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Valorization of Treated Sludge as a Sustainable Substrate for Rhizobial Biofertilizer Production

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Abstract: This study explored the feasibility of using sewage sludge as a carrier material for the production and preservation of rhizobia, with the aim of promoting its application as a sustainable biofertilizer. A preliminary microbiological analysis was performed to characterize the native microbial community of the sludge. Rhizobial inocula prepared with sterilized sludge were applied to plants under controlled conditions, and strain survival as well as plant growth responses were evaluated before and after inoculation. Preservation experiments were conducted using sludge and peat substrates, tested under two storage techniques at varying temperatures and durations. The sludge was found to harbor pathogenic microorganisms, highlighting the necessity of effective sterilization prior to its use. Once sterilized, sludge demonstrated its potential as a carrier material, maintaining the viability of *Rhizobium etli* CIAT 899 for up to four weeks at 4 °C and for one to two weeks at 25 °C. Inoculation with sludge-based formulations significantly enhanced the dry biomass of the tested plants. The findings suggest that sewage sludge, when properly treated, can serve as a promising carrier for rhizobial inoculants. This strategy not only contributes to sustainable sludge management but also supports eco-friendly alternatives to synthetic fertilizers.

Keywords: Sewage sludge, Biofertilizer, Culture, Inocula, Rhizobia, Support

1. Introduction

The wastewater produced by consumers undergoes a complex process before being discharged into the natural environment. This treatment process begins with the collection of wastewater and ends with the production of large quantities of solid waste, known as sewage sludge. The large-scale production of sewage sludge is a global phenomenon with negative repercussions on public health and the environment [1]. On the other hand, sludge is a valuable source of nutrients such as organic matter, nitrogen and phosphorus [2], as well as biomolecules such as lipids, proteins and enzymes [3].

Indeed, there are several possible approaches to sludge reclamation. Agricultural reclamation remains the most widely used method for regenerating soils helps reduce dependence on synthetic chemical fertilizers, while promoting resource recycling [4]. However, sludge must be treated to remove potential contaminants, such as heavy metals and pathogens, to ensure its safety and effectiveness in agricultural systems [5]. Biofertilizers derived from sewage sludge are of particular interest for their ability to improve soil structure, increase organic matter content and stimulate microbial activity, all of which are essential for sustainable agriculture [6].

Every year, around 70% of sewage sludge is recovered agronomically through land application or composting [7], which is emerging as a preferred option for sludge management [8]. This approach has undeniable ecological advantages, but also some drawbacks [9]. Other uses have merged [10], for example in the production of bricks [11], ceramics [12], biofuels [13] bioethanol [14] and Bio-Fuel [15].

This diversification of industrial applications for sludge not only makes it possible to add value to this waste, but also reduces dependence on other natural resources and mitigates the environmental impacts associated with its disposal. In addition, it contributes to a more sustainable circular economy by transforming waste into useful resources for different industrial sectors.

But with the overproduction of these sludges, the problem still remains in many countries, prompting the search for other solutions to convert them into valuable, environmentally-friendly products that comply with environmental standards. This article discusses the use of rhizobia as a growth medium for the production and conservation of biofertilizers, while seeking to identify the optimum conditions for the preservation of these microorganisms.

The production of biofertilizers from sewage sludge is an innovative approach with numerous advantages. Not only does it enable the sustainable management of these residues, it also improves the sustainability of agricultural systems. This method offers a competitive alternative to chemical fertilizers, whose harmful effects on the environment and human health have been widely documented. It promotes soil fertilization and helps reduce greenhouse gas emissions. It limits the use of synthetic nitrogen fertilizers, which are a major source of agricultural pollution. To guarantee the sustainability of sewage sludge management, it is essential that it is economically viable. Energy recovery solutions, such as biogas production, offer interesting prospects for investors keen to support projects that combine profitability and respect for the environment.

2. Materials and Methods

2.1. Sludge sampling

The sludge used in this study was collected from the Cap Falcon wastewater treatment plant (Figure 1), located 3 km from the commune of Aïn El Turk, northwest of Oran, Algeria (35°45'38.8" N latitude and 0°48'32.8" W longitude). The sludge studied was kept at a temperature of 4°C to maintain its viability. Before use, it was thoroughly agitated to ensure homogeneity.

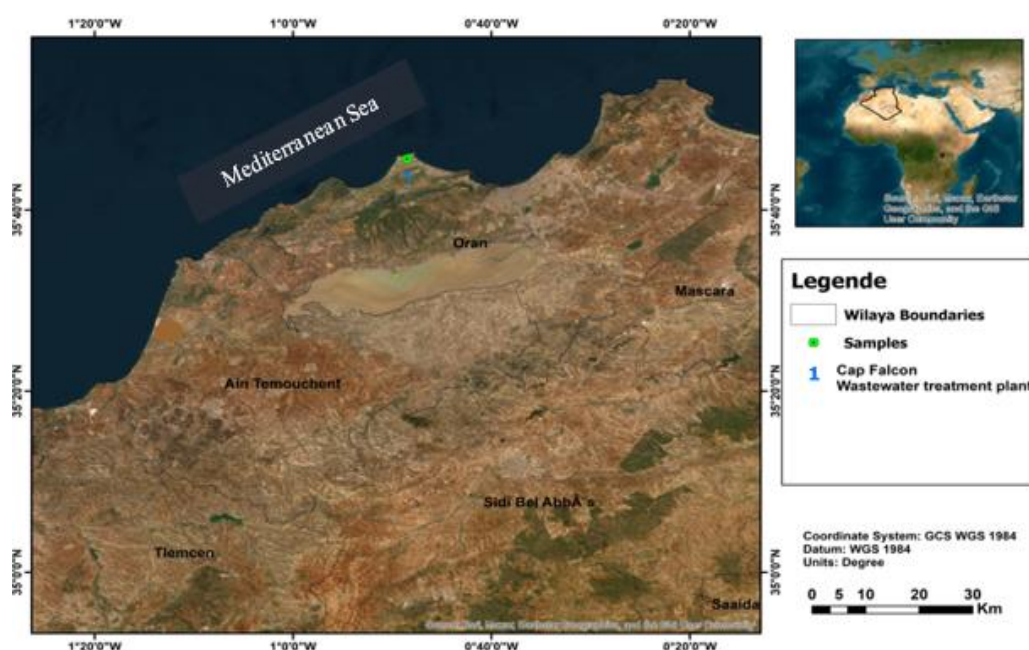


Figure1. Geographical location of sampling site

2.2. Research and enumeration of microbial flora in sludge

For this microbiological analysis, 5 g of the sludge studied is diluted in 45 mL of sterile physiological water, then homogenized for 5 minutes. Then, 1 mL of this stock solution is inoculated into the various culture media used (Figure 2).

