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Preface

We would like to present, with great pleasure, the inaugural volume-10, Issue-1, January 2024, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas.

Environmental Research:

Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.

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Improved Water Quality of Lake Ranisagar using Bioaugmentation Technology

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Abstract— *Bioaugmentation technology achieved rapid bioremediation to significantly improve the water quality of Ranisagar Chaupati Lake over the course of our 8-week study. At the beginning of our study, Ranisagar Chaupati Lake was graded on the Water Quality Index (WQI) as bad water quality and on the Trophic State Index (TSI) as highly eutrophic. Bioaugmentation technology was added to the lake as an all-natural, environmentally safe protocol to improve water quality. This resulted in overall improvement in the WQI from 36.6 (Bad quality) to 57.5 (Moderate quality) and the trophic status of the lake improved from hypereutrophic to eutrophic state.*

Keywords— Bioaugmentation, Ranisagar Chaupati, Water Quality, WQI, Eutrophic.

I. INTRODUCTION

The state of Chhattisgarh in central India was created in the year 2000 as a result of the Madhya Pradesh Reorganization Act. It is the 10th largest state in India (by surface) and with a population of over 25 million people it is one of the fastest developing states in India. The state is dotted with numerous lakes, ponds and other water bodies that serve as a major tourist attraction.[1] Most of the pilgrimage sites in Chhattisgarh are located in the vicinity of a lake or a pond. Human activities in the vicinity of the water bodies have caused deterioration of water quality. The Ranisagar Chaupati – a highly eutrophic lake, located in Rajnandgaon district of the state Chhattisgarh – was suggested by the local municipal corporation as a location to demonstrate Bioaugmentation technology capacity to improve water quality through an all-natural bioremediation protocol.

The Ranisagar Chaupati Lake situated at 21°5’28”N 81°1’22”E in Rajnandgaon district is surrounded by garden which is used for public recreation and is likely to receive a higher nutrient load than any other lake situated in its vicinity.

The lake has a surface area of 20100 m^2 (2.01 ha) and an average initial water depth of 1.3 m. Stagnant bodies of water such as lakes or ponds are typically more affected by surrounding human activities than flowing surface water (like streams or rivers).

Ranisagar Chaupati is used for public recreation, but the pond has been receiving discharges of domestic sewage and gray waters from an open canal which collects from surrounding settlements (nallah) for over a decade. The main objective behind the project was to improve the overall water quality of Ranisagar Chaupati Lake through the implementation of an all-natural, environmentally safe protocol using Bioaugmentation Technology.

II. MATERIALS AND METHODS

The bioaugmentation technology used for this study is a proprietary composite biocatalyst that enhances a broad range of hydrolytic, oxidative, and reductive biochemical reactions. It contains a novel consortium of metabolically cooperative microorganisms with endogenous and exogenous enzymes, and small molecule co-factors which support both biocatalytic and metabolic activity. They are composed of all-natural materials and non-genetically modified.

Ranisagar Chaupati is a small and shallow lake which continuously received inflow of domestic sewage and grey waters from the nearby households over the past decade. An eight-week bioremediation protocol using Bioaugmentation technology was implemented starting December 28th, 2015. Before starting the study, all of the pond’s inlets and outlets were closed and floating aquatic plants and debris were removed. Samples collected on December 13th, 2015 revealed the following starting water quality:

TABLE 1
DATA FOR INITIAL SAMPLING

Parameter	Unit	Lake condition before bioremediation with BIOWISH* Aqua*	Bathing Water Standards as per MoEf**
PH	mg/l	9.3	6.5-8.5
DO	mg/l	3.6	5 or more
COD	mg/l	88.8	<10
BOD	mg/l	34.7	3 or less
TSS	mg/l	58.5	<10

Table explaining initial sampling
****Results for samples drawn on December 13th, 2015***
*****Standards for Bathing water as per MoEF-Ministry of Environment, Forest and Climate Change (Government of India)***

Dosing: The lake was arbitrarily divided into 11 sections to facilitate dosing. An initial shock dose of 56 kg was sprayed on December 28. Lower maintenance doses of 14 kg (aiming to maintain a 0.5 ppm activity for the calculated water volume) were added once a week. The Bioaugmentation technology used for this study is a water-soluble powder and ships ready to dose. For each pond section, the required amount of product was dissolved in pond water and sprayed on the surface using a portable pump. The entire dosing event for the 2-ha pond took no longer than two hours each week.

FIGURE 1: Lake Ranisagar divided into 11 sections

Sampling: CSIR – NEERI: The National Environmental Engineering Research Institute (NEERI) established in Nagpur in 1958, was created and funded by the Government of India (GoI) reporting to the Ministry of Science and Technology (India) of Central Government. NEERI is a pioneer laboratory in the field of environmental science and engineering and part of Council of Scientific and Industrial Research (CSIR) having five zonal laboratories at Mumbai, Kolkata, Hyderabad, Chennai, and Delhi.[2] NEERI played a major role in auditing the entire project. Since the bioremediation project for Ranisagar Chaupati lake was approved by the State Government of Chhattisgarh, it was important that the sampling, chain of custody and water quality analysis be done by a reputable GoI certified lab.

TABLE 2
SUMMARY OF DOSING EVENT

Date	Description
December 28th	1st dosing (Shock Dose)
January 7th	2nd dosing - Maintenance Dose
January 14th	3rd dosing Maintenance Dose
January 21st	4th dosing- Maintenance Dose
February 4th	5th dosing Maintenance Dose
February 11th	6th dosing Maintenance Dose
February 18th	7th dosing - Maintenance Dose
February 25th	8th dosing - Maintenance Dose

NEERI's sampling protocol included 22 sampling locations distributed across the pond's area. Grab samples were collected from these locations on the dates shown in the table below. Surface water samples were collected directly without any filtration in acid-rinsed polypropylene bottles, depth water samples were collected approximately 1m from the surface using depth sampler and transferred to clean polypropylene bottles and sludge samples from the bottom of the lake were collected using a van-veen sediment sampler and then transferred into zip-lock polyethylene bags. Similarly, samples for phytoplankton and zooplankton were collected separately and preserved immediately on-site, while samples for chlorophyll a estimation were collected in acid-rinsed amber colored polypropylene bottles and kept away from direct sunlight until analyzed. Separate samples for fecal coliform analysis were collected in sterile polypropylene bottles. Sample pH, temperature and dissolved oxygen were immediately determined on-site, while the remaining samples were shipped for analysis at CSIR-NEERI at Nagpur after adequate preservation of the collected samples. Amongst the 5 sampling events conducted, 2 sampling events (2nd and 4th monitoring) were conducted to analyze 13 major parameters (pH, temperature, DO, COD, BOD, ammonia, nitrate, TKN, phosphate, suspended solids, total dissolved solids, fecal coliform and chlorophyll a) and 3 sampling events (1st, 3rd and 5th monitoring) were conducted to analyze 33 parameters for complete analysis of the lake including water characteristics, sediment characteristics, aquatic diversity and physio-chemical parameters inclusive of above mentioned 13 parameters.

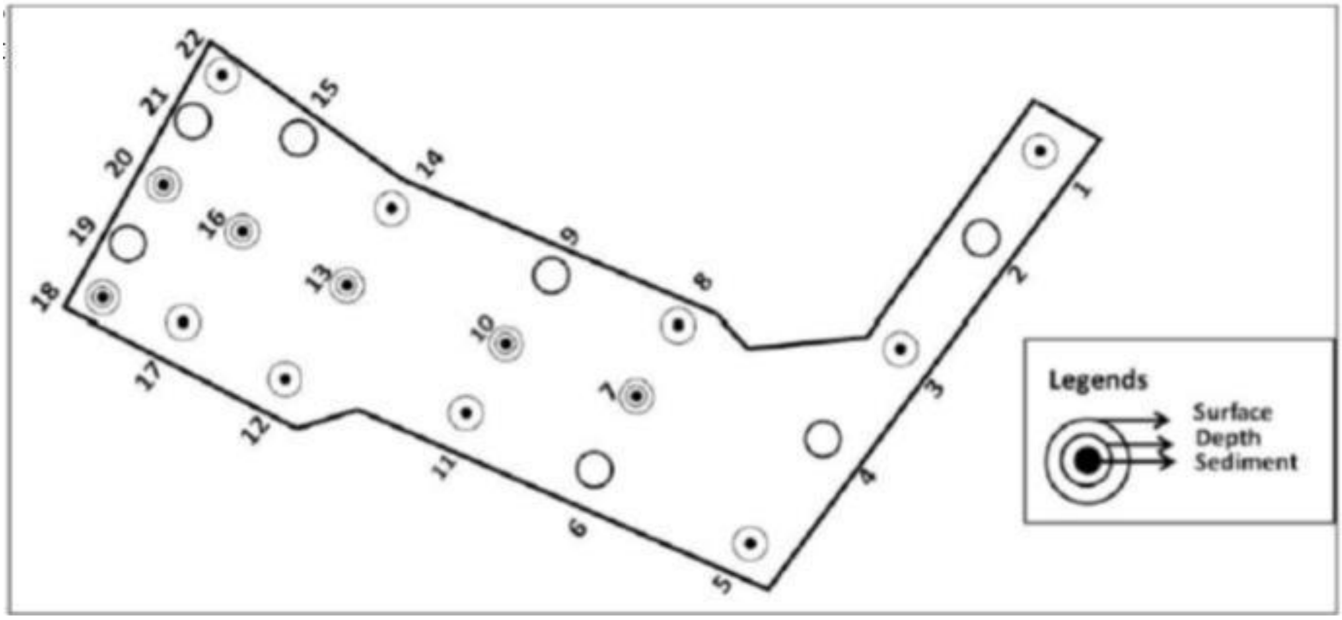

FIGURE 2: Schematic of sampling location in Ranisagar Lake

TABLE 3
SAMPLING DATES

Date	Description
December 13th	1st sampling before dosing (lesser parameters)
January 13th	2nd sampling (full parameters)
January 27th	3rd sampling (lesser parameters)
February 10th	4th sampling (lesser parameters)
February 24th	5th sampling (full parameters)

III. RESULTS AND DISCUSSION

The graphs below show the 22 surface water samples for each sampling event plotted in a "box graph" style. The top and bottom lines for the box represent upper and lower quartiles, while the center line shows the mean (arithmetic average for the 22 samples). The bars extending from the boxes show: Quartile +/- 1.5 * (Inter Quartile Range).

