round	Point	Soft	Flat	Soft	White	33	E
		Greenish White	Round	Small	Soft	Flat	Soft	Transparent	134	F
	2	Yellow	Round	Small	Soft	Flat	Soft	Yellow	4	G
		Transparent White	Round	Small	Soft	Flat	Soft	Transparent	26	H
		Transparent White	Round	Point	Soft	Flat	Soft	Transparent	228	I

Table 2. Morfologi Koloni Bakteri Pengenceran 10^{-6}

Sample	U	Colour	Shape	Size	Edge	Elevation	Texture	Pigmentation	Amm ount	Code
KMPb	1	Yellow	Round	Small	Soft	Flat	Soft	White	24	I
		White	Round	Medium	Soft	Flat	Soft	Transparent	18	J
		Greenish White	Round	Point	Soft	Flat	Soft	Transparent	133	K
	2	Red	Round	Point	Soft	Flat	Rough	Transparent	2	L
		White	Irregular	Medium	Wavy	Flat	Rough	Transparent	1	M
		Transparent	Round	Medium	Soft	Flat	Soft	Transparent	23	N
		White	Round	Small	Soft	Raised	Soft	White	88	O
KT1Pb	1	Yellowish White	Round	Small	Soft	Flat	Soft	Yellowish	15	H
		White	Round	Big	Soft	Flat	Soft	Transparent	4	I
		Transparent White	Round	Point	Soft	Flat	Soft	Transparent	23	J
	2	Yellowish White	Round	Small	Soft	Flat	Soft	White	26	K
		White	Irregular	Big	Wavy	Flat	Soft	Transparent	17	L
		White	Round	Medium	Soft	Flat	Soft	Transparent	7	M
KT2Pb	1	White	Round	Small	Soft	Flat	Soft	White	16	F
		White	Round	Small	Soft	Flat	Soft	Transparent	44	G
		Red	Round	Point	Soft	Flat	Rough	Transparent	2	H
	2	Red	Round	Point	Soft	Flat	Rough	Transparent	2	I
		White	Round	Small	Soft	Flat	Soft	White	36	J
		Transparent White	Round	Medium	Soft	Flat	Soft	Transparent	42	K
KB1Pb	1	Red	Round	Point	Soft	Flat	Rough	Transparent	3	J
		White susu	Round	Small	Soft	Flat	Soft	White	17	K
		White susu	Round	Point	Soft	Flat	Soft	White	44	L
		Transparent	Round	Point	Soft	Flat	Soft	Transparent	26	M
	2	White	Irregular	Big	Wavy	Convex	Rough	Transparent	1	N
		White	Irregular	Big	Wavy	Flat	Tangled	Yellow	1	O
		Yellowish White	Round	Medium	Soft	Flat	Soft	White	7	P
		Transparent White	Round	Point	Soft	Flat	Soft	Transparent	115	Q
KB2Pb	1	White	Round	Big	Irregular	Flat	Rough	Transparent	2	J
		Yellowish White	Irregular	Big	Soft	Flat	Tangled	Transparent	4	K

Sample	U	Colour	Shape	Size	Edge	Elevation	Texture	Pigmentation	Amm ount	Code
		Yellowish White	Round	Point	Soft	Flat	Rough	Yellow	37	L
		Yellowish White	Round	Point	Soft	Flat	Soft	Yellow	9	M
		White	Round	Point	Soft	Flat	Soft	White	137	N
	2	White	Round	Big	Soft	Flat	Soft	White	48	O
		White	Round	Point	Soft	Flat	Soft	White	21	P
		Transparent	Round	Point	Soft	Flat	Soft	Transparent	103	Q

Isolation is the activity of taking microbes from their original habitat and then bred on an artificial medium with the aim of separating one type of microbe from other types of microbes derived from a mixture of various microbes (Sabbathini et al., 2017). Bacterial isolation activities are also accompanied by purification to obtain bacterial isolates consisting of only one type of species (Sabbathini et al., 2017). The colony form of a bacterium is influenced by age and certain terms of growth. The variety of bacterial colony forms that occur is also influenced by the environment (biotic and abiotic factors), food factors (growing medium) and temperature (minimum, optimum and maximum). Sousa et al., (2013) explains that colony morphology can be an important indicator of phenotypic variation as a result of bacterial adaptation and biological strategies to stresses such as nutrient deficiencies, oxygen deprivation, and the presence of contamination.