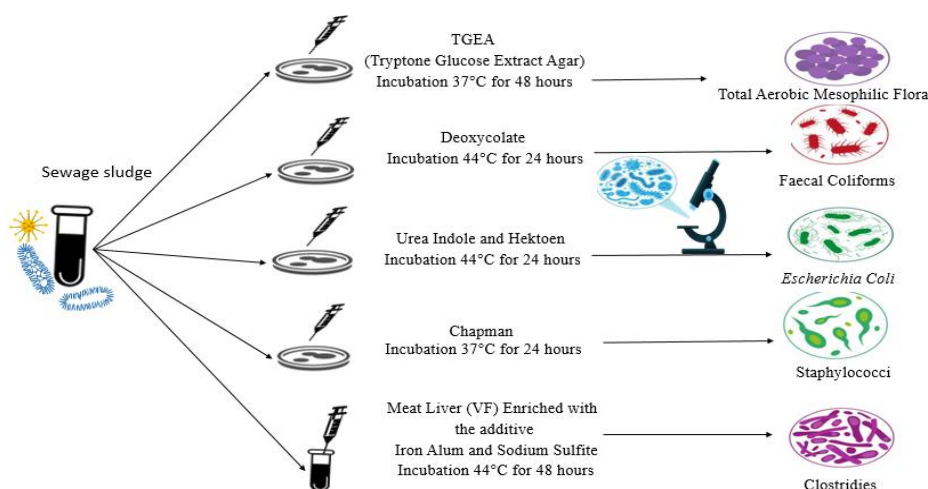


Figure 2. Research and enumeration of microbiological flora in sludge

Fecal coliforms, including *Escherichia coli*, are detected on deoxycolate medium, with confirmation on Hektoen agar and Urea Indole medium. Staphylococci are isolated on Chapman medium and confirmed by oxidase and catalase tests. Clostridia are tested on meat liver medium (VF) enriched with iron alum and sodium sulfite. Finally, Streptococci are identified on blood agar, with a distinction made according to their type of hemolysis.

2.3. Sludge sterilization

Microbiological analysis of the sludge showed that sterilization was necessary to eliminate all pathogenic microorganisms detected. To determine the optimum sterilization parameters for the sludge studied, different temperatures (80°C, 90°C and 100°C) and times (30mn, 1h, 1h30mn and 2h) were tested. The choice of these conditions was determined on the basis of preliminary tests. Sterilization was considered successful when all microorganisms present in the sludge were eliminated. To confirm this, a stock solution was prepared by diluting the sterile sludge (1 g of sludge in 9 mL of sterile physiological water). 0.1 mL of this solution was then spread on two solid media, nutrient agar and blood agar, and incubated at 25°C and 37°C respectively.

2.4. Experimental design

2.4.1. Preparation of sludge-based liquid culture media

Liquid culture media were prepared using sterile slurry at various concentrations, namely 5%, 10%, 15%, 20%, 25%, 50% and 75% (W/V). To do this, the quantity of sterile sludge designated according to concentration was diluted in 100mL of distilled water, then homogenized for 5 min. The solution was then filtered through a 32 mm PTFE syringe filter with a porosity of 0.45 µm to remove solid particles, and adjusted to pH 7. The pH was monitored throughout the experiment using a calibrated pH meter (INO 7320).

2.4.2. Preparing rhizobial pre-cultures

Three rhizobial strains, *Sinorhizobium meliloti* SM14, *Rhizobium etli* CIAT 899 and *Sinorhizobium meliloti* SM7 supplied by the Laboratory of Rhizobia Biotechnology and Plant Improvement (LBRAP) at Oran University¹, were the subject of this study. The choice of strains selected is based on their compatibility with host plants and their agronomic performance. These strains were grown on YEM agar medium and incubated at 28°C for 48 hours for optimal growth. From these rhizobial strains, pre-cultures were prepared in sterile liquid YEM medium at a volume of 3 mL per tube and then incubated at 28°C with agitation at 200 rpm.

2.4.3. Cultivation of rhizobia on sludge-based liquid media

These pre-cultures were used to inoculate the various media previously prepared with the sludge at different concentrations, as well as the liquid YEM medium taken as a control, at a rate of 25mL per flask, then incubated at a temperature of 28°C with agitation (200 rpm). The growth rate of the strains studied was estimated by spectrophotometry at a wavelength of 620 nm (Jenway 7305 spectrophotometre).

The specific growth rate (μ) is a parameter commonly used in microbiology to quantify the rate of increase of a microbial population during the exponential growth phase. It is generally expressed in h^{-1} . The specific growth rate is calculated using the following equation: $\mu = \ln X_2 - \ln X_1 / t_2 - t_1$. where: μ : specific growth rate (h^{-1}); X_1 : biomass or cell density at time t_1 ; X_2 : biomass or cell density at time t_2 ; $\ln$: natural logarithm; $t_2 - t_1$: time interval (hours) (Monod, 1949).

2.4.4. Cultivation

Our research focused on three legumes, *Phaseolus vulgaris*, *Cicer arietinum* and *Medicago sativa*, belonging to the *Fabaceae* family, due to their significant nutritional and economic importance. The selection of just three plant species: *Phaseolus vulgaris*, *Cicer arietinum* and *Medicago saliva* could have a significant impact on increasing agricultural production and reducing the need for imports into our country.

For cultivation, healthy *Phaseolus vulgaris* and *Cicer arietinum* seeds were subjected to disinfection using the method recommended by Tillard and Drevon (1988), while *Medicago sativa* seeds were grown using the technique of [16]. The pregerminated seeds were placed in pots containing sand. The seeds were then inoculated twice, once three days after potting and again one week after emergence. The test was carried out in triplicate for each case, and seedlings without any inoculation served as a control. This experiment was carried out over a three-month period in a greenhouse, under controlled conditions. To examine the impact of inoculation on crop development, a growth parameter was assessed by measuring the dry weight of the aerial and root parts of each plant.

2.5. Use of sludge as a medium for the conservation of *Rhizobium etli* CIAT 899 strain

The sludge was subjected to a preparation process that included crushing, sieving, then placing in opaque jars and polyethylene bags, followed by double moist heat sterilization for 1 hour 30 minutes at 100°C, with a 24-hour interval between the two sterilizations. This medium was compared with peat, widely used as a medium, which was also sterilized in the same way as sludge. An inoculum solution prepared from the *Rhizobium etli* CIAT 899 strain on sterile liquid YEM medium was sterile-applied to the supports in a quantity that perfectly matched the retention capacity of the supports, then homogenized to ensure even distribution of the bacterial cells throughout the support.

