

1 **Saline soil bioremediation using *Muntingia calabura* extracts reveals the first reported isolation**
2 **of halotolerant bacteria molecularly related to *R. marisflavi* and *C. firmus* in Central Luzon,**
3 **Philippines**

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21 **ABSTRACT**

22 Land salinization negatively affects soil quality and crop productivity. This can be countered
23 through bioremediation of salt-affected soil using microorganisms, which can be further enhanced
24 through biostimulation. In the present study, the biostimulatory effect of *Muntingia calabura* fruit
25 and leaf extracts as determined by soil salinity and bacterial load was explored. Representative
26 bacterial colonies were also characterized via full-length 16S rRNA (~1500 bp) analysis through
27 DNA barcoding. The results showed that the soil with fruit and leaf extracts (SS + FE and SS + LE,
28 respectively) demonstrated lower electrical conductivity (EC) values than those of the control
29 (applied with distilled water). Meanwhile, the bacterial load in all the treatments gradually decreased,
30 possibly due to the salt stress and the extracts' antimicrobial properties. Molecular characterization
31 revealed that the surviving species were closely related to halotolerant bacteria *Rosellomorea*
32 *marisflavi* with 100.00% percent identity and *Cytobacillus firmus* (98.97% and 98.84%),
33 documenting the first potential occurrence of these taxa in Central Luzon, Philippines. The study
34 suggests that the extracts, while lowering total bacterial load, might have served as a growth-
35 supporting nutrient source for the halotolerant species, corroborating the improved EC reduction.
36 Further investigations on the multifaceted bioremediation mechanisms using the extracts are strongly
37 recommended.

38 **Keywords:** Salinity bioremediation; Biostimulation; Green waste utilization; Soil amendment;
39 Electrical conductivity; Indigenous bacteria

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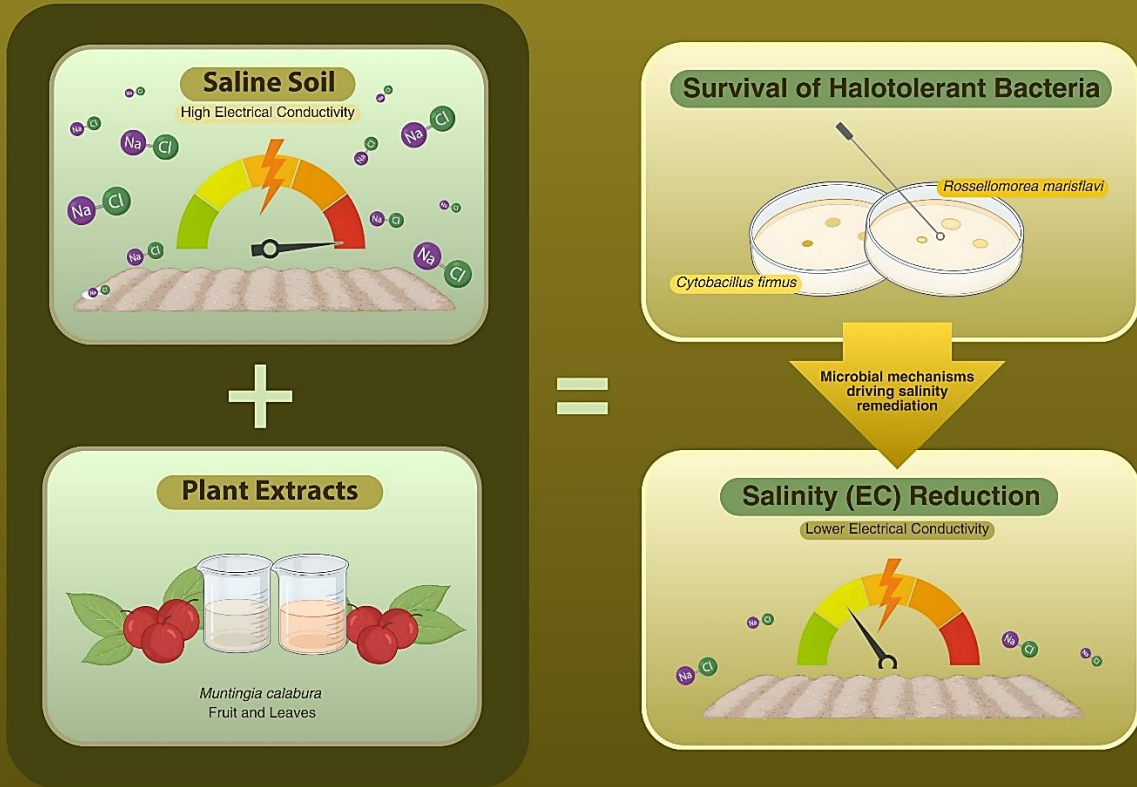
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Saresa (*Muntingia calabura*) fruit and leaf extracts enhance saline soil bioremediation



62 INTRODUCTION

63 The Philippines, as an agricultural country, highly depends on cultivating various crops for
64 livelihood, economic stability, and food security. With the vast land areas used in farming rice, corn,
65 sugarcane, and other essential crops, maintaining healthy soil quality is vital to maximize agricultural
66 productivity. In particular, different environmental factors affect not only soil health and quality, but
67 also the growth performance and yield of any plant/crop it anchors. Soil salinity, defined as the salt
68 and/or mineral content of the soil, is one of these influencing factors that, when at high levels,
69 becomes detrimental to the biological processes vital to the crop growth and development (Kamran
70 *et al.* 2019).

71 Defined as an increase in the salinity of the soil, land salinization covers over 118 countries
72 with more than 424 million hectares of topsoil and 833 million hectares of subsoil being salt-affected
73 (Food and Agriculture Organization, 2025). This process can be induced by climate change, the
74 massive introduction of intensive farming-associated irrigation, and the frequent use of groundwater,
75 as well as low-quality water, for irrigation (Machado & Serralheiro, 2017). External stress due to high
76 soil salinity impacts plant growth through osmotic shock and ionic toxicity, which both interfere with
77 photosynthesis, cellular metabolism, and uptake of water and nutrients (Safdar *et al.* 2019). As cited
78 by Shrivastava and Kumar (2015), more than 50% of the arable land is estimated to be salinized by
79 the year 2050 (Jamil *et al.* 2011).