Table 3. Total Plate Count (TPC)

Sample	Dilution	U1	U2	Average	TPC (cfu/ml)
KMPb	10^{-5}	298	164	231	23,1 x 10^6
	10^{-6}	175	114	144,5	
KT1Pb	10^{-5}	164	45	104,5	10,45 x 10^6
	10^{-6}	42	50	46	
KT2Pb	10^{-5}	279	299	289	28,9 x 10^6
	10^{-6}	62	80	71	
KB1Pb	10^{-5}	83	287	185	18,5 x 10^6
	10^{-6}	90	124	107	
KB2Pb	10^{-5}	178	258	218	21,8 x 10^6
	10^{-6}	48	172	110	

From table 3 data, it shows that Dukuh Krajan Timur II has the highest population, namely 28.9 x 106 CFU / ml, followed by Dukuh Kembang 23.1 x 106 CFU / ml, Dukuh Krajan Barat II 21.8 x 106 CFU / ml, Dukuh Krajan Barat I 18.5 x 106 CFU / ml, and Dukuh Krajan Timur I 10.45 x 106 CFU / ml. Based on table 2, it is known that the number of microbial populations in each region is different. According to Bais et al (2006), Plant roots whose growth has not been or are not intensive cause a decrease in the number of microbes, because root exudate is one of the places of soil microbial growth. Kaharu et al (2021) also explained that different environments affect soil microbial populations. A high population indicates that organic matter is sufficient, temperature is appropriate, water availability is sufficient, and soil ecology is supportive.

Purification of isolates is aimed at obtaining a pure culture. In the purification of these isolates, it is carried out twice so that completely pure isolates are obtained. Purification is carried out by taking bacterial isolates with different morphological types at each location, so that bacterial isolates are obtained, namely KMPb O, KT1Pb C, KT2Pb H, KB1Pb H, KB2Pb K.