The solid inocula thus prepared were stored at various temperatures: 4°C, 25°C and 40°C for four weeks. Viability and the quantity of inoculum introduced are monitored throughout this period.

3. Statistical analysis

Statistical analyses were performed using STATISTICA version 6.0 software. The one-way analysis of variance (ANOVA) and Duncan's test were used to compare the treatments and check for significant differences between the groups. ANOVA tested these differences under the assumption of normality, and Duncan's test then identified homogeneous groups. P-values were included to strengthen the results, with a significance threshold of 5% ($p < 0.05$) to ensure the reliability of the conclusions. Principal component analysis (PCA) was performed to group treatments with growth parameters.

3. Results

4.1. Determination of sludge microbial flora

Microbiological analyses revealed the presence of a wide range of pathogenic microbial populations (aerobic flora, faecal coliforms, *Escherichia coli* and *Staphylococcus*), requiring sterilization to eliminate all these microorganisms and prepare the sludge for cultivation and conservation of the *Rhizobium* microorganism used. In addition, the results obtained enabled us to determine the appropriate temperature for eliminating all pathogenic flora as well as the total flora present in the sludge studied, which is of the order of 100°C with a duration of 1h30 min. A temperature of 100°C maintained for 1 h 30min is considered effective for the elimination of bacteria present in sewage sludge. This method is based on the principle that moist heat is capable of destroying the majority of pathogenic microorganisms, including resistant bacterial spores. This sterilization results in a substrate devoid of all microorganisms, including pathogens, and enables it to be used as a culture medium and support, while preserving the organic matter present in the sludge and avoiding the formation of any toxic products that may hinder the growth of the rhizobia microorganisms studied

4.2. Evaluation of rhizobia growth after cultivation

Monitoring the growth of the strains studied on sludge-based media at increasing concentrations of 5%, 10%, 15% and 20%, 25%, 50% and 75% yielded the results shown in Figure 3.

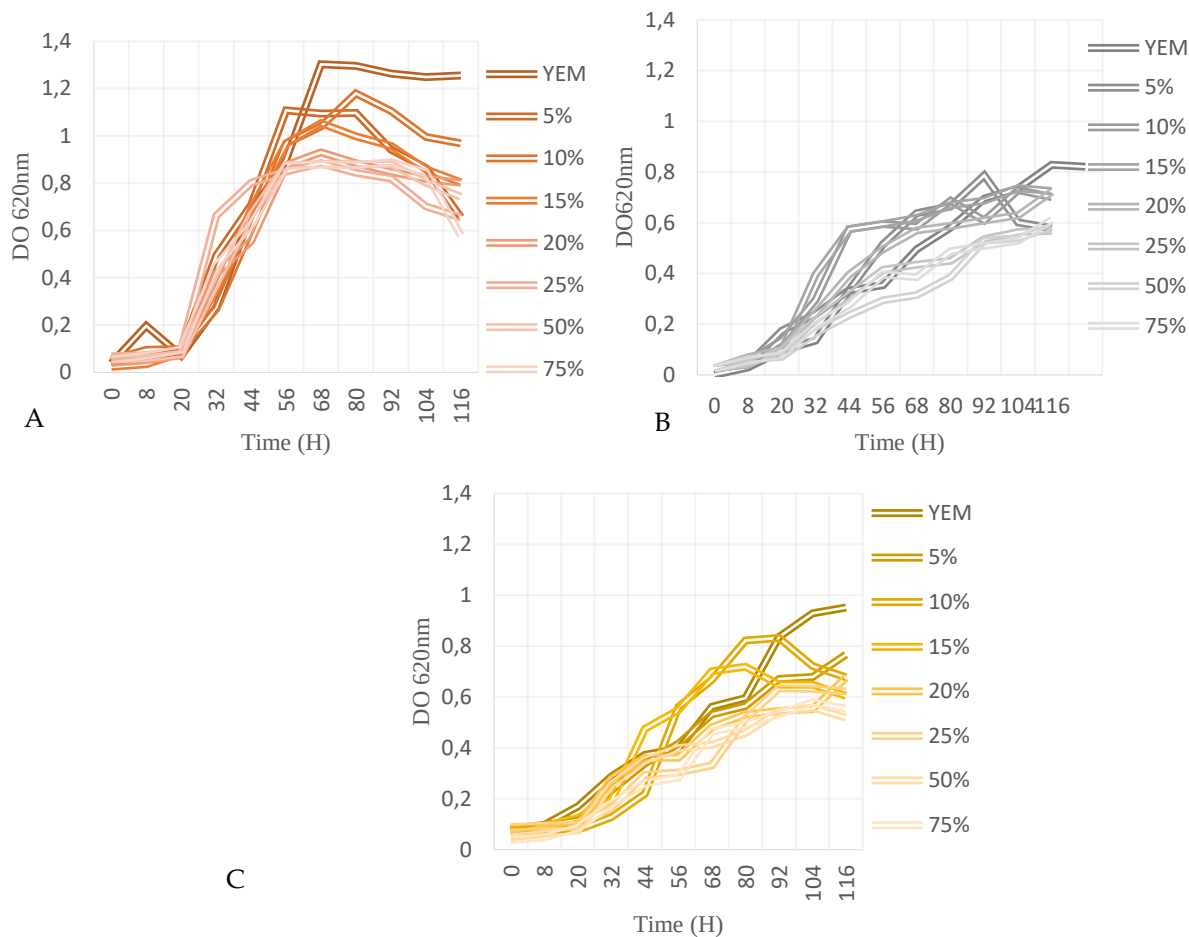


Figure 3. Kinetics of *Rhizobium etli* CIAT988 (A), *Sinorhizobium meliloti* SM7(B) and *Sinorhizobium meliloti* SM14(C) on YEM and sludge media at different concentrations.

The results obtained (Figure 3) show very interesting growth of *Rhizobium etli*, *Sinorhizobium meliloti* SM7 and *Sinorhizobium meliloti* SM14 on the culture medium prepared with sludge at concentrations of 5%, 10% and 15%, approaching that shown on YEM medium compared with the other concentrations. However, the lowest growth rates were obtained with other concentrations ranging from 20 to 75%. Following these results, the growth rate for each kinetic was calculated and illustrated (Fig 4) to enable an accurate assessment of this growth at different concentrations, in comparison with that observed in YEM medium. *Rhizobium etli* rates of $\mu=0.336 \text{ h}^{-1}$ at 5%, $\mu = 0.350 \text{ h}^{-1}$ at 10% and $\mu= 0.317 \text{ h}^{-1}$ at 15%.