80 In the Philippines, the Bureau of Soils and Water Management (2020) continuously works to
81 address soil salinity problem in the country. According to their consolidated technical reports, at least
82 17 provinces have areas with slightly saline to very severely saline conditions, characterized by high
83 electrical conductivity. Meanwhile, there are studies showing that some groundwater sources in
84 Tarlac, relatively utilized for irrigation, displayed high electrical conductivity (Franquera *et al.* 2019;
85 Inson *et al.* 2021). With the soil salinity challenges not only in the country, but also globally, it is
86 imperative to provide strategies for land preservation, ensuring maximum agricultural productivity.

87 Biostimulation is an ideal strategy to desalinate salt-affected soil. It refers to the addition of
88 nutrients to enhance the bioremediation ability of microorganisms, boosting the removal and/or
89 degradation of soil pollutants in the process. In biostimulation, the microorganisms are stimulated
90 through substrate modification (Adams *et al.* 2015). According to the study of Shahi *et al.* (2016),
91 both the pollutant degradation capability and growth of the bacterial community in soil were enhanced
92 after the addition of total organic carbon. Moreover, the reviews of Cherif-Silini *et al.* (2021),
93 Mokrani *et al.* (2022), Terzaghi *et al.* (2022), and Gupta *et al.* (2024) all emphasize the utilization of
94 microorganisms as a promising strategy in soil desalination. Furthermore, the application of
95 halophilic and halotolerant microorganisms can aid in the long-term production of crops under salt-
96 stressed conditions (Kumawat *et al.*, 2022). Hence, exploiting the desalinating ability of these
97 microorganisms and boosting it through biostimulation is an advantageous and eco-friendly approach.

98 *Muntingia calabura*, locally known as “aratis” or “saresa”, is a plant present in tropical areas
99 and is common in the Philippines. Its fruits and leaves are easily detached from the tree, generating
100 green waste. Typically, these fruits and leaves are of no use to the landowners who have *M. calabura*
101 trees in their premises and simply discard them. Langsdorf *et al.* (2021) emphasized that green waste,
102 when properly utilized, can be converted into functional materials. Therefore, to make use of these
103 fruits and leaves, the biostimulatory effects of their extracts towards the microbial activity of naturally
104 present bacteria in soil were evaluated to promote salinity reduction. Similarly, studies have already
105 reported the bioremediation of pollutants with the amendments of plant materials, including wheat
106 straw (Cai *et al.* 2023), corn stalk (Gideon *et al.* 2025), rice straw (Zhang *et al.* 2025), and sugarcane
107 residual waste (Umar *et al.* 2022). Moreover, *M. calabura* leaves and fruits contain sugars, organic
108 compounds (Pereira *et al.* 2018; Zolkeflee *et al.* 2021), high moisture, fiber, crude fiber, as well as
109 magnesium, sodium, potassium, phosphorus, and iron (Peter *et al.* 2020). All of these chemical
110 constituents of *M. calabura* leaves and fruits can be favorable factors for the growth of indigenous
111 bacteria, thereby increasing their bioremediation potential.

112 In this context, the study investigated the biostimulatory effects of *M. calabura* fruit and leaf
113 extracts on saline soil by evaluating salinity reduction through electrical conductivity; changes in soil
114 quality parameters (pH and dissolved oxygen), bacterial load, and the molecular characterization of
115 bacterial isolates to determine whether halotolerant species thrive, potentially contributing to salt
116 reduction.

117

118 **MATERIALS AND METHODS**

119 **Extraction of *M. calabura* fruits and leaves**

120 Detached *M. calabura* fruits and leaves were collected from around Tarlac State University –
121 Lucinda Campus, Tarlac City, Tarlac, Philippines. After collection, they were washed under running
122 water to remove dirt and other particles. Surface sterilization was carried out by dipping the fruits and
123 leaves in 70% ethanol for 5 minutes, followed by rinsing with sterile distilled water. Separately, fruits
124 and leaves were boiled in sterile distilled water (1:2 w/v, 50 g of fruits/leaves to 100 mL distilled
125 water) for 5 minutes. The resulting solution was filtered and dispensed into sterilized storage bottles.
126 The extracts were then stored at refrigerated conditions until the laboratory analyses.

127 **Pot experiment**

128 Soil samples from around a man-made lagoon (Figure 1) located on the same campus were
129 collected and sieved. The soil was mixed with autoclaved loam soil (1:1 w/w, 50 g each) inside sterile
130 storage bottles. As a pilot research, the setups were then spiked with a 50 mM NaCl solution to
131 simulate a critical ecological tipping point for salinity-induced stress in plants, as described by
132 Shrivastava & Kumar (2015) and Zhou *et al.* (2024). Afterwards, the fruit and leaf extracts were also
133 mixed into the soil mixture using spatulas. The setups were then stored in a room with a temperature
134 and illumination of approximately 27°C and 31 lux, respectively. Table 1 summarizes the

135 experimental groups, with DW + DW and SS + DW serving as the baseline and negative control
136 groups, respectively. Three (3) replicates were prepared for each treatment.



137
138 Figure 1. Man-made lagoon in Tarlac State University – Lucinda Campus

139

140 Table 1. Composition of the biostimulation setups

Treatment	Components
DW + DW	Soil + 20 mL distilled water + 10 mL distilled water
SS + DW	Soil + 20 mL salt solution + 10 mL distilled water
SS + FE	Soil + 20 mL salt solution + 10 mL fruit extract
SS + LE	Soil + 20 mL salt solution + 10 mL leaf extract

141 Note: DW = distilled water; SS = salt solution; FE = fruit extract; LE = leaf extract

142

143 **Physicochemical and microbiological analyses**

144 Soil samples from the biostimulation setups were obtained, mixed, and homogenized in sterile
145 distilled water in a 1:5 w/v ratio (Ismayilov *et al.* 2021; Kargas *et al.* 2022). The soil extracts were
146 then transported to the laboratory for physicochemical analyses. Using a multi-parameter meter, the

147 electrical conductivity (EC), as well as the dissolved oxygen (DO) and pH of the extracts, were
148 measured.

149 Another soil sample sets were transferred and mixed into test tubes containing 0.9% NaCl
150 solution up to 10^{-6} serial dilutions. Aliquots from these dilutions were spread-plated using Plate Count
151 Agar (PCA) enriched with nystatin (to inhibit fungal growth), and NaCl adjusted to 50 mM (to
152 simulate the saline conditions of the origin soil). The plates were then incubated at 30°C for 6 days.
153 After incubation, colony formations were observed and counted.