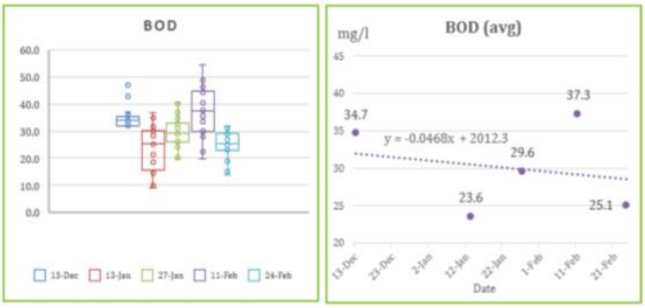

FIGURE 3: Graph 1 Five-point summaries of data collected and Graph 2 Average values of 22 sampling points for BOD

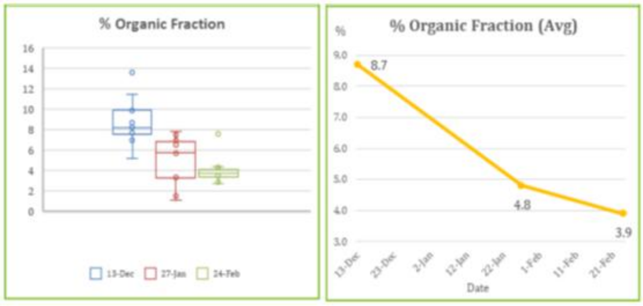

FIGURE 4: Graph 3 Three-point summaries of data collected and Graph 4 Average values of 13 sampling points for % organic fraction

The fluctuations in BOD can be attributed to the rapid degradation of organic matter in the system. This increased biological activity in the sediment may have resulted in changing the release of organic components which contributed to varying BOD readings.

Sludge in sewage treatment lagoons are likely to contain over 50% organic fraction. Old material which is allowed to mineralize for years may have 10 to 15% organic fractions. Chaupati pond sludge was found to have an initial 8.7% organic fraction, with very high levels of Fe and Mn – indicating iron rich sandy soils and a very advanced stage of mineralization, a very poor indication for bioremediation. Despite this, rapid action could be seen on the sludge by week 4. A 45% drop in sludge organic fraction was seen 4 weeks into the program. Increased biological activity is also reflected in the TSS spike. Achieving lower BOD and sludge organic fraction values in 8 weeks shows how rapidly Bioaugmentation technology can catalyze biological action in water systems.

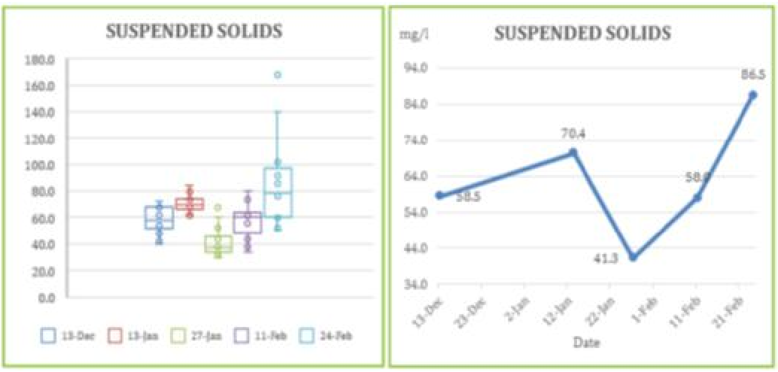

FIGURE 5: Graph 5 Five-point summaries of data collected and Graph 6 Average values of 22 sampling points

Bioaugmentation technology used for this study contains a consortium of microorganisms which can facilitate diverse nitrogen management metabolic pathway in water bodies. The microbial consortium in the bioaugmentation technology is able to promote ammonia (NH_3) oxidation directly to gaseous forms of nitrogen through Heterotrophic Nitrification and Denitrification (HND).[3] This results in lower ammonia nitrogen concentrations while avoiding peak formations of nitrite and nitrate (which may be toxic to aquatic life). The different oxidative/reductive pathways in HND by-pass the use of dissolved oxygen as an electron acceptor in aerobic nitrification, resulting in higher DO levels for the pond (as seen on Graph 14).

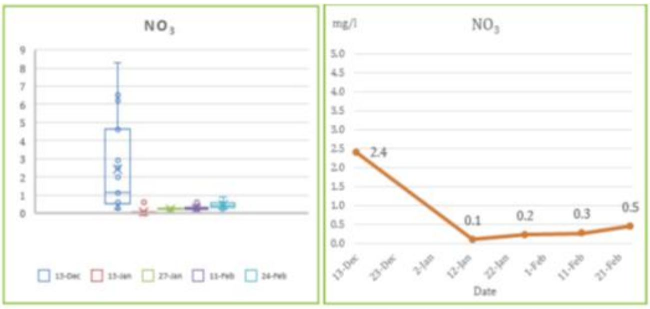

FIGURE 6: Graph 7 Five-point summaries of data collected and Graph 8 Average values of 22 sampling points for NO_3

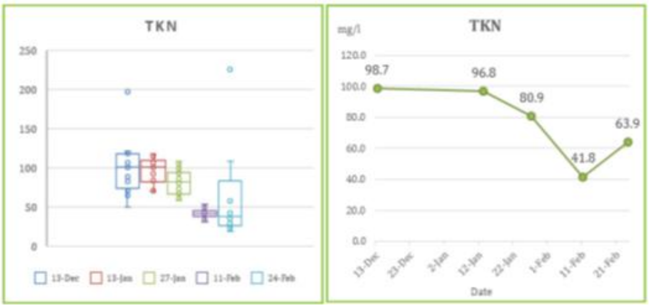

FIGURE 7: Graph 9 Five-point summaries of data collected and Graph 10 Average values of 22 sampling points for TKN

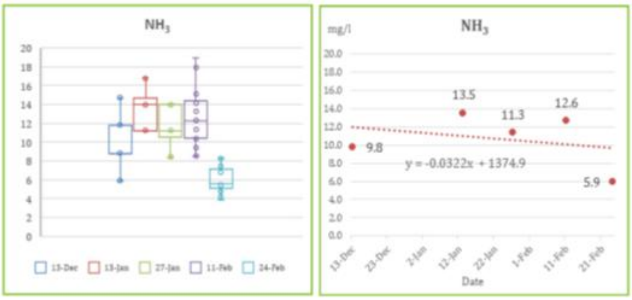

FIGURE 8: Graph 11 Five-point summaries of data collected and Graph 12 Average values of 22 sampling points for NH_3

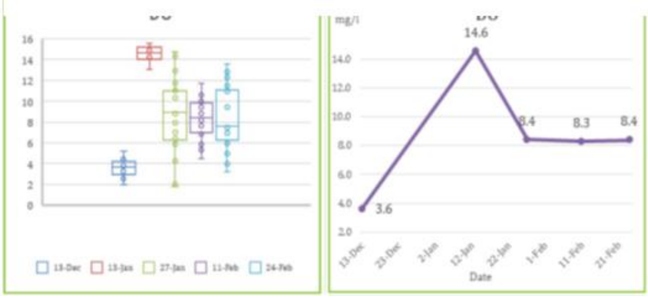

FIGURE 9: Graph 13 Five-point summaries of data collected and Graph 14 Average values of 22 sampling points for DO

We see an increase in phosphate concentrations which may be explained by the increased release of nutrients from the lake sediment via aggressive decomposition of organic fraction. A sharp drop in phosphate concentration could be observed after the 5th monitoring, indicating that the slower rate of organic degradation in the sludge allows for more effective phosphate settling.

During the study, the pH of the lake decreased consistently from an average value of 9.3 to 8.8. Decrease in pH indicates improved biological activity[4]. Bioaugmentation also showed great effect on restoring the pH values in the lake water.