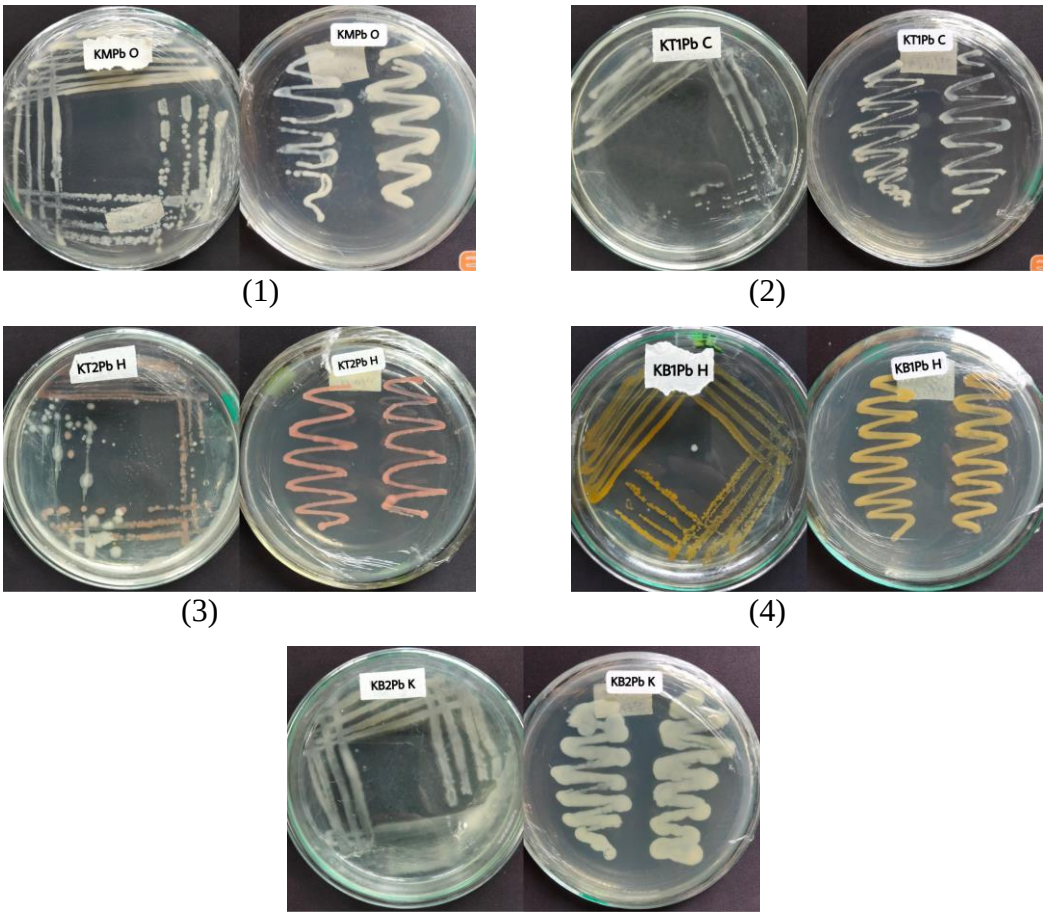

Figure 1. Pure Bacterial Isolates KMPb O (1), KT1Pb C (2), KT2Pb H (3), KB1Pb H (4), KB2Pb K (5)

4. Conclusion

Based on the results of the study, several bacterial isolates have been obtained from conventional rice fields in Pemalang Regency that are polluted with lead. The macromorphological characteristics of bacterial isolates from the five majority sampling sites have a rounded shape and flat elevation of varying sizes and colors.

The results of colony calculations show that KT2Pb bacterial isolates have the most number of colonies, reaching 28.9 x 106 CFU / ml, while the isolates with the least number of colonies are KT1Pb isolates.

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6. References

[1] BPS. 2022. Luas Panen, Produktivitas, dan Produksi Padi Menurut Kabupaten/Kota di Provinsi Jawa Tengah, 2019 dan 2020. BPS Provinsi Jawa Tengah.

[2] Gerdhart, K.E., Huang, X.D., Glick, B.R. & Greenberg, B.M. 2008. Phytoremediation and Rhizoremediation of Organic Soil Contaminants: Potential and Challenges. *Plant Science*, 176(1): 20-30.

[3] Hardiani, H., Kardiansyah, T., & Sugesty, S. 2011. Bioremediasi Logam Timbal (Pb) dalam Tanah Terkontaminasi Limbah *Sludge* Industri Kertas Proses *Deinking*. *Jurnal Selulosa*, 1(1): 31-41.

[4] Hartini, E. 2011. Kadar Plumbun (Pb) dalam Umbi Bawang Red di Kecamatan Kersana Kabupaten Brebes. *Jurnal Visikes*, 10(1): 69-75.

[5] Irfan, M. 2014. Isolasi dan Enumerasi Bakteri Tanah Gambut di Perkebunan Kelapa Sawit PT. Tambang Hijau Kecamatan Tambang Kabupaten Kampar. *Jurnal Agroteknologi*, 5(1): 1–8.

[6] Fahruddin, F., Eva, J. & Zaraswati, D. 2019. Antifouling Potential of *Thalassia hemprichii* Extract Against Growth of Biofilm-forming Bacteria. *ScienceAsia.* 45(1): 21–27.

[7] Sabbathini, G.C., Pujiyanto, S., Wijanarka & Lisdiyanti, P. 2017. Isolasi dan Identifikasi Bakteri Genus *Sphingomonas* dari Daun Padi (*Oryza sativa*) di Area Persawahan Cibinong. *Jurnal Biologi*, 6(1): 59-64.

[8] Nurjana. 2001. Isolasi, Identifikasi, dan Penentuan Ammount Bakteri Asal Tambak Tanah Gambut. *Buletin Teknik Pertanian*. 6 (2):77-80.

[9] Purwaningsih, S. 2005. Isolasi, Enumerasi, dan Karakterisasi Bakteri *Rhizobium* dari Tanah Kebun Biologi Wamena. *Jurnal Biodiversitas*. 6(2): 82-84.

[10] Kaharu, P., Tanggapo, A.M. & Mambu, S. 2021. Dinamika Populasi Mikroba Tanah dan Respon Pertumbuhan Vegetatif Tanaman Jagung (*Zea mays* L.) terhadap Aplikasi Amelioran Pupuk Organik. *Jurnal Bios Logos*, 11(2): 102-108.