154 **Isolation and molecular characterization of bacterial colonies**

155 To further understand the effect of the extracts on the bacterial activity, the most abundant
156 bacterial colonies from the PCA plates were isolated. Three colonies were randomly selected and
157 subcultured onto Nutrient Agar (NA) plates, which were also supplemented with NaCl and nystatin.
158 For initial characterization, biochemical tests were done to determine whether the colonies produce
159 catalase, oxidase, coagulase, and indole.

160 For the molecular characterization, the bacterial cultures were submitted to Apical Scientific,
161 Selangor, Malaysia, through the Noveaulab Asia Corporation, Quezon City, Metro Manila,
162 Philippines. Bacterial DNA Barcoding was employed to analyze the full-length of 16S rRNA (~1500
163 bp). The processes involved were (1) extraction of the genomic DNA, (2) PCR amplification and
164 purification, and (3) bidirectional sequencing of the PCR products. Sequence similarities were
165 assessed to determine the top 10 matches, and phylogenetic trees were subsequently generated.

166 **Data analysis**

167 The research utilized a completely randomized design to lay out the four (4) treatments with
168 three (3) replicates. One-way analysis of variance (ANOVA) and Tukey Post-Hoc Test at 5% level
169 of significance were used to analyze and compare the data per treatment in terms of the
170 physicochemical and microbiological parameters. Meanwhile, the computational tool Basic Local

171 Alignment Search Tool (BLAST) was used for analyzing sequence similarity for molecular
172 characterization. The top ten (10) matching sequences were obtained from the National Center for
173 Biotechnology Information (NCBI) database.

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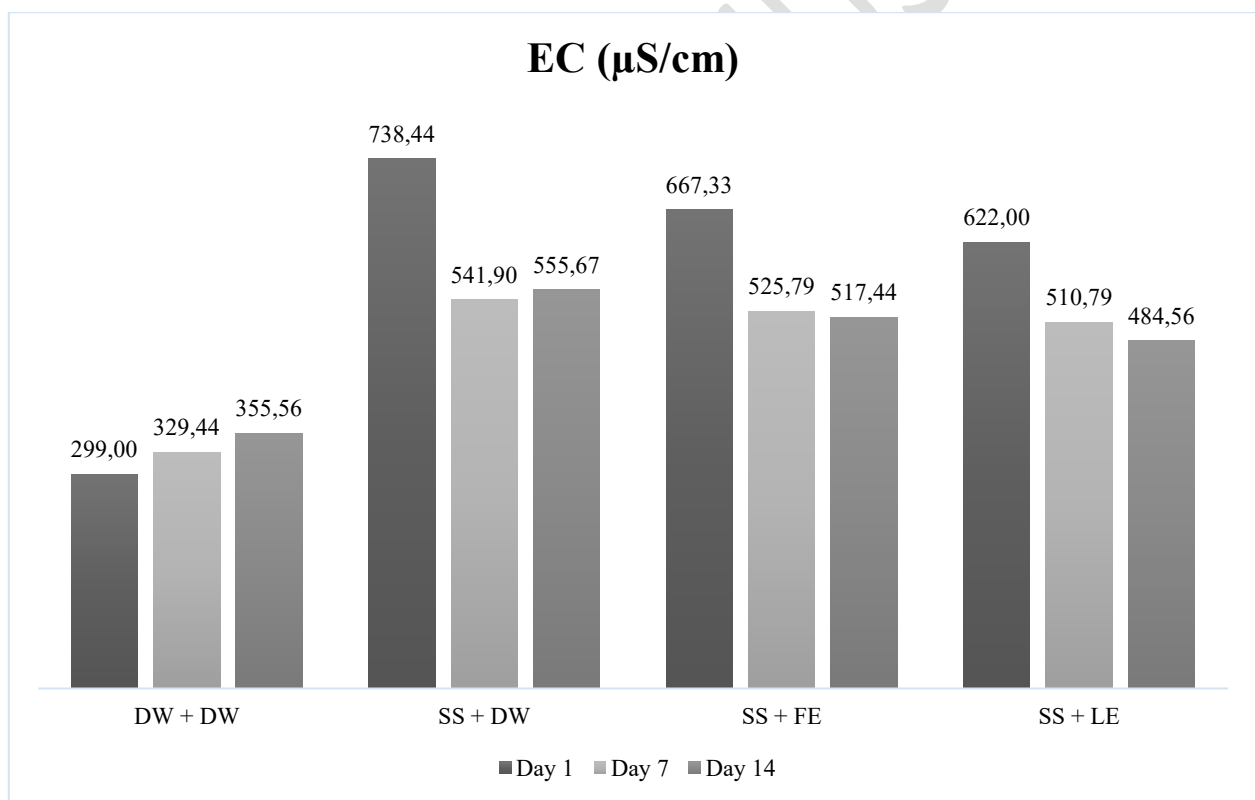
175 **RESULTS AND DISCUSSION**

176 **Salinity of the soil samples as determined by their electrical conductivity**

177 The presence of NaCl in soil increases its ability to conduct electricity, leading to a higher
178 electrical conductivity (EC). In the present pilot research, soil samples spiked with 50 mM NaCl
179 solution were utilized to simulate the transition from productive to unsustainable agricultural land
180 (Shrivastava & Kumar 2015; Zhou *et al.* 2024), where microbial and/or chemical interventions can
181 be economically impactful. As displayed in Figure 2, the soil samples with *M. calabura* fruit and leaf
182 extracts exhibited lower ECs compared to those of the control SS + DW, with SS + LE demonstrating
183 the best reduction effect. The latter was able to decrease the EC of the saline soil down to 484.56
184 $\mu\text{S}/\text{cm}$ by Day 14, while SS + FE had 517.44 $\mu\text{S}/\text{cm}$; both values were lower than that of the control
185 SS + DW (555.67 $\mu\text{S}/\text{cm}$). Moreover, as shown in Table 2, no significant difference was observed
186 among SS + DW, SS + FE, and SS + LE on Day 7, suggesting a temporary parity in their EC reduction
187 performances. Nevertheless, there was a significant difference between the control SS + DW and SS
188 + LE on Days 1 and 14, supporting the leading effectiveness of the leaf extract in reducing EC during
189 these testing points.