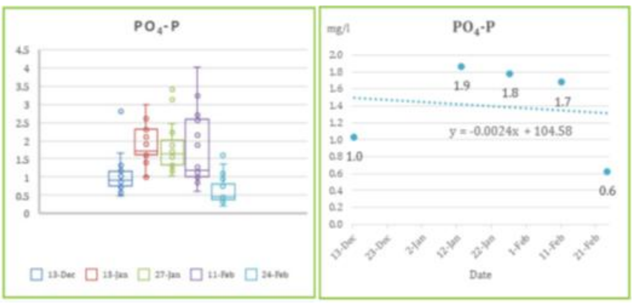

FIGURE 10: Graph 15 Five-point summaries of data collected and Graph 16 Average values of 22 sampling points for PO_4-P

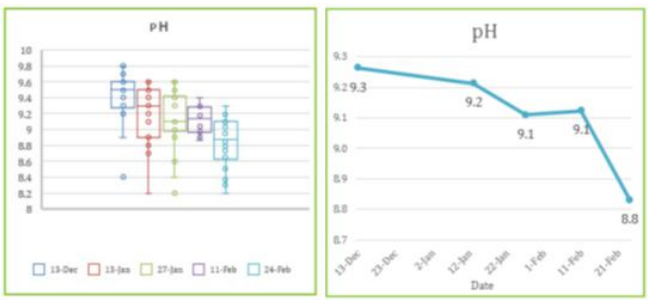

FIGURE 11: Graph 17 Five-point summaries of data collected and Graph 18 Average values of 22 sampling points for pH

3.1 Water Quality Index (WQI)

WQI, developed by National Sanitation Foundation (NSF-WQI), was used to quantify the change in the quality of water in response Bioaugmentation. NSF-WQI categorizes water quality into five categories: 0-25 Very Bad, 25-50 Bad, 50-70 Moderate, 70-90 Good and 90-100 Excellent. In this method, nine different physio-chemical parameters are used to determine the WQI (dissolved oxygen, fecal coliform, pH, BOD, temperature, total phosphate, nitrate, turbidity, and total solids).[5]

The overall changes in the WQI of the lake during 1st monitoring to 5th monitoring is depicted as follows:

TABLE 4
CHANGES IN WQI OF LAKE DURING 1ST MONITORING TO 5TH MONITORING

Sampling	1st	2nd	3rd	4th	5th
NSF-WQI	36.6	46.6	55.1	56.1	57.5
Water Quality	Bad	Bad	Moderate	Moderate	Moderate

3.2 Trophic State Index (TSI)

The Trophic State Index (TSI) classifies the trophic status of a lake or any water body using a measurable value. The most commonly used numerical classification method of the trophic status of a lake was that developed by Robert Carlson in the year 1977 and is known as Carson's Trophic State Index (TSI). Carson's TSI uses three dependent variables which have direct correlation with the trophic status of the lake, namely, algal biomass, transparency of the lake water (which enables light penetration), and the nutrient concentration to classify the trophic status of the lake into hyper oligotrophic, oligotrophic, mesotrophic, eutrophic, and hypereutrophic conditions.[6]

TABLE 5
CLASSIFICATION OF LAKES BASED ON TSI VALUES[6]

TN values	Trophic Status
<30	Hyperoligotrophic
30—40	Oligotrophic
40-50	Mesotrophic
50-70	Eutrophic
>70	Hypereutrophic

Based on the TSI values, Ranisagar Chaupati Lake was classified as hypereutrophic before starting the Bioaugmentation. The water quality improved significantly after Bioaugmentation and resulted in the trophic status of the lake to shift from hypereutrophic to eutrophic state in 8 weeks.

IV. CONCLUSION

Bioaugmentation in Ranisagar Chaupati Lake helped to remarkably improve the water quality. We saw an overall improvement in the water quality index (WQI) from 36.6 (Bad quality) to 57.5 (Moderate quality) and the trophic status of the lake improved from hypereutrophic to eutrophic state. The concentration of chlorophyll a, an algal pigment that imparts green color to the water[7], decreased by 40%. Decrease in concentration of Chlorophyll a was due to significant change in the population density (size) and dynamics of phytoplankton (algal) species. 21% reduction was observed in the phytoplankton population after Bioaugmentation. The reduction in population of phytoplankton was reflected in reduction in pH of the lake water from an average value of 9.3 to 8.8. Very high pH in lake bodies results due to high photosynthetic activity[8]. Bioaugmentation also caused significant reduction in the concentrations of organic ammonia (represented as TKN), inorganic ammonia and nitrate by 65%, 40% and 79%, respectively. The organic carbon concentration of lake water, as represented in terms of BOD concentrations also showed an average reduction of 29% within two months of Bioaugmentation.

Similarly, the lake sediment also showed a 72% reduction in organic fraction during this period. Improvement in the water quality is also reflected by an overall decrease in the amount of pollution indicating algal species in lake water as represented by Palmer's Pollution Index (PPI). The PPI of the lake decreased by 47% indicating improvement of lake water quality in terms of reduced nutrient concentration.

TABLE 6
EFFECT OF BIOAUGMENTATION ON SEVERAL PARAMETERS BEFORE AND AFTER DOSING

Parameters	Before Dosing (13th Dec 2015)	After Dosing (24th Feb 2016)	Observation	Remarks
pH	9.3	8.8	Decreased by 5%	Improved
Dissolved Oxygen	3.6	8.4	Increased by 133%	Improved
Chlorophyll a	0.30	0.18	Decreased by 40%	Improved
TKN (as N)	98.7	34.5	Decreased by 65%	Improved
Ammonia (as N)	9.8	5.9	Decreased by 40%	Improved
Nitrate (as NO_3)	2.4	0.5	Decreased by 79%	Improved
Phosphate (as P)	1.0	0.6	Decreased by 40%	Improved
BOD (3d, 27^{o}C	35	25	Decreased by 29%	Improved
Sediment Organics (%)	8.7	2.4	Decreased by 72%	Improved
Phytoplankton (Density/L)	7.7×10^6	6.1×10^6	Decreased by 21 %	Improved
Palmer's Pollution Index (PPI)	19	10	Decreased by 47%	Improved
Zoopankton (Density/L)	81333	81	Decreased by 99.9%	Improved

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Nutrient use Efficiency of different Organic Fertilizer and its Effect on Soil Properties and Yield of *Brassica Alboglabra*

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Abstract**—** *In organic farming, homemade organic fertilizer such as compost or enriched organic material had become one of crucial factors for nutrient management. Other than the limited source of N for organic fertilizer as the key factor for growth-limiting nutrients in organic farming, the cost of fertilization is also affected by different organic fertilizer application rates. Therefore, the objectives of this study were (i) to determine the effect of these organic fertilizers on the soil properties (chemical and biological properties) and (ii) to evaluate the efficiency and growth performance of Kailan (Brassica alboglabra) as influence by the application of different organic fertilizer in the field. A study was conducted at an integrated organic farming research area in the Malaysian Agricultural Research and Development Institute (MARDI) at Serdang, Selangor for two consecutive cycles. The experimental treatments were focused on the different rates of organic fertilizer inputs (3-33 t/ha from organic sources) with soil alone as a control. The treatments were applied 14 days after transplanting using Brassica alboglabgra as a test crop. The study result found that a BIOC at 2 tonne kg/ha give significant different in nutrient use efficiency (NUE), however NC at 33 tonne kg/ha rate of organic input had improved most of soil properties NF showed a great yield as well as their growth performances. Study findings have shown that the range of application rate of organic input depends on applicability of the organic fertilizer whether it can promotes nutrient uptake in plants and or can be scaled up to farm level to sustain organic system productivity.*

Keywords**— *Organic Fertilizer, Soil Properties, Yield, Nutrient Use, Organic Farming, Compost.***

I. INTRODUCTION

In organic vegetable cultivation, inorganic fertilizer is not allowed with exception of naturally occurring mineral sources. The substitutes for inorganic fertilizer are mostly in organic nutrient sources that can be grouped into such as animal sources, plant sources, green manures and compost (Aini *et al.*, 2005) which can serve as alternative practice in organic crop production. To improve soil fertility, application of organic fertilizer has become an important practical measure. Organic fertilizers can enhance soil microbial activity by promoting the utilization of nutrients and restoration of microbial community structure and function in the rhizosphere soils. They can increase the mass and diversity of the soil microbiota, improve soil fertility, and stimulate soil enzyme activity, ultimately driving the carbon and nitrogen cycles (Huang et al., 2023). Additionally, organic fertilizers can introduce beneficial microorganisms into the soil, increase microbial diversity, and influence the structure and function of nitrifying and denitrifying microorganisms. Therefore, organic fertilizers play a significant role in enhancing soil microbial activity, ultimately contributing to improved soil health and agricultural productivity (Wang et et ., 2013; Pu et al., 2022; Sivojiene et al., 2021).

Organic fertilizer is able to enhance soil microbial activity (Ren *et* al., 1996), increased microbial biomass (Suresh *et al.*, 2004) and also improved soil structure (Bin, 1983; Dauda *et al.*, 2008). Though high amounts of manure or composts are required initially at organic farm, the quantity of subsequent application may be reduce as soil physical, chemical and biological improve with time (Aini *et al.*, 2005). Organic fertilizers can have varying levels of nutrient use efficiency depending on the type of fertilizer and the farming system. Organic farming systems are highly dependent on organic fertilizers, such as manure and compost, which can provide a range of nutrients to the soil (Chmelikova et al 2021). However, the nutrient content of organic fertilizers can be highly variable, and their release rates can be slower than synthetic fertilizers, which can impact nutrient uptake efficiency (Barlog, 2023). Additionally, organic fertilizers can contribute to soil organic matter, which can improve soil structure and water-holding capacity, ultimately contributing to improved nutrient uptake efficiency (Xingxua et al., 2023).