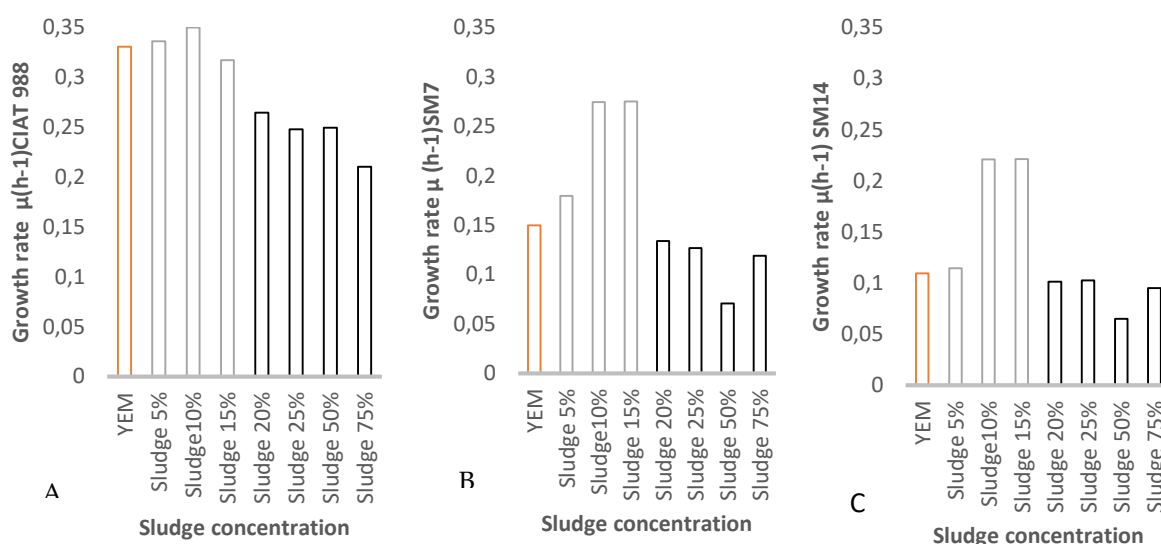


Figure 4. Growth rates of *Rhizobium etli* CIAT988 (A), *Sinorhizobium meliloti* SM7 (B) and *Sinorhizobium meliloti* SM14(C) from different kinetics.

The latter are close to those obtained on the YEM medium, in particular 10%, which shows a very interesting rate exceeding that of the control. As for the growth rate of *Sinorhizobium meliloti* SM7, we can see that the rates largely exceed those obtained with the YEM medium, in particular 10% and 15%, which show very interesting rates of $\mu = 0.274 h^{-1}$ and $\mu = 0.275 h^{-1}$. The same trend was recorded for the *Sinorhizobium meliloti* SM14 strain, with rates of around $\mu = 0.220 h^{-1}$ and $\mu = 0.221 h^{-1}$ respectively. The culture medium prepared with the sludge studied at a concentration of 5% provides 0.9 g organic matter, 0.7 g phosphorus, 0.5 g nitrogen and 0.49 g carbon. At concentrations of 10% and 15%, the levels of these elements are multiplied by two to three. It was therefore deemed appropriate to select these sludge concentrations to meet the nutrient requirements of the rhizobia studied.

4.3. Evaluation of crop growth after inoculation

The liquid inoculums prepared with the sludge studied and the reference inoculum prepared with YEM medium were used to inoculate cultivated plants, namely: *Phaseolus vulgaris*, *Cicer arietinum* and *Medicago sativa* to assess the influence of these different inoculums on the latter (Figure 5).