190 The occurrence may be preliminarily explained with the organic compounds potentially
191 present in the extracts used in SS + FE and SS + LE. Through the process of chelation, the compounds
192 may have bound with the soluble salt ions, mobilizing them in the process (Xiao *et al.* 2022; Anderson
193 *et al.* 2023). Moreover, soil bacteria might have also taken part in the remediation through the
194 production of organic acids and chelating agents (Ribeiro *et al.* 2020), as well as ion sequestration
195 and the consequent EC reduction (Hernández-Canseco *et al.* 2022).

196 The aforementioned concepts may also be linked with the EC decrease observed in the control
 197 SS + DW, as soil naturally has present microorganisms and organic compounds (Lehmann & Kleber
 198 2015; Paul 2016; Kleber *et al.* 2021). However, a fluctuation took place in this treatment with a
 199 slightly increased EC by Day 14, which may be due to the minerals that took longer to dissolve. These
 200 minerals might have broken down and released weathering products, elevating the concentration of
 201 dissolved ions in the process (Rieder *et al.* 2024); hence, the small increment demonstrated in SS +
 202 DW. Furthermore, the consistent increase of EC in the control DW + DW (non-saline soil) may also
 203 be due to the breakdown of minerals over time. This further substantiates the effectiveness of *M.*
 204 *calabura* extracts, as the setup in the treated groups seemingly defied this occurrence, evident in the
 205 continued lowering of the EC values in SS + FE and SS + LE by Day 14.



206
 207 Figure 2. Electrical conductivity of the soil sample from different treatments

208
 209 Table 2. Salinity of the soil sample extracts as described by their electrical conductivity

Treatment	Electrical Conductivity (µS/cm)		
	Day 1	Day 7	Day 14

DW + DW	299.00	329.44	355.56
SS + DW	738.44	541.90 ^{ab}	555.67 ^a
SS + FE	667.33 ^a	525.79 ^{ac}	517.44 ^{ab}
SS + LE	622.00 ^a	510.79 ^{bc}	484.56 ^b

210 Note: DW = distilled water; SS = salt solution; FE = fruit extract; LE = leaf extract; Means with the
 211 same superscripts, within a column, have no significant difference ($p > 0.05$).

212

213 **Influence of *M. calabura* fruit and leaf extracts on oxygen availability and pH**

214 To further determine the quality of the soil after 14 days, the dissolved oxygen (DO) and pH
 215 of the soil extract samples were tested. Table 3 summarizes the DO and pH values of the soil samples.
 216 Despite the differences among the means, all of the treatments tested showed no significant difference
 217 from one another in both parameters. Taking these findings into consideration, SS + FE and SS + LE,
 218 both of which were not significantly different from the control DW + DW (non-saline soil), were able
 219 to aid the remediation of saline soil without compromising its DO and pH.

220 The two parameters, supplemental to the reduced EC, can provide insight as to whether the
 221 soil can still be potentially viable for plant cultivation and microbial growth after the remediation
 222 process. Oxygen availability affects the growth and metabolism of plants, particularly during seed
 223 germination (Ray *et al.* 2016); influences microbial processes involved in nutrient cycling and soil
 224 fertility (Li *et al.* 2021); and aids in the root function and nutrient uptake (Li *et al.* 2021). Meanwhile,
 225 pH impacts nutrient cycling, plant nutrition, soil remediation, and microbial activity in soil (Neina,
 226 2019; Wang *et al.* 2019).

227 Table 3. Dissolved oxygen and pH of the soil samples by Day 14

Treatment	Dissolved Oxygen (mg/L)	pH
DW + DW	7.10 ^a	7.81 ^a
SS + DW	6.73 ^a	7.72 ^a
SS + FE	5.56 ^a	7.71 ^a
SS + LE	6.38 ^a	7.65 ^a

228 Note: DW = distilled water; SS = salt solution; FE = fruit extract; LE = leaf extract; Means with the
229 same superscripts, within a column, have no significant difference ($p > 0.05$).

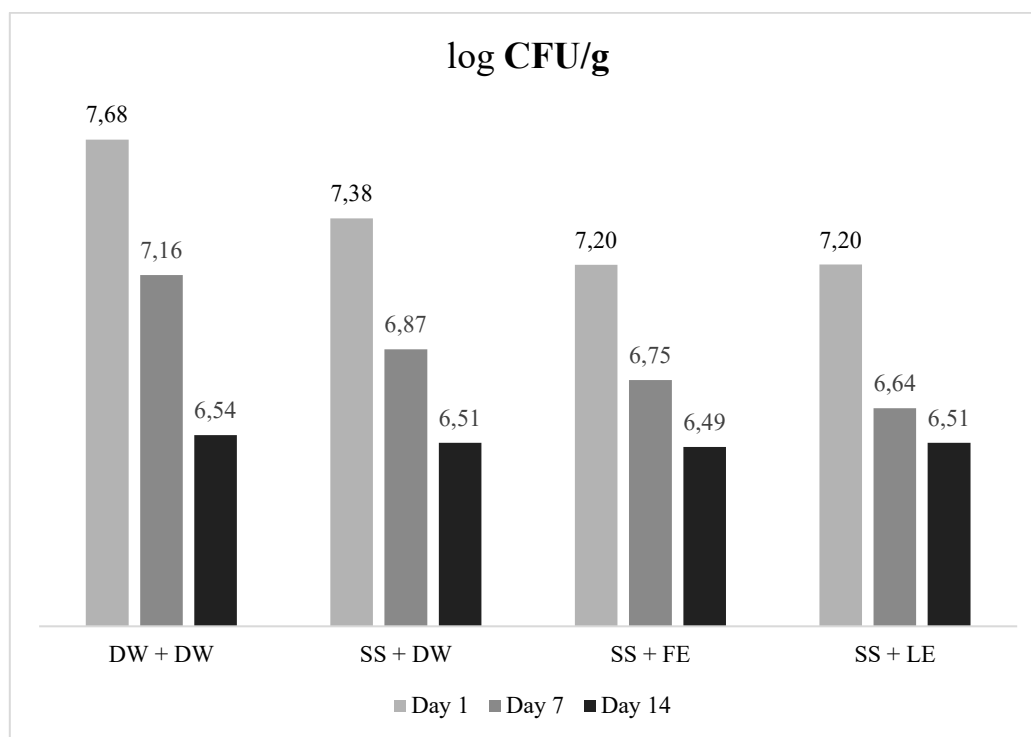
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231 **Bacterial load of soil samples**