Overall, the nutrient use efficiency of organic fertilizers can be improved through careful management practices, such as optimizing fertilizer application rates, timing, and placement, and through the use of complementary nutrient sources, such as cover crops and crop rotations (Oluwadunsin et al., 2022). Invariably, many cultivated soils of the world are scarce in one or more of the essential nutrients required supporting vigorous plants. Estimates of overall efficiency of applied fertilizer have been reported to be around or lesser than 50% for N, less than 10% for P, and about 40% for K (Baligar *et al*., 2001). Plants that are capable in absorption and utilization of nutrients prominently enhance the efficiency of applied fertilizers, decreasing cost of inputs, and avoiding losses of nutrients to ecosystems. To increase fertilizer use efficiency (FUE) and to reduce its negative influence on the atmosphere has been an important point in the world for a long time. It can be particularly affected by fertilizer management as well as by soil and irrigation Management. There is increased demand for fertilizer nutrients to meet the global demand for food, However there are inadequate fertilizer resources available and rising public concern related to nutrient use side effects. This has led to calls for Nutrient use efficiency (NUE) to be improved, but not at the expense of decreased crop productivity. NUE depends on the plant's capacity to take up nutrients efficiently from the soil, but also depends on inner transport, storage and remobilization of nutrient.

II. MATERIAL AND METHODS

2.1 Field experiment and treatment used:

The experiment was conducted at Integrated Organic Farm, MARDI Serdang. The test crop is Kailan was grown under rainshelter and treated with seven different application of organic fertilizer. There were chicken dung (CD), indigenous microorganism compost (IMO), vermicompost (V), normal compost (NC), rice husk charcoal compost (BIOC), nature farming (NF), depleted oil bleaching earth compost (DOBE) and plus untreated soil served as CONTROL. The organic fertilizers were applied according to manual and nutrient requirement for kalian and as shown in Table 1. The following treatments were made in a randomized block design (RCBD) in five replicates.

2.2 Soil sampling and Laboratory Analyses:

Soil at depth 0-20 cm were sampled before organic fertilizers application in composite soil samples and at after harvesting in six replicates for chemical analyses for soil quality. The soils were air dried and ground to pass a 2 mm sieve for analyses. Total nitrogen in soils was analyzed by Micro Kjedahl digestion followed by distillation and titration with 0.1 N HCL. Organic carbon was determined by Walkey and Black rapid titration method. Available phosphorus in soils was extracted based on the Bray and Kurtz no.2 procedure. The soil acidity was measured on pH meter using 1:2.5 ratio and water. The exchangeable cations were extracted by leaching with 1 N ammonium acetate and their concentration was determined with Inductively Coupled Plasma (ICP) Optical Emission Spectrophotometer. Wet samples of soil were used for microbial population studies of total colony forming unit (cfu-$\log_{10}$) using total plate count on nutrient agar.

2.3 Nutrient use efficiency calculation

Nutrient use efficiency (NUE) may be defined as yield per unit fertilizer input or in terms of recovery of applied fertilizer. Nutrient use efficiency (NUE) is a critically important concept in the evaluation of crop production systems. The equation used as follows;

$$\text{Partial Factor Productivity (PFP)} = Y/F \qquad (1)$$

Whereas Y=yield of harvested portion of crop with nutrient applied and F = amount of nutrient applied

2.4 Statistical Analysis

All data were subjected to a one-way analysis of variance (ANOVA). Treatment means were compared using Duncan Multiple Range test at a significance level of $P<0.05$ using SAS 9.4.

III. RESULTS AND DISCUSSION

Six local made organic fertilizers (NF, CD, NC, VC, IMO, BIOC, DOBE) with its nutrient properties showed in Table 1. Soil properties of chemical characteristic before the application of organic fertilizer to the soil shown in Table 2. At the end of experiment, several properties of soil nutrient status such as in pH, nitrogen content, CEC and all exchangeable cation (K, Na, Ca and Mg) in those treatment increased significantly as showed in Table 3. Treatment with CD (chicken dung) gave most significant effect in soil nutrient properties. While, the carbon content in all treatment showed no significant different even though there quite high amount of carbon source added in the soil. This might be due to the availability of nutrients especially nitrogen and could be due to improvement of soil water holding capacity.

TABLE 1
SOME CHEMICAL CHARACTERISTIC OF SEVEN LOCAL MADE ORGANIC FERTILIZER USED IN THE EXPERIMENT

Types of fertilizer	pH (H_2O)	Organic carbon (%)	Total N (%)	P_2O_5 (%)	K_2O (%)	Application tonne/ha
V	6.69	21.32	1.79	1.02	1.75	3
IMO	6.36	20.87	1.983	0.25	0.98	20
DOBE	5.58	4.45	0.44	2.5	0.7	20
NC	6.35	24.49	1.57	0.77	2.83	33.33
CD	5.66	3	1.72	1.82	2.18	33.33
BIOCHAR	6.5	19.2	4.1	3.3	2.7	2
NF	6.03	1.17	0.27	0.25	0.98	13.33

TABLE 2
SOME CHEMICAL CHARACTERISTIC OF THE SOIL BEFORE APPLICATION OF ORGANIC FERTILIZER

Soil parameters	Values
Soil pH (H_2O)	6.75
CEC	6.21
Conductivity (µs/cm)	1108
Organic carbon (%)	3.27
Nitrogen (%)	0.118
P (ug/gm)	406.05
Exchangeable K (meq/100gm soil)	101.41
Exchangeable Na (meq/100gm soil)	257.18
Exchangeable Ca (meq/100gm soil)	11.8
Exchangeable Mg (meq/100gm soil)	52.48
Total microbial count (log_{10} CFU)	5.792

The total microbial count in Table 3 does not reflect any variation and had no significant different effect from any organic fertilizer type that had been used compared to control. Generally, the plate culture method normally can only separate 0.1-1.0% of soil microbes and cannot reflect the original status of soil microbial diversity (Cai and Liao, 2002; Vigdis and Lisc, 2002; Luo *et al.* 2003). While, according to vanZwieten (2006), it explained as variable effect of a given amendment on different organism may change the composition of the microbial community without changing the total population or activities.

Management in the use of fertilizer even organic fertilizer can be increase through few recommendation such as use it at right time which is the interrelated to site specific nutrient management (SSNM). Greater synchronization between crop demand and nutrient supply is necessary to improve nutrient use efficiency, especially for N (Giller et al., 2004). Split applications of N during the growing season, rather than a single, large application prior to planting, are known to be effective in increasing N use efficiency. Besides that, integrated nutrient management (INM) involves optimum use of indigenous nutrient components i.e. crop residues, organic manure, biological N fixation as well as chemical fertilizer and their balancing interactions to rises N and P recovery. The appropriate understanding and exploitation of these positive interactions among the plant nutrient is keys for increasing returns to the farmers in terms of yield as well as soil quality and NUE of applied N (Aulakh and Malhi, 2004).

TABLE 3
EFFECT OF DIFFERENT ORGANIC FERTILIZERS ON SOIL PROPERTIES (CHEMICAL AND BIOLOGICAL) AFTER HARVEST PERIOD

Treatments	pH H_2O	Conductivity µs	%		SOIL - P	Total microbial count
			Nitrogen	Carbon	uq/qm of soil	(Log_{10} CFU)
NF	7.06 b	397.7 c	0.09 b	2.69 a	334.34 ab	6.275 a
BIOC	7.03 bc	861.2 b	0.09 b	2.85 a	341.56 ab	6.270 a
NC	7.46 a	1615.8 a	0.14a	2.77 a	244.70 b	6.340 a
CONTROL	7.06 b	1152.1 b	O.10 b	2.59 a	315.18 ab	6.363 a
IMO	7.13 b	720.0 bc	0.09 b	2.40 a	292.22 ab	6.318 a
V	7.16 b	804.0 bc	0.11 ab	2.59 a	296.10 ab	6.248 a
CD	7.13 b	909.2 b	0.12 ab	2.46 a	363.24 a	6.521 a
DOBE	6.78 c	963.0 b	0.11 ab	2.68 a	305.80 ab	6.027 a

Treatments	meq/100q soil				
	CEC	Exch K	Exch Ca	Exch Na	Exch Mg
N F	11.14 ab	65.32 b	163.64 a	5.02 c	34.03 c
BIOC	10.96 ab	106.48 b	150.97 a	8.61 bc	34.46 c
NC	14.37 a	238.37 a	145.07 a	18.02a	47.84 ab
CONTROL	8.19 b	133.56 b	193.53 a	10.03 bc	43.40 ab
IMO	12.15 ab	112.79 b	142.79 a	6.60 c	31.97 b
V	11.56 ab	119.79 b	174.99 a	8.75 bc	39.41 ab
CD	14.09 a	137.55 b	226.56 a	14.27 ab	56.29 a
DOBE	9.72 ab	82.45 b	238.54 a	8.17 bc	43.88 ab

Values are given as a mean of five replicates. Means with the same letter in a column are not significantly different ($P<0.05$) as determined by Duncan Multiple Range Test