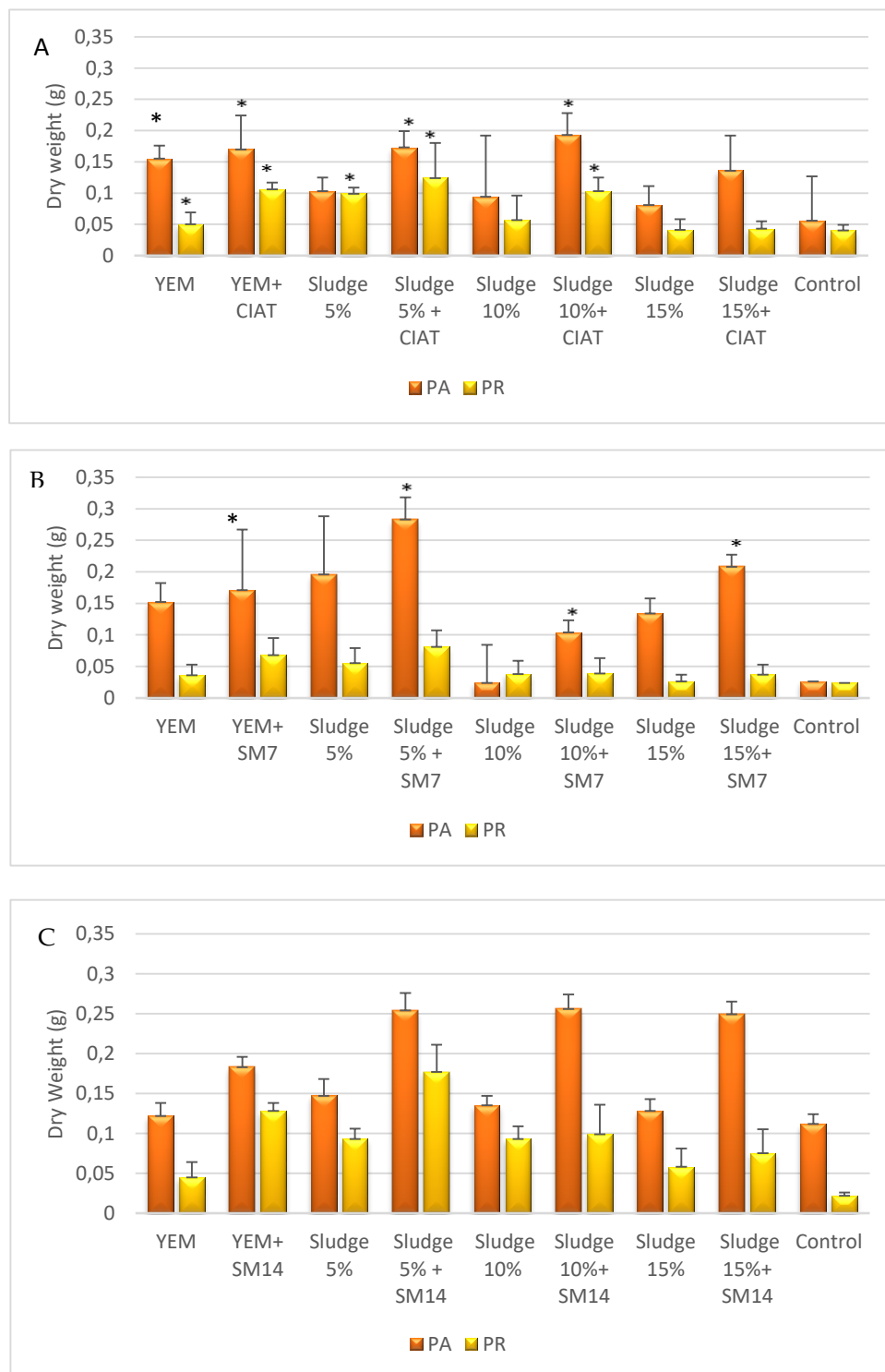


Fig. 5: Dry weights of aerial and root parts of *Phaseolus vulgaris* (A), *Cicer arietinum* (B) and *Medicago sativa* (C) after inoculations

PA: Dry weight of aerial part; PR: Dry weight of root part; T: non-inoculated control.

Means followed by an asterisk (*) indicate a significant difference between the different inoculums at $p < 0.05$ according to the Duncan test.

ANOVA was appropriate for analyzing differences between multiple treatment groups (inoculum concentrations) because it helps determine if observed differences in plant growth are statistically significant. Following ANOVA, Duncan's test was applied to identify homogeneous groups among treatments, ensuring that only statistically different treatments are reported. Given the multiple inoculum concentrations and plant types

tested, these methods are well-suited for the experimental design, providing a robust statistical framework for the comparisons.

The results shown in figure 5 (A) show that compared with the non-inoculated control, the effect of sludge inoculation on *Phaseolus vulgaris* dry biomass ranged from 46% to 68%. This is due to their richness in essential nutrients such as nitrogen, phosphorus and potassium, which promote plant growth. The highest dry weight percentage was obtained after the application of the inoculum prepared with sewage sludge on the tested plant, particularly at a concentration of 10%, resulting in a 51% increase in shoot dry biomass compared with the non-inoculated control.

This inoculation was carried out with the different media used to prepare these inoculums, in order to cancel out their effects, which proved to be highly significant on the aerial dry biomass of the plant studied with a coefficient $P=0.00001$. This explains why inoculation with *Rhizobium etli* CIAT had a positive influence on the dry biomass of the cultivated plant, compared with non-inoculated control plants. This effect remains weak compared with the effect of the YEM medium and decreases significantly at 10% of the sludge studied. The YEM medium contains mineral salts, mannitol and yeast extract; these nutrients constitute a good source of nutrients for the plants, which probably supports the positive effect of this medium on the dry biomass of the plant studied. The sludge studied is a non-negligible source of fertilizing elements. These nutrients provide the Rhizobia studied with optimal conditions for their growth and metabolic activity. These results (Fig. 5 B) also show the effect of inoculation on *Cicer arietinum*, which led to an increase in dry biomass ranging from 32% to 56% compared with the non-inoculated control. This observation underlines the effect highly significant of sludge ($p=0.00004$) on the growth of this plant, probably due to its richness in fertilizing elements, which favoured this increase in biomass. This effect is better than the effect of the YEM medium, and increases with increasing sludge concentration. The most pronounced effect was observed at the 5% concentration, with a 38% increase in root biomass compared with the non-inoculated control. The sludge studied is a non-negligible source of nutrients, satisfying the nutrient requirements of *Cicer arietinum* and translating this increase into the dry biomass of the plant studied.

These results (Figure 5 C) also show the effect of *Medicago sativa* inoculation, which was better with its specific strain, *Sinorhizobium meliloti* SM14 this improvement in dry weight ranged from 34% to 48%. This inoculation was carried out with the different media used to prepare these inoculums, in order to cancel out their effects, which proved to be significant. This explains why this inoculation stimulated the dry biomass of *Medicago sativa*, which is shown by the improvement in the dry biomass of this plant. This is linked to the large quantities of fertilizing elements, particularly nitrogen, contained in the sludge, which contributed to the vegetative development of *Medicago sativa*. This study highlights the importance of sludge as an effective nutrient for rhizobia growth, while underlining its significant impact on crops, indicating that inoculums prepared with sludge could be an interesting and economical alternative for improving agricultural productivity while reducing dependence on chemical fertilizers.

A principal component analysis (PCA) (Fig 6) was carried out to assess the correlation between the contribution of inoculums made with sludge at concentrations of 5%, 10% and 15% compared with the inoculate prepared with YEM medium on the growth parameters that present the dry weight of the aerial and root parts of the cultivated plants.

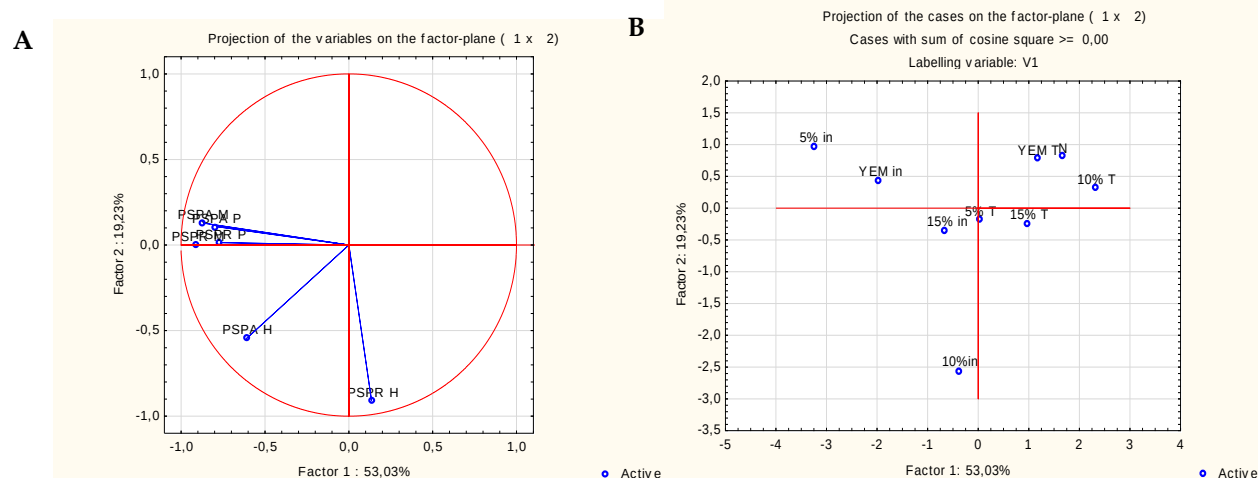


Figure 6. Principal component analysis of crop dry biomass

A: Contribution of analyzed parameters on the two second axes of the PCA. **B:** Distribution of inoculated sludge on the two second PCA axes.