232 The quantification of colony-forming units (CFUs) from the samples provided insights on
233 how the extracts influenced the activity of the microbial community indigenous in the soil as
234 determined by the total culturable bacteria. Figure 3 presents all the bacterial loads of the soil samples
235 from the treatments, demonstrating a gradual decrease across the three time points. It is also quite
236 noticeable that the treated groups, SS + FE and SS + LE, had the lowest bacterial load on Day 1 (both
237 7.20 log CFU/g), Day 7 (6.75 log CFU/g and 6.64 log CFU/g, respectively), and Day 14 (6.49 log
238 CFU/g and 6.51 log CFU/g). Moreover, as shown in Table 4, statistical analysis revealed that the
239 baseline control DW + DW showed no significant difference compared to the other treatments on
240 Day 1. While these three treatments exhibited intermediate similarities, a significant difference was
241 observed between SS + DW and SS + LE. Meanwhile, no significant difference was demonstrated
242 between the two control treatments by Day 7, as well as the two experimental groups. Furthermore,
243 all of the treatments tested no significant difference from one another at the last testing point.

244 Salinity, being a major soil stressor (Zhang *et al.* 2019), might cause the bacterial community
245 to decrease over time, in addition to the declining nutrient availability in the substrates. As for the
246 treated groups, the occurrence might be due to the natural antimicrobial properties of *M. calabura*
247 fruits and leaves, known to contain bioactives including phenols, tannins, alkaloids, and flavonoids,
248 among others (Ansori *et al.* 2021; Ariffin *et al.* 2022), which all exhibit inhibitory effects against
249 various microorganisms (Arora *et al.* 2024). Along with this inhibitory activity, the lower values of
250 the treated groups can also be rooted in the salinity stress itself (Zhang *et al.* 2021), as well as the
251 influence of pH on microbial activity (Naz *et al.* 2022), making the soil environment slightly less
252 favorable for the bacterial community over time. Nevertheless, the treated groups still managed to
253 exhibit the leading EC reduction (Figure 2 and Table 2). The phenomenon might have taken place

254 due to the diminishing of general bacteria, while paving the way for the thriving and survival of
 255 halophilic and/or halotolerant bacteria that could tolerate, and ultimately, help remediate the saline
 256 soil environment.



257
 258 Figure 3. Bacterial load of soil samples from different treatments

259

260 Table 4. Bacterial load of the soil samples expressed in colony-forming units per gram (CFU/g)

Treatment	log CFU/g		
	Day 1	Day 7	Day 14
DW + DW	7.68 ^{abc}	7.16 ^a	6.54 ^a
SS + DW	7.38 ^{ad}	6.87 ^a	6.51 ^a
SS + FE	7.20 ^{bde}	6.75 ^b	6.49 ^a
SS + LE	7.20 ^{ce}	6.64 ^b	6.51 ^a

261 Note: DW = distilled water; SS = salt solution; FE = fruit extract; LE = leaf extract; Means with the
 262 same superscripts, within a column, have no significant difference ($p > 0.05$).

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266 **Molecular characterization of the bacterial colonies**

267 To characterize the culturable members of the microbial community, representative colonies
 268 were selected from each treatment group through biochemical characterization. While this targeted
 269 selection provides a taxonomic snapshot of the bacterial isolates capable of thriving under the soil
 270 conditions, it was intended as an exploratory identification rather than a comprehensive profiling of
 271 the microbial community.

272 Prior to molecular characterization, three (3) colonies from each treatment were selected and
 273 subcultured for biochemical tests. As summarized in Table 5, all colonies tested positive for catalase.
 274 Meanwhile, oxidase, coagulase, and indole production were not detected on the colonies. Considering
 275 the results of the biochemical tests, one (1) representative colony from each treatment was subjected
 276 to bacterial DNA barcoding for the full-length 16S rRNA (~1500 bp) analysis.

277 **Table 5. Biochemical test results of the isolated colonies from each treatment**

Treatment	Soil isolates per treatment	Biochemical tests			
		Catalase	Oxidase	Coagulase	Indole
DW + DW	Colony 1	+	-	-	-
	Colony 2	+	-	-	-
	Colony 3	+	-	-	-
SS + DW	Colony 1	+	-	-	-
	Colony 2	+	-	-	-
	Colony 3	+	-	-	-
SS + FE	Colony 1	+	-	-	-
	Colony 2	+	-	-	-
	Colony 3	+	-	-	-
SS + LE	Colony 1	+	-	-	-
	Colony 2	+	-	-	-
	Colony 3	+	-	-	-

278 Note: DW = distilled water; SS = salt solution; FE = fruit extract; LE = leaf extract; (+) = Detected;
279 (-) = Not detected

280

281 Table 6 highlights the top 10 sequence matches of the bacterial cultures subjected to DNA
282 barcoding. For DW + DW, it was revealed that strains with 100.00% percent identity (PI) belong to
283 *Bacillus cereus*. Colonies from both SS + DW and SS + FE were revealed to have 100.00% PI with
284 the strains of *Rosellomorea marisflavi*. Lastly, the culture from SS + LE had the highest matches with
285 *Cytobacillus firmus* strains (98.97% and 98.84%). The relatedness of these species and/or strains,
286 along with other sequence matches of the bacterial colonies, is summarized using phylogenetic trees
287 (Figure 4).

288

289 Table 6. Sequence matches of the bacterial colony isolated from each treatment

Treatment	Aligned Region	Sequence Length	Homologous Sequences (Percent Identity)	Sequence Length (Linear rRNA)	NCBI Accession Number
DW + DW	1486 bp	1488 bp	<i>Bacillus cereus</i> ATCC 14579 (100.00%)	1512 bp	NR_074540.1
			<i>Bacillus cereus</i> strain CCM 2010 (100.00%)	1535 bp	NR_115714.1
			<i>Bacillus proteolyticus</i> strain MCCC 1A00365 (99.93%)	1509 bp	NR_157735.1
			<i>Bacillus albus</i> strain MCCC 1A02146 (99.93%)	1509 bp	NR_157729.1
			<i>Bacillus sanguinis</i> strain BML-BC004 (99.93%)	1555 bp	NR_175555.1
			<i>Bacillus wiedmannii</i> strain FSL W8-0169 (99.87%)	1540 bp	NR_152692.1
			<i>Bacillus paramycoides</i> strain MCCC 1A04098 (99.87%)	1509 bp	NR_157734.1