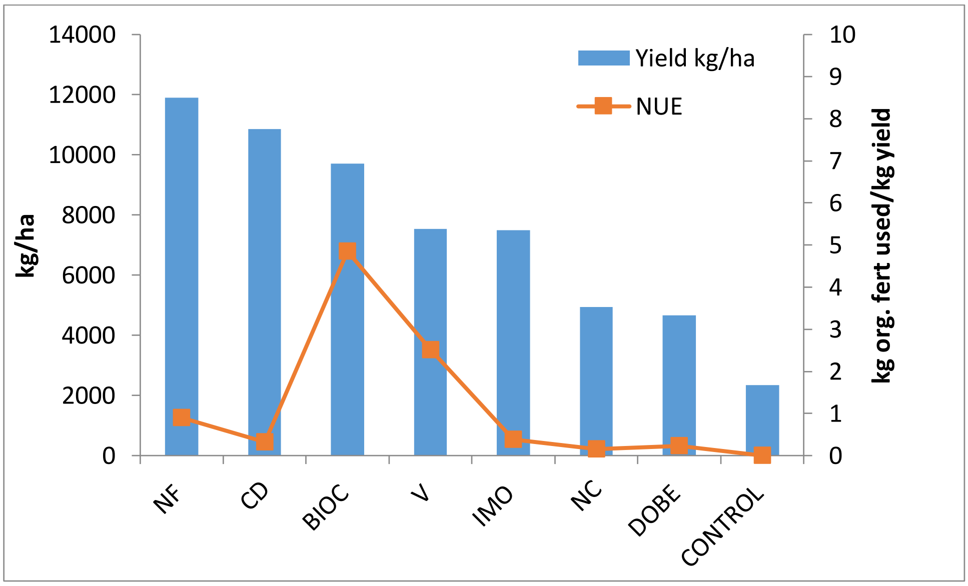

FIGURE 1: Graph on yield of kalian and fertilizer use efficiency (NUE)

The projected yield of kalian as shown in Figure 1 is estimated as medium-low production per hectare of season. The total average yield is about 8490 kg/ha below the targeted medium production 9000 kg/ha. Yield of kailan per plot were higher in treatment with NF (Nature Farming) with estimation of up to 12000 kg per hectare. In term of nutrient use efficiency (NUE) with partial factor of productivity (kg crop yield per kg nutrient applied) as described by Mosier *et al* 2004, it showed treatment with BIOC (biochar compost) and V (vermicompost) gave higher value index compared to other treatment.

The use of organic fertilizers can have several positive effects on soil properties as showed in Table 3. Organic fertilizers can improve soil structure, texture, and aeration, leading to better water retention and healthy root growth (Joaqim et al., 2023; Zhou et al, 2022). They also contribute to the long-term availability of nutrients, add beneficial microorganisms to the soil, and increase soil organic carbon and other nutrients (Sissay, 2019). Additionally, organic fertilizers are environmentally friendly, provide micronutrients, and are less likely to cause burning to plants. Overall, the application of organic fertilizers can lead to improved soil quality, increased crop yields, and sustainable agricultural.

IV. CONCLUSION

The use of different organic fertilizers in the cultivation of *Brassica alboglabra* can significantly influence nutrient use efficiency, soil properties, and crop yield. The effectiveness of these fertilizers depends on their composition and the soil's existing conditions. Organic fertilizers typically improve soil health by enhancing its physical structure, increasing microbial activity, and supplying essential nutrients. This leads to better nutrient availability and uptake by plants, often resulting in improved growth and yield of *Brassica alboglabra*. Moreover, the use of organic fertilizers is also beneficial for long-term soil fertility and environmental sustainability. However, specific outcomes can vary based on the type of organic fertilizer used, application rates, and the particular soil and environmental conditions. Application with organic fertilizer to the soil had increased most of its nutrients properties. Leafy vegetables such as for this experiment, Kailan (*Brassica alboglabra)* can be grown better in soil amended with organic fertilizer but the application rate and availability of all minerals should be considered. Furthermore, organic leafy vegetables are expected to be a quality product for human to consume and maybe profitable to producer and even environmental than those from conventional production system. Further studies are needed to determine optimal rates of organic fertilizer for proper growth and production of organic leafy vegetables.

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The Effect of Inoculation Phosphate Solubilizing Bacteria and *Rhizobium sp.* on Plant Growth and Production of Cowpea (*Vigna unguiculata*)

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Abstract— *The constraints of Alfisol or Mediterranean soils pose unique management challenges for maintaining soil fertility and agricultural productivity. The aim of the research was to study the effect of couple inoculation Rhizobium and Phosphat Solubilizing Bacteria (PSB) on plant growth and production of Cowpea in Alfisol or Mediterranean soils. The experiment used a Randomized Block Design with treatment as combination concentration of PSB and Rhizobium sp. The treatment consist of : control; PSB of 10 ml/L and Rhizobium sp 10 ml/kg seed; PSB of 10 ml/L and Rhizobium sp 20 ml/kg seed; PSB of 10 ml/L and Rhizobium sp 30 ml/kg seed; PSB of 20 ml/L and Rhizobium sp 10 ml/kg seed; PSB of 20 ml/L and Rhizobium sp 20 ml/kg seed; PSB of 20 ml/L and Rhizobium sp 30 ml/kg seed; PSB of 30 ml/L and Rhizobium sp 10 ml/kg seed; PSB of 30 ml/L and Rhizobium sp 20 ml/kg seed; PSB of 30 ml/L and Rhizobium sp 30 ml/kg of seed. Each treatment was repeated with three repetitions. Data analysis used analysis of variance at the 5% level, followed by LSD test at the 5% level. The results showed that couple inoculation PSB and Rhizobium sp. increase significantly N and P soil, plant growth of Cowpea i.e the plant height, number of root nodules, fresh weight, shoot dry weight and total dry weight, the average 100 seed weight, population of PSB in soils and Rhizobium in root cowpea compared by control.*

Keywords— Alfisol, Mediterranean soil, Phosphat Solubilizing Bacteria, Rhizobium sp., Vigna unguiculata.

I. INTRODUCTION

In Malang Regency, Mediterranean soil develops from limestone, calcareous sandstone, marl and basalt andesite. The Mediterranean land is found in Karst plains, tectonic plains, tectonic hills, lower volcanic slopes, old volcanic plains and old volcanic hills. Soil cross section is medium to very deep, good drainage, slightly fine to fine texture, slightly acidic to alkaline pH, medium to very high CEC and high to very high base saturation (BS), low to medium in C-organic and total N content., P_2O_5 (medium to high) and total K_2O low to moderate (Yatno *et al.*, 2017). Mediterranean soil has problems with being too alkaline and low in organic matter, macro and micro nutrient levels as well as low water retention capacity and poor drainage (Tri Pamungkas, 2021). Red Mediterranean soil derived from limestone has a higher soil pH value than soil derived from sandstone. The main problem with Mediterranean soil is the high soil pH which will give rise to direct problems, namely suitability for plants and availability of nutrients, namely low availability of microelements and phosphate because they are bound by Ca^{2+} (Supriyadi, 2007). Soil quality and fertility in Mediterranean calcareous agricultural soils are very important to be managed so that soil productivity can be sustainable. The development of Mediterranean land requires pioneer plants that can increase soil fertility, one of which is legumes. Legume crops can be a practical solution for improving calcareous soils because they contribute to nitrogen fixation, organic matter accumulation, and improved soil health. The results of Tri Pamungkas' research showed that the addition of organic fertilizer significantly increased the growth of plant height, number of leaves, and stem diameter of sugar cane seedlings planted in Mediterranean soil. Legume crops are useful as human and animal feed, wood energy, and as soil-improving components of agricultural and agro forestry systems through its association with bio-fertilizers (Havugimana *et al.*, 2016).

Cowpea is included in the minor legume group which has prospects for future development as a source of food, animal feed, green manure, industry, and health. It belongs to the Leguminosae family and as important legume crop growing across the world mainly intropical and subtropical regions (Papa et al., 2020; Kebede and Bekeko, 2020). It is classified as food crops, feed and industrial raw materials. According to Safitri et al. (2016) stated that cowpea seeds contain a lot of nutrition, every 100 g contains 24.4 g protein, 56.6 g carbohydrates, 1.9 g fat, 481 mg calcium, 399 mg phosphorus, and 2.68 g phytic acid. Cowpea also has a higher vitamin B1 content than green beans. The potential for cowpea seeds is quite high, which can reach 1.5 - 2 tons/ha depending on the variety, location, growing season and cultivation applied. Cowpea has the advantage of having a low fat content so it can minimize the negative effects of fatty food products (Saputro et al., 2015).

Cowpea is a leguminous plant that has also the ability to bind N_2 from the atmosphere. The ability of cowpea to fix nitrogen through biological nitrogen fixation is a cheap and sustainable alternative to inorganic fertilizers. This is because the performance of cowpea depends on the rhizospheric characteristics; hence, it is able to form a beneficial association with microorganisms present in the rhizosphere (Abdel-Fattah *et al.*, 2016). The potency of N_2 fixation by cowpea can be optimized by inoculation of *Rhizobium sp.* Morphologically, cowpea roots spread to a depth of soil between 30-60 cm and can be in symbiosis with *Rhizobium sp*. to bind free Nitrogen (N_2) from the air which then forms root nodules. Inoculation of Rhizobia isolates significantly increased the number of root nodules, compared to plants that were not inoculated (Kebede et al., 2020). Symbiosis between cowpea and *Rhizobium sp.* can increase the availability of N to support plant growth and production. *Rhizobium sp* is one of the bacteria that is very useful for agriculture which acts as a nitrogen fixer from the atmosphere. Nitrogen is one of the main elements in the production of food crops, especially legumes. By fixing nitrogen symbiotically, a cheap source is obtained and can help reduce production costs, especially on infertile soils (Suryantini, 2015). The development of cowpea plants in calcareous areas has several obstacles related to the level of soil fertility. In the calcareous soil, high levels of calcium can also cause problems with the presence of soil potassium. Clay colloids can adsorb and exchange base cations depending on their valence. Cations with three and two valences are more strongly adsorbed by colloids compared to cations with one valence (Putra and Hanum, 2018). As a result, potassium which has a valence of one, will be suppressed by high Ca in calcareous soils which has a valence of two. In calcareous or normal soils, P gets immobilized by cations such as Ca^{2+} to form a complex calcium phosphate ($Ca_3(PO_4)_2$). Nearly, 70 to 90% of phosphorus fertilizers applied to soils is fixed by cations and converted inorganic P (Walpola and Yoon, 2012).