TN: non-inoculated control; YEM: YEM medium ;YEM in: inoculated YEM medium; 5% T: medium prepared with sludge at a concentration of 5%; 5% in: medium prepared with sludge at a concentration of 5% inoculated; 10% T: medium prepared with sludge at a concentration of 10%; 10% in: medium prepared with sludge at a concentration of 10% inoculated; 15%T: medium prepared with sludge at a concentration of 15%; 15% in : medium prepared with sludge at a concentration of 15% inoculated; PSPAH: dry weight of *Phaseolus vulgaris* aerial part; PSRRH: dry weight of *Phaseolus vulgaris* root part; PSPAM: dry weight of *Medicago sativa* aerial part; PSPPRM: dry weight of *Medicago sativa* root part; PSPAP: dry weight of *Cicer arietinum* aerial part; PSPPRP: dry weight of *Cicer arietinum* root part.

PCA was employed to analyze the contribution of different sludge concentrations to plant growth and identify the main factors driving variation. The first two PCA axes explain 72% of the variation in growth, with the first axis capturing 53%. This allows us to visualize which inoculum concentrations have the greatest impact on specific growth parameters. By grouping treatments based on their contribution to aerial and root dry weight, PCA aids in visualizing the nutrient-driven growth trends in plants, emphasizing the potential of certain sludge-based inoculums over others.

The second axis accounts for 19% of information. It is negatively correlated with the dry weight of the aerial and root parts of *Phaseolus vulgaris*. However, there was a positive correlation between the dry weight of the above-ground and root parts of *Medicago sativa* and *Cicer arietinum*.

These latter growth parameters were improved by the application of inoculum made with sludge at a concentration of 5% to the cultivated plants, as well as by inoculum made with YEM medium. However, the dry weight of the aerial part of *Phaseolus vulgaris* was

improved by the addition of inoculum made with 10% of the sludge, which can be attributed to the acquisition of nutrients provided by the sludge studied.

PCA was applied to explore the relationship between the different sludge inoculum concentrations and the growth parameters, particularly the dry weight of aerial and root parts. As explained in the manuscript, PCA helps reduce the dimensionality of the data, highlighting which inoculum concentrations (e.g., 5%, 10%, 15%) had the most significant influence on plant growth. The PCA plot (Figure 6) shows how these variables group, indicating a positive correlation between specific inoculum concentrations and dry weight. For instance, the dry weight of the root and aerial parts of *Medicago sativa* and *Cicer arietinum* showed a strong positive correlation with inocula made with sludge at a concentration of 5%, which provides insights into nutrient acquisition from these treatments.

In Algeria, the use of sewage sludge for the production of crop inoculants remains poorly investigated. However, the exploitation of sludge as a fertilizer offers a dual benefit: it contributes to the valorization of this waste material while reducing the production cost of microbial inoculants. Moreover, it can help decrease dependence on chemical fertilizers and mitigate their negative impacts on the environment and human health.

4.4. Evaluating the use of sludge as a substrate

Figure 7 shows the viability of the *Rhizobium etli* CIAT 899 strain on solid support prepared with the sludge studied, stored at different temperatures (4°C, 25°C and 40°C) for four weeks, compared with the reference support (peat). The results obtained show that the number of *Rhizobium etli* CIAT 899 strains was well maintained over the four weeks at 4°C on both sludge and peat, in contrast to the other temperatures where numbers were stable only during the first week.