			<i>Bacillus cereus</i> strain IAM 12605 (100.00%)	1486 bp	NR_115526.1
			<i>Bacillus paranthracis</i> strain MCCC 1A00395 (99.80%)	1509 bp	NR_157728.1
			<i>Bacillus fungorum</i> strain 17-SMS-01 (99.80%)	1576 bp	NR_170494.1
SS + DW	1489 bp	1491 bp	<i>Rossellomorea marisflavi</i> strain TF-11 (100.00%)	1506 bp	NR_025240.1
			<i>Rossellomorea marisflavi</i> strain TF-11 (100.00%)	1466 bp	NR_118437.1
			<i>Bacillus haikouensis</i> strain C-89 (98.51%)	1473 bp	NR_148273.1
			<i>Rossellomorea aquimaris</i> strain TF-12 (98.25%)	1507 bp	NR_025241.1
			<i>Rossellomorea arthrocnemi</i> strain EAR8 (98.44%)	1474 bp	NR_181775.1
			<i>Heyndrickxia acidicola</i> strain 105-2 (97.32%)	1548 bp	NR_041942.1
			<i>Mangrovibacillus cuniculi</i> strain R1DC41 (97.18%)	1549 bp	NR_181118.1
			<i>Bacillus salis</i> strain ES3 (96.64%)	1515 bp	NR_179406.1
			<i>Falsibacillus albus</i> strain GY 10110 (96.51%)	1533 bp	NR_171509.1
			<i>Heyndrickxia shackletonii</i> strain LMG 18435 (96.36%)	1503 bp	NR_025373.1
SS + FE	1487 bp	1489 bp	<i>Rossellomorea marisflavi</i> strain TF-11 (100.00%)	1506 bp	NR_025240.1
			<i>Rossellomorea marisflavi</i> strain TF-11 (100.00%)	1466 bp	NR_118437.1

			<i>Bacillus haikouensis</i> strain C-89 (98.51%)	1473 bp	NR_148273.1
			<i>Rossellomorea</i> <i>aquimaris</i> strain TF- 12 (98.25%)	1507 bp	NR_025241.1
			<i>Rossellomorea</i> <i>arthrocneimi</i> strain EAR8 (98.44%)	1474 bp	NR_181775.1
			<i>Heyndrickxia</i> <i>acidicola</i> strain 105-2 (97.31%)	1548 bp	NR_041942.1
			<i>Mangrovibacillus</i> <i>cuniculi</i> strain R1DC41 (97.17%)	1549 bp	NR_181118.1
			<i>Bacillus salis</i> strain ES3 (96.64%)	1515 bp	NR_179406.1
			<i>Falsibacillus albus</i> strain GY 10110 (96.51%)	1533 bp	NR_171509.1
			<i>Heyndrickxia</i> <i>shackletonii</i> strain LMG 18435 (96.36%)	1503 bp	NR_025373.1
SS + LE	1461 bp	1463 bp	<i>Cytobacillus firmus</i> strain NBRC 15306 (98.97%)	1477 bp	NR_112635.1
			<i>Cytobacillus firmus</i> strain IAM 12464 (98.84%)	1483 bp	NR_025842.1
			<i>Cytobacillus gottheilii</i> strain WCC 4585 (97.74%)	1512 bp	NR_108491.1
			<i>Cytobacillus</i> <i>oceanisediminis</i> strain H2 (99.57%)	1393 bp	NR_117285.1
			<i>Cytobacillus</i> <i>depressus</i> strain BZ1 (97.52%)	1459 bp	NR_146034.1

<i>Robertmurraya dakarensis</i> strain Marseille-P3515 (97.25%)	1472 bp	NR_147382.1
<i>Mesobacillus harenae</i> strain Y40 (97.13%)	1550 bp	NR_178928.1
<i>Mesobacillus subterraneus</i> strain COO13B (97.13%)	1539 bp	NR_104749.1
<i>Cytobacillus massiliigabonensis</i> strain Marseille-P2639 (96.93%)	1514 bp	NR_179554.1
<i>Mesobacillus thioparans</i> strain BMP-1 (97.12%)	1472 bp	NR_043762.1