Optimizing the role of cowpeas as a pioneer crop planted in Mediterranean soil requires agronomic practise, one of which is the application of biological fertilizer to help increase the availability of nutrients in the soil, especially elements of N and P to support growth and production. However, the availability of P in the soil is lacking, even though the total amount of P in the soil is high through repeated fertilization applications. This is due to the nature of P which is easily fixed by metals, colloids and calcium which makes it unavailable for plants. Agricultural soils in general also face constraints on the availability of P elements for plants. Even though fertilization has been done many times for each planting, because P is tightly bound by soil colloids, the availability of P element in the soil is low. Likewise with soils that have high Al-dd content, generally also have constraints on the availability of P elements. Low availability of phosphorus (P) in calcareous soils can effect sustainability of improvement in cereals crops yield. A higher amount of calcium in calcareous soils precipitates the P, thus making it immobile in soil. Inoculation of phosphate solubilizing bacteria (PSB) could be helpful in the sustainable management of immobile P in soil (Wahid *et al.*, 2020).

An alternative to increase the efficiency of fertilization in overcoming P fixation by Al, Fe and Ca is by utilizing phosphate solubilizing microorganisms. Phosphate solubilizing bacterial inoculants are microbes given to plants for the process of dissolving P which is bound to become a form available to plants. It play an important role in the biogeochemical cycle of soil P, including dissolution, mineralization, and immobilization of P, thereby increasing P bioavailability and plant nutrition. Root-associated PSB, can modulate plant root systems and stimulate root absorptive capacity and nutrient uptake, including P acquisition (Bargaz *et al.*, 2021). PSB facilitates the conversion of stable phosphorus to active phosphorus and increases the residual phosphorus fraction in compost (Sun *et al.*, 2023). *Bacillus subtilis* is one of PSB which is able to decompose P bonds in the soil and can enhance plant P uptake through remobilization of recalcitrant forms of P in soil. The results of research conducted by Batool and Iqbal (2019) on *Triticum aestivum* L., recommend the use of PSB as biofertilizer, as an alternative to chemical fertilizer.

Providing N and P nutrients in calcareous soil requires technology in the form of the use of biofertilizers which can support the availability of nutrients needed by Cowpea plants to support their growth and production. This study aims to determine the optimal concentration of application of Phosphate solubilizing bacteria and *Rhizobium sp.* to increase biology soil fertility and the growth of cowpea cultivated in Alfisol or Mediterranean soils.

II. MATERIALS AND METHODS

2.1 Study area and soil characteristic

The research was conducted at the Screenhouse of the Faculty of Agriculture, Wisnuwardhana University, Madyopuro Village, Kedungkandang District, Malang City, which is located at an altitude about 500 m above sea level. The type of soil used is the Alfisol or Mediterranean taken from the Bantur area of Malang Regency. The soil used has the following characteristics (primary data from laboratory analysis): pH (H_2O)=7.6 (slightly alkaline), C-Org= 0.73% (very low), N-tot= 0.08% (very low); P-Olsen= 34 ppm (medium); K-dd= 0.24 cmol(+)/kg (low); Ca-dd= 10.20 cmol(+)/kg (high).

2.2 Data collection

Growth parameters observed included plant height, number of root nodules, plant fresh weight, shoot and root dry weight, total dry weight, and weight of 100 seeds, soil PSB population and *Rhizobium sp.* population. Plant height was measured from the base of stem to the top of plant shoot using ruler. The number of root nodules was measured by removing roots at the end of the vegetative phase. The number of effective root nodules is calculated from the pink nodules in color. At the age of 70 days after planting, three samples cowpea plants were taken in each treatment in each replication, then were measured the total fresh weight, dry weight of roots and shoots, and total dry weight of plants. The plant samples were washed then rinsed with distilled water to remove dust and soil on the surface. After the total fresh weight was calculated, then the roots and shoots plant were dried in the oven at 70 ºC for 48 hours and weighed to measure the root dry weight and shoot dry weight (Rahayu et al., 2022). The weight of 100 seeds was measured by weighing 100 cowpea seeds taken from each sample plant on each experimental unit after harvest. Calculation of microbial populations of Rhizobium used the Selective Medium Total Plate Count (TPC) method. A total of 10 grams of sample was suspended in 90 ml of solution sterile physiological saline, followed by serial dilution using 9 ml of sterile physiological saline to level dilution 10-5. A total of 100 l of the suspension then spread into Yeast Mannitol (YM) Agar selection medium added with Congo Red, then incubated 3-10 days at room temperature room (28-30 ºC). Its growth is observed and its population calculated based on the Most Probable method Number (MPN). Calculation of the population of Phosphate Solubilizing Bacteria using the Selective Medium Total Plate Count (TPC) method. A total of ten grams of sample was suspended in 90 ml of solution sterile physiological saline, then serial dilutions were carried out using 9 ml of sterile physiological saline to a 10-5 dilution. A total of 100 l of the suspension was then spread over the selection medium Pikovskaya Agar, and incubated for 7 days at room temperature (28 + 2 ºC). Growth was observed and the number of colonies counted has a clear zone around the colony based on the MPN method (Peraturan Menteri Pertanian, 2011). Soil chemical properties were analyzed consist of pH (H2O 1:1), C organic (Walkley and Balck), Nitrogen (Kjeldahl), P (Olsen), K dan Ca (NH4OAC pH 7.0) (Agricultural Research ad Development Agency, 2012).

2.3 Data analysis

This experiment used a randomized block design, consisting of 9 treatments, namely: control (without PSB and *Rhizobium sp.* innoculants); PSB inoculant concentration of 10 ml/L and *Rhizobium sp.* 10 ml/kg seed; PSB innoculant concentration of 10 ml/L and *Rhizobium sp.* 20 ml/kg seed; PSB innoculant concentration of 10 ml/L and *Rhizobium sp.* 30 ml/kg seed; PSB inoculant concentration of 20 ml/L and *Rhizobium sp.* 10 ml/kg seed; PSB innoculant concentration of 20 ml/L and *Rhizobium sp.* 20 ml/kg seed; PSB inoculant concentration of 20 ml/L and *Rhizobium sp.* 30 ml/kg seed; PSB inoculant concentration of 30 ml/L and *Rhizobium sp.* 10 ml/kg seed; PSB inoculant concentration of 30 ml/L and *Rhizobium sp.* 20 ml/kg seed; PSB inoculant concentration of 30 ml/L and *Rhizobium sp.* 30 ml/kg seed. Each treatment combination was repeated three times. Observational data were analyzed at the 5% level of variance, followed by the 5% BNT test if there was a significant effect of the treatment.

III. RESULTS AND DISCUSSION

3.1 Plant Growth and Production of Cowpea

The results of the varians analysis showed that there was a significant effect on the concentration of Phosphate Solubilizing Bacteria (PSB) and *Rhizobium sp.* on cowpea plant height. Table 1. shows that the height of cowpea plants was significantly different between those treated with phosphate solubilizing bacteria (PSB) and Rhizobium bacteria and those without treatment (control). From the first to the ninth week of observation, the average height of cowpea plants in the control showed significantly different and lower than the cowpea applied with PSB and Rhizobium. Until the end of the observation, which was 63 days after planting (DAP), the cowpea plants grown on media with the inoculation of PSB 20 ml/l and Rhizobium 30 ml/kg, produced the highest growth in plant height (155.33 cm) compared to the other treatments. In fast growth, especially in the vegetative phase, plants need N elements which play an important role in cell division, the formation of amino acids and proteins, chlorophyll and many potential enzymes that are important for photosynthesis and plant growth (Fitriatin et al., 2017), as well as important for stimulating root growth and increasing the uptake of other nutrients for plants (Nget *et al.*, 2022). So that with higher Rhizobium inoculation it helps the availability of N in the soil.

TABLE 1
PLANT HEIGHT (CM) OF COWPEA (*VIGNA UNGUICULATA*) AS INOCULATION OF PHOSPHATE SOLUBILIZING BACTERIA AND *RHIZOBIUM SP.*

PSB and Rhizobium Concentration	Plant height (cm)																	
	7 dap		14 dap		21 dap		28 dap		35 dap		42 dap		49 dap		56 dap		63 dap	
0 ml/L and 0 ml/kg	10,17	a	13,17	a	19,17	a	26,50	a	30,83	a	40,83	a	56,67	a	62,67	a	69,33	a
10 ml/L and 10 ml/kg	17,67	b	26,17	b	59,50	c	74,50	b	91,00	b	112,33	bc	106,17	b	110,50	b	116,00	b
10 ml/L and 20 ml/kg	18,17	b	25,33	b	61,50	c	68,17	b	94,50	b	111,83	bc	127,17	c	132,83	bc	136,83	bc
10 ml/L and 30 ml/kg	18,83	b	28,33	b	63,33	c	75,83	b	96,00	b	113,17	bc	120,67	bc	126,83	bc	134,67	bc
20 ml/L and10 ml/kg	16,67	b	25,33	b	49,83	bc	76,83	b	91,33	b	111,83	bc	133,50	c	136,83	bc	146,17	c
20 ml/L and 20 ml/kg	17,50	b	27,33	b	57,00	bc	79,33	b	96,17	b	92,17	b	131,17	c	136,17	bc	149,50	c
20 ml/L and 30 ml/kg	18,00	b	27,67	b	61,50	c	81,17	b	101,17	b	117,83	c	136,17	c	141,17	c	155,33	c
30 ml/L and 10 ml/kg	18,00	b	24,67	b	39,00	b	75,50	b	101,83	b	126,33	c	134,67	c	138,33	c	144,67	c
30 ml/L and 20 ml/kg	18,50	b	24,83	b	52,67	bc	75,33	b	94,17	b	117,67	c	125,50	c	128,67	bc	142,33	c
30 ml/L and 30 ml/kg	16,50	b	29,00	b	56,83	bc	77,67	b	98,33	b	119,50	c	127,83	c	133,17	bc	143,83	c
BNT 5%	3,005		6,68		18,41		19,13		20,44		22,52		16,45		27,24		25,28	