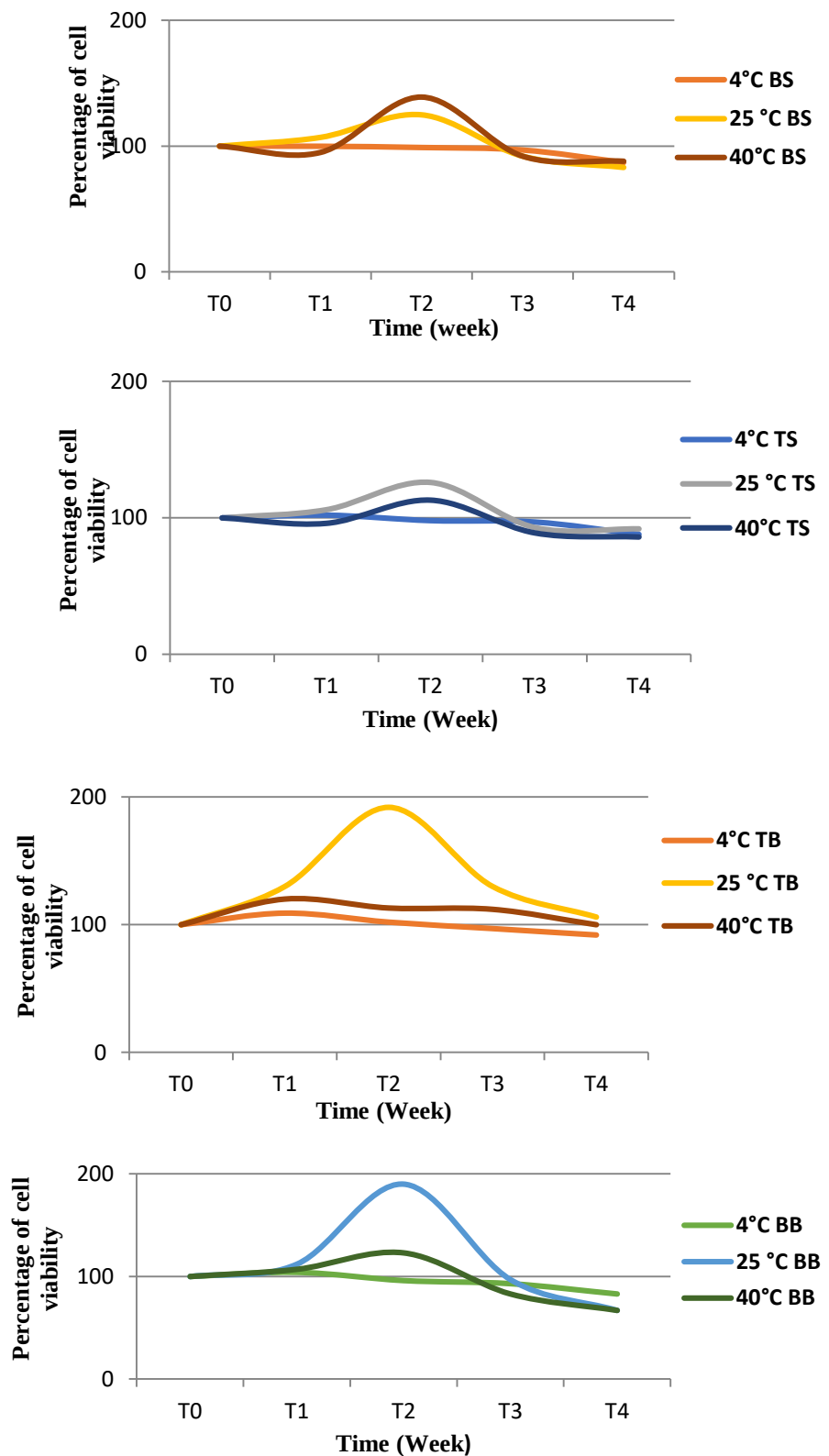


Figure 7. Percentage of cell viability with two media (Peat and sludge) at different storage times and temperatures

TS: Peat packed in polyethylene bags; BS: Sludge packed in polyethylene bags; TB: Peat packed in sterile jars; BB: Sludge packed in sterile jars.

The viability of *Rhizobium etli* at 4°C for four weeks on the sludge, compared with other temperatures, suggests that cool, stable conditions favor the conservation of the micro-

organisms. However, the stability observed only during the first week at higher temperatures could indicate thermal stress mechanisms affecting the survival of rhizobia in the long term.

5. Discussion

5.1. Microbial properties of sludge

The assiduity of bacterial flora in sludge can be explained by the presence of organic compounds in the sludge, which provide the food necessary for the development of these microorganisms. The presence of faecal coliforms in sewage sludge is evidence of contamination of faecal origin. The value of these germs recorded in the sludge studied was 2.7×10^4 CFU /mL. This bacterial load is still lower than that reported by [17], whose count is of the order of 6.2×10^5 CFU/mL. Taking into account the bacterial load of *Escherichia coli*, it reaches 4×10^5 CFU/mL. [18] reported the absence of *Escherichia coli*, while [19] mentioned the presence of this species with a content of 1.6×10^7 UFC/mL. [20] reported a level of 6.3×10^5 CFU/mL. The presence of Staphylococci in sludge is an excellent indicator of fecal contamination. The concentration of these germs in the sludge analyzed is 4.8×10^4 CFU /mL. In contrast, the sludge studied by [21] has a value of around 3.8×10^6 CFU/mL. The assiduity of *Clostridium perfringens* in sewage sludge indicates contamination of fecal origin [22]. Indeed, this germ was not detected in the sludge studied, which is consistent with the study carried out by [23]. Sewage sludge can be loaded with other bacterial communities such as *Nostocoida limicola* [24], *Salmonella* [25], *Enterococcus* sp [20], *Acinetobacter calcoaceticus* STP14 [26], Micromycetes [27], *Pseudomonas* [28] and *Escherichia coli* [29]. The use of sludge in agriculture raises concerns about the levels of pathogenic microorganisms and their compliance with safety thresholds. Before application to farmland, sludge must contain less than 1000 CFU of *Clostridium* and coliforms per gram of dry matter. As for *Staphylococcus*, their concentration must not exceed 100 CFU/g of dry matter [30].

5.2. Rhizobia growth after cultivation

Sewage sludge is known for its high carbon and organic matter content [31] The liquid culture medium prepared with sterile sludge at a concentration of 5% provides 0.9g of organic matter, 0.7g of phosphorus, 0.5g of nitrogen and 0.49 of carbon. At 10% and 15%, it provides two to three times the concentration of these elements. For this reason, it proved appropriate to select these sludge concentrations to meet the nutrient requirements of these microorganisms. It is important to note that the pH of the media prepared with the sludge studied is considered optimal, which was in favor of the growth of these bacteria.

Our results are in perfect agreement with those of the literature as reported by [32] and [33] indicating that the sludge supports rhizobia survival well. It is well recognized that the YEM medium is rich in nutrients, providing carbon sources, such as mannitol, as well as vitamins, minerals and yeast extracts, which are essential for rhizobia growth [34]. Furthermore, sludge also contains macronutrients and micronutrients that promote rhizobia development [35]. Consequently, using sludge as a culture medium could provide an optimal environment for the growth of these microorganism.

5.3. Effect of inoculation on crop growth

Sludge management remains a crucial challenge for the protection of public health and the successful implementation of sanitation projects. The effect of inoculums prepared with sludge on the growth of the plants tested, namely *Phaseolus vulgaris*, *Cicer arietinum* and *Medicago sativa*, showed an improvement in the dry biomass of the plants tested at 5% and 10% sludge. Inoculation of legumes with suitable rhizobia has been widely used to ensure the availability of a sufficient rhizobia population in the plant rhizosphere [36]. At present, a very limited number of studies have investigated the contribution of inoculum made with sludge on crops. One example is the study by [37], who demonstrated that sludge-based inoculums are effective for nodulation and dry weight in alfalfa

The concentrations of organic and metallic micropollutants in the studied sludge were well below the limits established by AFNOR, confirming its suitability for use as an organic amendment. Application of the sludge promoted the uptake of heavy metals by the tested plants (*Phaseolus vulgaris*, *Cicer arietinum*, and *Medicago sativa*), while their accumulation in plant tissues and soil remained limited. Heavy metal concentrations measured in both the shoots and roots remained below the regulatory thresholds, with no apparent phytotoxic effects on the tested plants. Furthermore, sludge application significantly enhanced the dry biomass production of both shoots and roots, indicating a beneficial effect on plant growth [38].

5.4. Effect of using sludge as a support

A good inoculum carrier must be highly absorbent, non-toxic and economical [39]. Sewage sludge meets these criteria, making it a promising option for inoculum production, representing a new option for inoculum valorization and considerably reducing the cost of inoculum production.

However, its use as a storage medium depends on the nature of the packaging and, above all, the storage temperature. Although peat is the preferred medium for solid inoculum production [40] due to its water retention capacity and high organic matter content [41]. Consequently, it is important to find other alternatives with similar advantages. The results obtained with sludge are very similar to those obtained with peat. This underlines the importance of these results in advancing the use of sludge, which is readily available, at low cost, and in reducing environmental pollution. Sludge, as a by-product of wastewater treatment plants, is generally inexpensive, sustainable and can be used to improve soil quality, although contaminant management is essential to reduce health risks. Peat, on the other hand, although effective as a soil improver, has higher extraction costs and is often unsustainable.