290 Note: DW = distilled water; SS = salt solution; FE = fruit extract; LE = leaf extract

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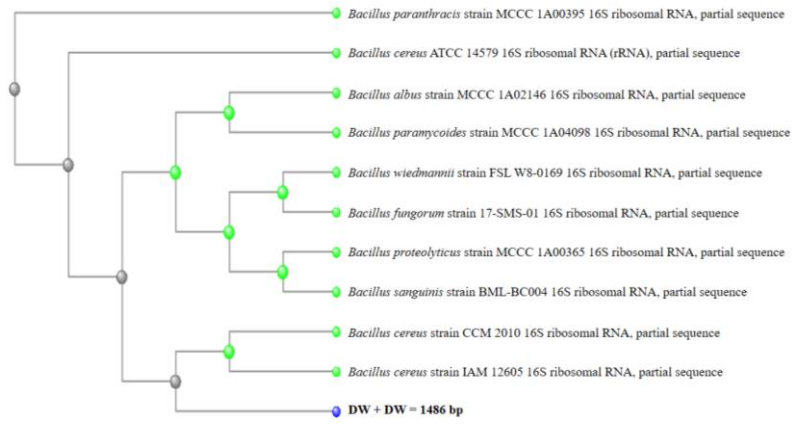
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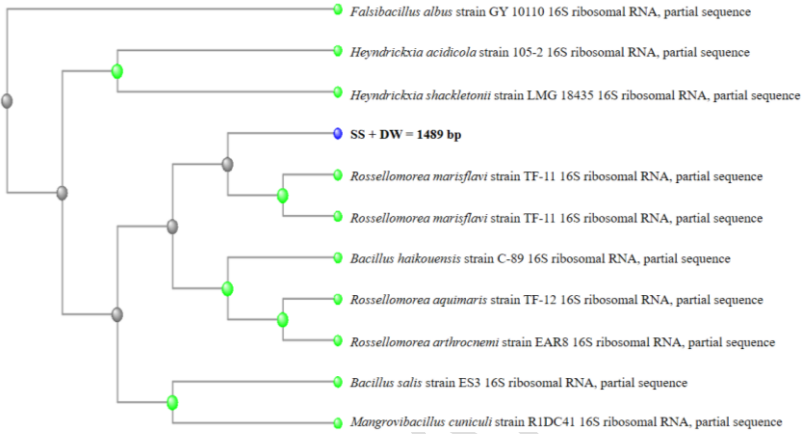
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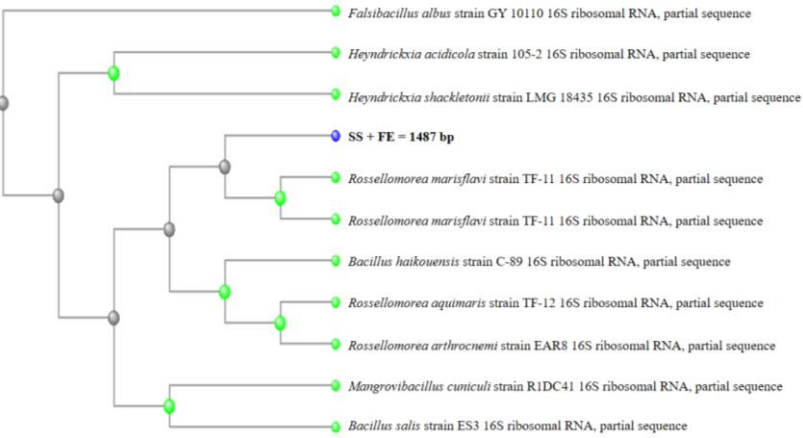
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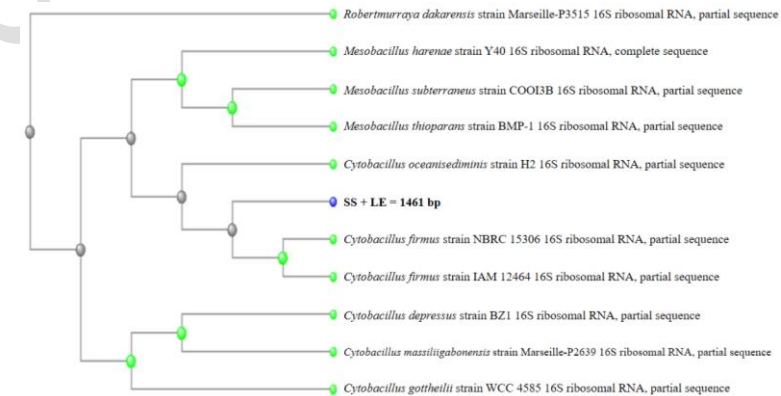
304



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306



307

Figure 4. Phylogenetic trees describing the relationships among bacterial species

308

309 While DNA barcoding enabled characterization of the aforementioned halotolerant species,
310 the bacterial load observed across treatments only reflects the total culturable bacteria. Nevertheless,
311 the isolation of *B. cereus* from DW + DW (non-saline soil) is discernible, given that the bacterium is
312 ubiquitous in soil environments (Hassan *et al.* 2018; Kulkova *et al.* 2023). Meanwhile, *R. marisflavi*
313 is a bacterium that is saline resistant, as highlighted in the studies of Li *et al.* (2024), Bai *et al.* (2024),
314 and Ali *et al.* (2025). Likewise, *C. firmus* is also resistant to salt stress (Gao *et al.* 2024; Chu *et al.*
315 2025), corroborating its dominance in SS + LE, and its consequent isolation.

316 Furthermore, outlined in Table 7 are the reported isolation origins of the aforementioned
317 bacterial species, some of which harbor saline environmental conditions. In the present study, the
318 bacterial species observed are most likely indigenous to the soil, which is a known reservoir of diverse
319 microbial communities (Philippot *et al.* 2024). The addition of *M. calabura* extracts, especially in SS
320 + LE, served as a key survival nutrient source that enhanced the growth and detectability of *C. firmus*.
321 On the other hand, although *R. marisflavi* was detected in both the control SS + DW and SS + FE, the
322 *M. calabura* fruit extract, probably through its nutrient and/or biochemical composition, might have
323 enhanced the remediation capability of the species. This is evident as the EC values demonstrated by
324 SS + FE, like those by SS + LE, were lower than those of SS + DW. Overall, the addition of the
325 extracts might be associated with reduced growth of non-halotolerant bacteria and a lower overall
326 bacterial load. At the same time, the extracts may have served as a nutrient source for halotolerant
327 species, potentially supporting their growth, accounting for the observed lower total bacterial load yet
328 improved EC reduction in the treatments. This nutrient source may comprise sugars, fiber, minerals,
329 and other biochemical constituents present in *M. calabura* fruits and leaves (Krishnaveni &
330 Dhanalakshmi, 2014; Pereira *et al.* 2018; Peter *et al.* 2020; Upadhye *et al.* 2021; Zolkeflee *et al.* 2021).
331 Furthermore, the results may also indicate that *C. firmus*, with the influence of *M. calabura* leaf extract,
332 was the most potent in remediating the saline soil, as evidenced by the lowest ECs observed in SS +
333 LE.

334

335 Table 7. Reported origins of the closest related species of the bacterial isolates

Closest related species	Origin and site of isolation
<i>Bacillus cereus</i>	Mongo sprouts, silage, and soil samples in the Philippines (Venturina <i>et al.</i> 2016)
	Rice paddy fields in the Philippines (Bañares <i>et al.</i> 2019)
	Muddy sediments of mangrove swamps in Thailand (Chantarasiri, 2015)
	Halophytic weed <i>Cenchrus ciliaris</i> growing in Khewra Salt Range of Pakistan (Hassan <i>et al.</i> 2018)
<i>Rosellomorea marisflavi</i>	Rice grown in acid sulfate soils in Vietnam (Lam <i>et al.</i> 2024)
	Hindon River (in proximity to Gautam Buddha Nagar in Uttar Pradesh of India) (Kumar <i>et al.</i> 2025)
	Rhizosphere of <i>Zea mays</i> L. (cultivar Xianyu 335) grown in Beijing, China (Li <i>et al.</i> 2025)
	Rhizosphere soil samples from maize and vegetable farms at the Centre for Agricultural Technology and Entrepreneur Studies (CATES), Bells University of Technology, Ota, Ogun State, Nigeria (Ogunsola <i>et al.</i> 2025)
<i>Cytobacillus firmus</i>	Rhizosphere of pepper (<i>Capsicum annuum</i> L.) from the greenhouse of Kyungpook National University (Republic of Korea) (Jung <i>et al.</i> 2023)
	Hot springs located in the southern region of Saudi Arabia (Al-Harthy <i>et al.</i> 2024)
	Soil samples contaminated with heavy metals from disposal sites in Pantnagar, Uttarakhand, India (Ghosh <i>et al.</i> 2025)
	Chromium slag dump in Xinxiang, Henan Province, China (Yin <i>et al.</i> 2025)
	<i>Avicennia marina</i> (Gray Mangrove) in La Union, Philippines (Gutierrez <i>et al.</i> 2025)