Note: the numbers followed by the same letter in the same column are not significantly different in the Least Significant Difference Test (LSD) at the 5% level. PSB = Phosphate Solubilizing Bacteria; PSB = Phosphate Solubilizing Bacteria; hst = days after planting; dap = day after planting

Table 2 shows that the control treatment and lower concentration of PSB and Rhizobium, namely 10 ml/l and Rhizobium 10 ml/kg, tended to produce an average weight of 100 cowpea seeds lower than cowpea plants which were inoculated with the higher concentration of PSB (20 ml/l and 30 ml/l) and Rhizobium concentration (20 ml/kg and 30 ml/kg). The results of (Nyaga and Njeru, 2020) research showed that rhizobia inoculation significantly increased cowpea yields where inoculation with native isolates recorded 22.7% increase in yield when compared to uninoculated control in the first season and 28.6% increase in yield in the second season.

TABLE 2
WEIGHT OF 100 SEEDS, NUMBER OF ROOT NODULES, ROOT LENGTH OF COWPEA (*VIGNA UNGUICULATA*) AS INOCULATION OF PHOSPHATE SOLUBILIZING BACTERIA AND *RHIZOBIUM SP.*

PSB and Rhizobium Concentration	100 seed weight (g)		The Number of root nodule		Root length (cm)	
0 ml/L and 0 ml/kg	7,03	A	1,83	A	9,00	a
10 ml/L and 10 ml/kg	7,17	A	2,17	A	9,33	ab
10 ml/L and 20 ml/kg	7,75	abc	4,00	Ab	12,00	bcde
10 ml/L and 30 ml/kg	8,32	bc	5,00	Bc	12,83	cde
20 ml/L and 10 ml/kg	7,20	Ab	7,00	Cd	10,00	abc
20 ml/L and 20 ml/kg	10,45	D	10,00	E	11,00	abcd
20 ml/L and 30 ml/kg	12,14	E	11,00	Ef	11,00	abcd
30 ml/L and 10 ml/kg	8,65	C	9,00	De	10,83	abcd
30 ml/L and 20 ml/kg	11,77	E	10,00	E	13,00	de
30 ml/L and 30 ml/kg	12,27	E	13,33	F	14,00	e
BNT 5%	1,13		2,34		2,88	

Note: The numbers followed by the same letter in the same column are not significantly different in the Least Significant Difference Test (LSD) at the 5% level. PSB = Phosphate Solubilizing Bacteria.

Observation of the root nodules number showed that the control treatment and the low concentration of PSB and Rhizobium respectively 10 ml/l and 10 ml/kg, tended to produce number of cowpea nodules as 1-2 root nodules. This result showed lower than the higher concentration of PSB (20 ml/l and 30 ml/l) and Rhizobium (20 ml/kg and 30 ml/kg). The highest number of root nodules was produced in the inoculation of PSB 30 ml/l and Rhizobium 30 ml/kg and the application of PSB 20 ml/l by soaking the seeds in Rhizobium 30 ml/kg. The results of this study are in line with the results of Habete and Buraka (2016) research, that showed the presence of rhizobium bacteria inoculation increased the number of nodules per plant which was significantly different for the two varieties of beans. Furthermore, both N fertilizer application and rhizobium inoculation increased 100-seeds weight and seed yield per ha. For better soil health and reduced production costs, it is advisable to use bacteria treated seeds. The root length parameter showed that the cowpea plants that were inoculated with PSB 30 ml/l and Rhzobium sp. 30 ml/kg produced an average root length longer than the other treatments, while the control cowpea plants produced short root lengths. The result of (Chandra and Kumar, 2008) research showed that combined inoculation treatment of *Rhizobium sp.*+ Phosphate Solubilizing Bacteria, PSB (Bacillus megaterium) + PGPRs LK-786 (Kurthia sp.) produced the highest and significantly more number and dry weight of nodules and plant dry weight of Lentil (Lens culinaris L.) than *Rhizobium sp.* alone at different intervals. Combined inoculation of chickpea with Rhizobia and Phosphate Solubilizing Bacteria (PSB), Bacillus sp., and Enterobacter aerogenes showed a significant enhancement of chickpea nodulation, biomass production, yields and N, P, and protein content in grains as compared to single inoculation or single application of N or P. Formulation of biofertilizers based on tasted strains could be used for chickpea co-inoculation in P-deficient soils (Benjelloun *et al.*, 2021). Rhizobia in association with a legume host have the exceptional ability to form root nodules. PGPR with rhizobial inoculation enhanced nodulation, growth and yield of lentil (Lens culinaris) (19.8 nodules/plant, 70.6 mg nodule dry weight/plant, 1 605kg/ ha) as compared to Rhizobium alone (17.8 nodules/plant, 64.3mg/plant and 1 546kg/ha resp.) and non-inoculated control (13.9nodules/plant, 47.7mg/plant and 1 401kg/ha resp.) (Khanna and Sharma, 2011).

TABLE 3
PLANT FRESH WEIGHT, SHOOT DRY WEIGHT, ROOT DRY WEIGHT, AND TOTAL DRY WEIGHT OF COWPEA (*VIGNA UNGUICULATA*) AS INOCULATION OF PHOSPHATE SOLUBILIZING BACTERIA AND *RHIZOBIUM SP.*

PSB and Rhizobium Concentration	Fresh Weight (g)		Root Dry Weight (g)		Shoot Dry Weight (g)		Total Dry Weight (g)	
0 ml/L and 0 ml/kg	65,17	a	0,61	a	18,79	a	19,39	a
10 ml/L and 10 ml/kg	72,00	ab	0,65	a	24,56	b	25,22	b
10 ml/L and 20 ml/kg	73,67	ab	0,47	a	25,31	b	25,78	b
10 ml/L and 30 ml/kg	75,50	ab	0,60	a	25,84	b	26,43	b
20 ml/L and 10 ml/kg	78,67	bc	0,79	a	26,73	b	27,53	b
20 ml/L and 20 ml/kg	80,67	bc	0,53	a	26,78	b	27,30	b
20 ml/L and 30 ml/kg	91,33	c	0,63	a	31,34	c	31,97	c
30 ml/L and 10 ml/kg	71,33	ab	0,44	a	24,54	b	24,97	b
30 ml/L and 20 ml/kg	74,33	ab	0,58	a	25,34	b	26,02	b
30 ml/L and 30 ml/kg	79,83	bc	0,53	a	27,26	bc	27,94	bc
BNT 5%	12,84		0,35		4,37		4,11	

Note: the numbers followed by the same letter in the same column are not significantly different in the Least Significant Difference Test (LSD) at the 5% level. PSB = Phosphate Solubilizing Bacteria.

Table 3 shows that the fresh weight of cowpea was significantly different between those treated with phosphate solubilizing bacteria and Rhizobium bacteria and those without treatment (control). The control showed plant fresh weight (65 g) lower than cowpea inoculated with PSB and Rhizobium. In cowpea plants inoculated with 20 ml/l PSB and *Rhizobium sp* 10 ml/kg, 20 ml/l PSB and 20 ml/kg *Rhizobium sp.*, 20 ml/l PSB and 30 ml/kg Rhizobium resulted in a higher average fresh weight than those inoculated with 10 ml/l PSB at all of Rhizobium concentrations level.

The result showed that there were no significant difference on root dry weight between the plants inoculated with PSB and Rhizobium and the control. Meanwhile, there were significant differences on shoot dry weight and total plant dry weight between control and those applied with PSB and *Rhizobium sp*. The highest shoot dry weight and total dry weight were shown in plants that were inoculated with PSB 20 ml/l and Rhizobium 30 ml/kg. The plant growth needs Nitrogen and Phosphorus as a mineral nutrient. Although Phosporus is abundance in soil, both in organic and inorganic forms, its availability is limited because P mostly occurs in insoluble forms. The average soil P content is about 0.05% (w/w) but only 0.1% of the total P is available to plants due to its low solubility and soil fixation. An adequate supply of phosphorus during the early phases of plant development is important for supporting the development of the reproductive parts of the plant. This is because P plays an important role in increasing root branching and increasing sturdy plants and resistance to disease. Phosphorus accounts for about 0.2 - 0.8% of the plant's dry weight. With the application of phosphate solubilizing microorganisms (PSM) it is the best environmentally friendly means to help the availability of plant P nutrients (Sharma *et al.*, 2013). The combined inoculation of Arbuscular Mycorrhizal Fungi (AMF) and Phosphate Solubilizing Bacteria (PSB) with ground rock phosphate RP had more potential to improve maize-wheat yields and P uptake comparable to those obtained by using expensive phosphatic fertilizers in P deficient calcareous pH soils (Wahid *et al*., 2020). The research results of Hashem et al. (2019) showed that inoculation of plant roots with rhizobia may result in a small number of nodules depending on the root nodulation process which is based on the exchange of signals between the host and bacteria leading to the formation of rhizobia in the host tissue, nodulation and plant growth through increased day element uptake from the soil.