[11] Ishaq, M., Rumiati, A.T. & Permatasari, E.O. 2016. Analisis Faktor-faktor yang Mempengaruhi Produksi Padi di Provinsi Jawa Timur Menggunakan Regresi Semiparametrik Spline. *Jurnal Sains dan Seni ITS*, 5(2): 420–425.

[12] Bais, H.T., Weir, T.L., Perry, l.G., Gilroy, S. & Vivanco, J.M. 2006. The Role of Exudates in Rizhosphere Interactions with Plants and Other Organisms. *Annual Review of plant Biology*, 57(1): 233-266.

[13] Sousa, A. M., Machado, I., Nicolau, A. & Pereira, M. O. 2013. Improvements on Colony Morphology Identification Towards Bacterial Profiling. *Journal of Microbiological Methods*. 95(3): 327-335.

[14] Safrida, Y. D., Yulvizar, C. & Devira, C. N. 2012. Isolasi dan Karakterisasi Bakteri Berpotensi Probiotik pada Ikan Kembung (*Rastrelliger* sp.). *DEPIK: Jurnal Ilmu- Ilmu Perairan, Pesisir dan Perikanan.* 1(3): 200-203

[15] Juhri, D.A. 2017. Pengaruh Logam Berat (Kadmium, Kromium, dan Timbal) terhadap Penurunan Berat Basah Kangkung Air (Ipomoea aquatic Forsk) sebagai Bahan Penyuluhan Bagi Petani Sayur. Jurnal Lentera Pendidikan Pusat Penelitian LPPM UM Metro, 2(2): 219-229.

[16] Kandi, V. 2015. Bacterial Colony: First report of donut colony morphology among Diphtheroids isolated in blood. Cureus, 7(11): 1-7.

[17] Karyadi. 2005. Akumulasi Logam Berat Pb sebagai Residu pada Lahan Pertanian (Studi Kasus pada Lahan Pertanian Bawang Red di Kecamatan Gemuh Kabupaten Kendal). Tesis. Universitas Diponegoro, Semarang.

[18] Pratiwi, A. A., Suprihadi A., Raharjo, B., Wahyudi, P. & Parmiyatni, S. 2012. Isolasi dan Karakterisasi Bakteri Pendegradasi Pestisida Dicofol dari Tanah Sawah di Kabupaten Karawang. Jurnal Biologi, 1(1): 23-32.

[19] Priadie, B. 2012. Teknik Bioremediasi sebagai Alternatif dalam Upaya Pengendalian Pencemaran Air. Jurnal Ilmu Lingkungan, 10(1): 38-48.

[20] Safitri, F.D., Lukiwati, D.R. & Mansur, I. 2020. Inokulasi Berbagai Spesies Cendawan Mikoriza Arbuskular (CMA) terhadap Pertumbuhan dan Kemampuan Akumulasi Logam Pb pada Tanaman Marigold (Tagetes erecta L.). Jurnal Agro Complex, 4(1): 60-68.

[21] Saragih, A. M., Dermiyati, D., Buchari, H. & Arif, M.S. 2015. Pengaruh Sistem Olah Tanah terhadap Total Bakteri Tanah pada Pertanaman Kedelai Musim Tanam Kedua Setelah Pertanaman Jagung di Lahan Bekas Alang-alang (Imperata cylindrica. L). Jurnal Agrotek Tropika, 3(1): 174-179.

[22] Suastawan, G., Satrawidana, I.D.K. & Wiratini, N.M. 2016. Analisis Logam Pb dan Cd pada Tanah Perkebunan Sayur di Desa Pancasari. Jurnal Wahana Matematika dan Sains, 9(2): 44-51.
[23] Sukmawati. & Hardianti, F. 2018. Analisis Total plate Count (TPC) pada Ikan Asin Kakap di Kota Sorong Papua Barat. Jurnal Biodjati, 3(1): 72–78.
[24] Widowati, H. 2011. Pengaruh Logam Berat Cd, Pb terhadap Perubahan Colour Batang dan Daun Sayuran. El-Hayah, 1(4): 167-173.
[25] Wisnawa, P.D.P.K., Siaka, I.M. & Putra, A.A.B. 2016. Kandungan Logam Pb dan Cu dalam Buah Stroberi serta Spesiasi dan Bioavailabilitas dalam Tanah Tempat Tumbuh Stroberi di Daerah Bedugul. Jurnal Kimia, 10(1): 23-31.