336

337 Fundamentally, the bacterial quantity was not behind the reduction of the ECs, given the
338 gradually decreasing CFUs. Rather, it is possible to deduce that the biological processes and activities
339 of the bacterial species might have played the key role in the soil remediation. There can be a wide
340 range of potential mechanisms behind the saline soil remediation as exhibited by the three bacterial

341 species. Extracellular polymerase substances (EPS) might have been employed by bacteria to bind
342 sodium ions, sequestering them within the matrix (Pawar *et al.* 2013; Choudhary *et al.* 2016; Bhagat
343 *et al.* 2021). Moreover, production of organic acids and chelating agents by the bacteria might have
344 contributed to the salinity reduction (Ribeiro *et al.* 2020). Another mechanism may be through
345 synergism among multi-strain bacterial consortia, which was emphasized by Afzal *et al.* (2023) as a
346 tool for salinity reduction. This was also highlighted in the study of Cui *et al.* (2025), whereby
347 promotion of microbial interactions and symbiotic relationships drives soil quality improvement.
348 Furthermore, the transporters of the bacteria in their structure play a vital role in the remediation by
349 regulating specific ions in their environment. Through the salt-in strategy, which involves the
350 accumulation of ions in the bacterial cytoplasm, the bacteria in the soil samples might have maintained
351 their osmotic balance with the surrounding environment (Neagu & Stancu, 2025). While the presence
352 of halotolerant bacteria likely contributed to the observed bioremediation, as indicated by electrical
353 conductivity reduction, further studies are needed to confirm their mechanisms under the influence of
354 the *M. calabura* fruit and leaf extracts.

355 On the other hand, the bacterial species were also reported in the studies as endophytes
356 inhabiting plant tissues (Table 7). Moreover, the ability of *M. calabura* to accommodate microbial life
357 within its tissues is emphasized in the studies of Bimantara *et al.* (2022) and Simha *et al.* (2025),
358 reporting the isolation of endophytic fungi from the plant. Although the plant extracts might have
359 introduced the bacteria into the soil environment, this cannot be conclusively established yet under the
360 current experimental conditions and setup. Hence, future studies are needed to elucidate the
361 plausibility of the plant as a potential origin for both *R. marisflavi* and *C. firmus*.

362 Furthermore, to the best of the authors' literature search and collection, the study marks the
363 first documented potential occurrence of the latter two taxa in Central Luzon, Philippines. The
364 keywords "*Rosellomorea marisflavi* + Philippines" and "*R. marisflavi* + Central Luzon" were used
365 to search research publications from 2000 to 2025 in PubMed, Google Scholar, and Scopus. Gutierrez
366 *et al.* (2025) already reported the isolation and identification of *C. firmus* in La Union, Philippines.

367 However, the aforementioned province is located in the Ilocos Region, which is geographically distinct
368 and apart from Central Luzon. Meanwhile, to date, no reports of *R. marisflavi* in Central Luzon,
369 Philippines have been identified, supporting the novelty of the present finding. Nonetheless, this study
370 advances scientific understanding of the microbial diversity within different environmental settings in
371 the country.

372

373 CONCLUSION

374 In the present study, SS + FE and SS + LE demonstrated EC values lower than those of the
375 control SS + DW. By Day 14, the treatments, including DW + DW (non-saline soil), showed no
376 significant difference among one another in terms of DO and pH. This indicates the effectiveness of
377 *M. calabura* extracts in reducing EC, particularly in SS + LE, without compromising the other two
378 physicochemical parameters. While the 14-day pot experiment spiked with 50 mM NaCl solution
379 provided valuable initial insights into the biostimulatory effects of the extracts, future studies using
380 longer time points and higher salt concentrations can further build on these findings to fully capture
381 sustained effects.

382 Despite the gradual decrease of bacterial load in the treatments, molecular characterization
383 revealed that representative cultures of the surviving species were closely related to two halotolerant
384 bacteria: *R. marisflavi* and *C. firmus*. This may indicate that although the addition of extracts was
385 associated with a lower overall bacterial load, it may have supported the growth of halotolerant
386 bacteria, potentially accounting for the improved saline bioremediation observed in the treatments.
387 While current methods identified only representative isolates, future extensive microbiological and
388 molecular analyses must be conducted to validate microbial community dynamics, specifically the
389 halotolerant species across the treatments. On the other hand, the findings suggest that the addition
390 of the extracts, while resulting to lower total bacterial load, might have served as a nutrient source for
391 the halotolerant species, accounting for the improved saline bioremediation in the treatments. Overall,

392 exploring the multifaceted mechanisms behind the saline bioremediation using *M. calabura* extracts
393 must be further explored. Moreover, to the best of the authors' literature search and collection, the
394 study marks the first documented potential occurrence of the two aforementioned taxa in Central
395 Luzon, Philippines.

396 Beyond the ecological benefits, the effectiveness of *M. calabura* extracts to reduce EC and
397 promote halotolerant bacteria presents a potentially cost-effective pathway for land reclamation. With
398 the research objectives and findings being aligned with the circular bioeconomy, saline soil
399 bioremediation using the extracts can help in restoring soil productivity, supporting agricultural
400 livelihoods and food security. Policymakers can take part by subsidizing the development and
401 production of plant-derived soil amendments/conditioners as an approach for environmental and
402 agricultural sustainability.

403

404 **AUTHORS' CONTRIBUTIONS**

405 MANUCDOC: Conceptualization, Methodology, Experimentation, Data Analysis and
406 Interpretation, Writing (Original Draft), Writing (Review and Editing), Project Administration,
407 Funding Acquisition; MARCOS: Conceptualization, Funding Acquisition; VILLANUEVA:
408 Methodology, Experimentation, Writing (Review and Editing)

409

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418

419 **CONFLICT OF INTEREST**

420 The authors declare no conflict of interest.

421

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