Rhizobium and phosphate solubilizing bacteria are important for plant nutrition. These microbes also have an important role as plant growth-promoting rhizobacteria (PGPR) in plant biofertilizers. Inoculation of Rhizobium and BPF either singly or both with the use of phosphate fertilizer significantly increased root and shoot weight, plant height, seed yield, seed P content, leaf protein content and leaf sugar content. Inoculation of Rhizobium and BPF either singly or both with the addition of phosphate fertilizer improves seed production 30% - 40% better than using only phosphate fertilizer . The synergistic relationship between rhizobium inoculation and BPF which produces growth regulators such as IAA and GA can improve root growth both in length and root weight which in turn can increase water and nutrient uptake which helps improve plant canopy growth (Afzal and Bano, 2008). The role of Bacillus subtilis which plays a direct role in P solubilization, it can help the availability of P nutrients in the soil, and can increase plant growth. Among microbial inoculants, the Rhizobium + PSB was found most effective in terms of nodule number (27.66 nodules plant^{-1}), nodule fresh weight (144.90 mg plant^{-1}), nodule dry weight (74.30mg plant^{-1}), shoot dry weight (11.76 g plant^{-1}), and leghemoglobin content (2.29mg g^{-1} of fresh nodule) and also showed its positive effect in enhancing all the yield attributing parameters, grain and straw yields of Chickpea (Tagore *et al.*, 2013).

3.2 Population of Soils Phosphate Solubilizing Bacteria and Population of Cowpea Roots *Rhizobium sp.*

The results of laboratory analysis showed that inoculation of Phosphate Solubilizing Bacteria (PSB) *Bacillus subtilis* increases the soil PSB population (Table 4). The highest soil PSB population of 2.31 x 10^7 CFU/g was obtained from the PSB treatment at concentration of 20 ml/L compared to the control i.e population of 1.98 x 10^6 CFU/g (Table 4). This is in line with the results of Fitriatin et al. (2017) that the PSB population in the Rhizosphere of corn plants can be increased by application of PSM biofertilizer (Phosphate Solubilizing Microorganisms) to 2.0 x 10^{11} CFU/g compared to control (without PSM biofertilizer) as PSB population of 1 .2 x 10^{11} CFU/g. The use of PSB could significantly increase pH, available phosphorus and several kinds of trace elements both in the rhizosphere and non-rhizosphere soil. The PSB secreted small molecular organic acids to dissolve inorganic phosphorus and changed the soil properties, which changed the rhizosphere microbial community indirectly. PSB can produce various organic acids with low molecular weight, which alter the soil pH, solubilizing the phosphate from acid or alkaline soils (Liu *et al.*, 2020).

Alfisols or Mediterranean soils are a group of red soils caused by high iron content and low humus content (Wijanarko et al., 2007). Organic matter is a source of energy for soil macro and micro fauna. Soils with low organic matter content have an effect on low soil microbiological activity and population. Biological fertility (microbiological population) of soil can be increased by addition of organic matter as well as the addition of biological fertilizers. The results showed that increasing the concentration of Rhizobium to 30 ml/kg of seed could increase the soil Rhizobium population up to 1.29 x 10^7 CFU/g compared to no application of Rhizobium (control) as a population of 2.87 x 10^6 CFU/g. The increase in microbial population in the soil can be due to the application of bioinoculants which induce better root proliferation and are responsible for high soil enzyme activity resulting in higher biomass production and extended root exudates (Prathima *et al.*, 2022).

TABLE 4
POPULATION OF SOIL PSB AND POPULATION OF COWPEA ROOT *RHIZOBIUM SP.*

Treatment	Type of Bacteria	Population of Bacteria (CFU/g)
R0 (Control)	*Rhizobium sp.*	$2,87 \times 10^6$
R1 (10 ml/kg seeds)	*Rhizobium sp.*	$5,60 \times 10^6$
R2 (20 ml/kg seeds)	*Rhizobium sp.*	$1,01 \times 10^7$
R3 (30 ml/kg seeds)	*Rhizobium sp.*	$1,29 \times 10^7$
B0 (Control)	PSB *Bacillus subtilis*	$1,98 \times 10^6$
B1 (10 ml/L)	PSB *Bacillus subtilis*	$3,14 \times 10^6$
B2 (20 ml/L)	PSB *Bacillus subtilis*	$2,31 \times 10^7$
B3 (30 ml/L)	PSB *Bacillus subtilis*	$9,61 \times 10^6$

3.3 Nitrogen and Phosporus of Soils

Table 5 shows that the Nitrogen total in soils was significantly different between those treated with phosphate solubilizing bacteria and Rhizobium bacteria and those without treatment (control). Planting media and plants inoculated with PSB 10 ml/L and 30 ml/kg *Rhizobium sp.*, PSB 20 ml/L and 30 ml/kg *Rhizobium sp.*, PSB 30 ml/L and 30 ml/kg *Rhizobium sp.*, resulted N total in the soils was higher compared to other treatments. The research result of Wei et al. (2023) showed that long-term co-application of rhizobium and PK (phosphorus and potassium fertilization) promoted soybean nodule dry weight by 33.94% compared with PK + N (nitrogen and PK fertilization), and increased soybean yield by average of 32.25%, 5.90%, and 5.00% compared with CK (non-fertilization control), PK, and PK + N, respectively. The main effect of two rhizobium strain (Faba bean, Fb17 and Fb18) positively improved soil porosity, but soil bulk density was negatively infuenced. Soil chemical parameters such as organic carbon, total N, available P, available sulfur, Ca, Mg, and K were positively influenced (Chimdi et al., 2022). Inoculation Rhizobium can increase the availability of N in the soils through nitrogen fixation capability. The research of Oktaviani et al. (2017) showed that combination of Rhizobium and compot 200 g resulted highest value on the total soil N. The highest N soil is produced from combination treatment of 0.245 g, 200 g and Rhziobium compost as much as 25g.

Table 5 also shows that application of 30 ml/L PSB resulted in significantly higher P-total compared to controls and lower PSB concentrations. Total soil P levels in the media and plants applied were 30 ml/L PSB and 20 ml/kg *Rhizobium sp.* showed higher results than the control (without PSB and Rhizobium application) and other treatments. This is very possible because in calcareous soil Ca fixation of soil P becomes a form that is insoluble in the soil solution, and as a consequence is not available to plants. It could be mobilized, converted into soluble P forms using of PSM (Kalayu, 2019). Soil phosphate solubilizing bacteria (PSB) inoculation with mineral P can improved postharvest soil fertility relative to pre-harvest by improving soil organic matter from 0.61% to 0.70%, lowering pH from 7.74 to 7.68, and improving soil total N from 0.04 to 0.09%, ABDTPA-extractable P from 2.07 to 3.44 $mg.kg^{-1}$, and potassium (K) concentrations from 100.27 to 129.45 $mg.kg^{-1}$ (Khan *et al.*, 2022).

TABLE 5
THE CONTENT OF NITROGEN AND PHOSPORUS SOILS AS INOCULATION OF PHOSPHATE SOLUBILIZING BACTERIA AND *RHIZOBIUM SP.*

PSB Bacillus subtilis* and *Rhizobium sp. concentration	**N-Tot (%)**		**P-Tot (mg.kg-1)**	
0 ml/L and 0 ml/kg	0,07	a	208	a
10 ml/L and 10 ml/kg	0,21	b	421	b
10 ml/L and 20 ml/kg	0,28	b	454,67	bc
10 ml/L and 30 ml/kg	0,42	c	611,67	bcd
20 ml/L and 10 ml/kg	0,21	b	470,33	bc
20 ml/L and 20 ml/kg	0,28	b	623,33	cd
20 ml/L and 30 ml/kg	0,42	c	616	cd
30 ml/L and 10 ml/kg	0,21	b	604	bcd
30 ml/L and 20 ml/kg	0,28	b	943	e
30 ml/L and 30 ml/kg	0,42	c	723	d
BNT 5%	0,07		192,85	

Note: the numbers followed by the same letter in the same column are not significantly different in the Least Significant Difference Test (LSD) at the 5% level. PSB = Phosphate Solubilizing Bacteria.

IV. CONCLUSION

Inoculation of Phosphate Solubilizing Bacteria (PSB) and *Rhizobium sp.* to cowpea (*Vigna unguiculata*) grown on Alfisol or Mediterranean soils, has an influence on the growth and yield of cowpea plants, namely cowpea plant height, number of root nodules, fresh weight per plant, shoot dry weight and total plant dry weight and average weight of 100 seeds. It's also influence on increasing of soils PSB population and cowpea roots Rhizoobium. Total of N and P soils increased by inoculation of PSB and Rhizobium.

The inoculation of PSB 20 ml/l and 30 ml/l and Rhizobium 10 ml/kg, 20 ml/kg and 30 ml/kg resulted in higher plant height, plant fresh weight, shoot dry weight, total dry weight, and weight of 100 seeds of cowpea higher than the other treatments. The highest number of root nodules was produced in the treatment of PSB 30 ml/l and Rhizobium 30 ml/kg. The highest soil PSB population was resulted in the treatment of 20 ml/L and the highest population of cowpea roots *Rhizobium sp.* were produced in the 30 ml/kg treatment. The inoculation of Rhizobium 30 ml/kg produced the highest N-total soil, meanwhile inoculation of PSB 30 ml/L produced the highest P-total soils.

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