Although small-scale experimentation has provided interesting results on the viability of *Rhizobium etli* CIAT 899, it could potentially contribute to the validation of large-scale commercial applications. However, larger-scale trials, taking into account the heterogeneity of the sludge and the conditions under which it is treated, are needed to assess its industrial viability. Furthermore, the methods used have certain limitations, such as the restriction to

four temperatures tested, without taking into account other environmental factors (such as humidity and pH), or a comparison with different types of sludge. A more in-depth study of the biological mechanisms could enrich these results.

6. Conclusion

This study fills a gap in the literature by examining the use of sludge for rhizobial inoculum production, an area that has yet to be fully explored. Although much research has focused on the application of rhizobial inoculants, few studies have evaluated the effectiveness of sludge as a carrier for these bacteria. Furthermore, this research evaluates the effect of sludge-based inoculants on various crops, providing essential data on their potential to improve plant growth while reducing dependence on chemical fertilizers.

The valorization of sewage sludge, an unavoidable byproduct, has become a significant concern due to its environmental and socio-economic implications. To address these challenges, this study was conducted to explore innovative applications of sludge, particularly as a growth medium and carrier for rhizobia, a biofertilizer that is increasingly appealing to farmers for enhancing nitrogen inputs and improving crop yields. This biofertilizer is also utilized in the rehabilitation of degraded sites for the same reasons. By adopting a strategy centered on the recycling and reuse of sludge, this research aims to capitalize on available resources while mitigating the environmental impacts associated with sludge disposal.

The data obtained reveal that *Rhizobium etli* CIAT 899 can be effectively preserved on sewage sludge, particularly at a temperature of 4°C, where its viability remains stable for several weeks. This suggests a promising potential for the use of such sludge as a carrier for the production and preservation of biofertilisers. However, higher temperatures rapidly reduce the viability of rhizobia, highlighting the need for optimal storage conditions to maximize their effectiveness.

This study lays the groundwork for sustainable waste management and soil restoration practices. By promoting the rehabilitation of degraded soils, this approach aids in preserving the fertility of agricultural land and fostering more sustainable agricultural practices. Although sludge-based inoculants show promising potential for improving soil fertility and reducing dependence on chemical fertilizers, it is crucial to carry out field trials to assess their effectiveness on a wider range of crops. Further research is needed before they can be considered as viable alternatives. These lines of research will help to establish sustainable agricultural practices and promote efficient resource management.

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Author contributions

Mesbah Nadjet: Sampling, Methodology, Data collection and writing articles; Rezki-Bekki Meriem Amina; Bekki Abdelkader: Supervision. All authors reviewed the manuscript

Conflict of interest statement

The authors declare no conflict of interest.

References

1. Balkrishna, A., Singh, S. K., Pathak, R., Arya, V. 2022. Sludge Management: Available Solutions and Way Forward. Authorea. <https://doi.org/10.22541/au.164899217.73875491/v2>.
2. Charbonneau, A., Lucotte, M., Moingt, M., Blakney, A.J., Morvan, S., Bipfubusa, M., Pitre, F.E. 2024. Fertilization of agricultural soils with municipal biosolids: contributions of glyphosate and aminomethylphosphonic acid to soils of major crops in Quebec. *Total Environ. Sci.* 171290. <https://doi.org/10.1016/j.scitotenv.2024.171290>.
3. Sichler, T.C., Adam, C., Montag, D., Barjenbruch, M. 2022. Future nutrient recovery of sewage sludge under three different scenarios - German case study. *J. Cleaning. Prod.* 333, 130130. <https://doi.org/10.1016/j.jclepro.2021.130130>.
4. Zraïbi, L., Chaabane, K., Berrichi, A., Sbaa, M., Badaoui, M., Zarhloule, Y., Georgiadis, M. 2015. Assessment of the agronomic value of compost from the sludge of the wastewater treatment plant of the city of Nador. *J. Mater. Environ. Sci.* 6 (10), 2975-2985.
5. Kumar, A., Bhattacharya, J. 2020. Utilization of sewage sludge in agriculture: A review on the prospective and challenges. *Waste Manag.* 98, 182-190. <https://doi.org/10.1016/j.wasman.2019.12.013>.
6. Singh, S., Yadav, R., Singh, A.N. 2022. Applications of waste economics practices in the urban wastewater sector: implications for the ecosystem, human health and the environment. in *waste management and resource recycling in developing countries*. 625-646. <https://doi.org/10.1016/B978-0-323-90463-6.00010-5>.
7. Tarrago, M., Royo I., Martinez, S., Garcia-Valles, M., Neuville, D. R. 2021. Incorporation of calcium in glasses: A key to understand the vitrification of sewage sludge. *Int. J. Appl. Glass Sci.* 12(3): 367-380. <https://doi.org/10.1111/ijag.15920>.
8. Yaser, A.Z., Lamaming, J., Suali, E., Rajin, M., Saalah, S. 2022. Composting and anaerobic digestion of food waste and sewage sludge for campus sustainability: A review. *Int. J. Chem. Eng.* <https://doi.org/10.1155/2022/6455889>.
9. Ongun, A., Tepecik, M., Kayıkçıoğlu, H.H., Delibacak, S., Birişçi, T.; Aktaş, E., Kalaycı Önaç, A., Balik, G. 2023. Effects of sewage sludge treatments on plant nutrients, heavy metals and tall fescue (*Festuca arundinacea* Schreb.). *Turk. J. Agric. For.* 47: 828-841 Available from <https://doi.org/10.55730/1300-011X.3131>.
10. Singh, R., Gupta, A., Chauhan, S. 2022. Potential of biofertilizers derived from sewage sludge in sustainable agriculture. *Environ. Sci. Pollut. Res.* 29, 15567-15579. <https://doi.org/10.1007/s11356-022-18351-9>.
11. Zhang, W., Alvarez-Gaitan, J.P., Dastyar, W., Saint, P. C., Zhao, M., Short, D. M. 2018. Value-added products derived from waste activated sludge: a biorefinery perspective *Water* 10(5), 45.
12. Zhang, X., Jiao, Y., Yu, L., Liu, L., Wang, X., Zhang, Y. 2022. Effect of Sewage Sludge Addition on Microstructure and Mechanical Properties of Kaolin-Sewage Sludge Ceramic Bricks. *Coatings*, 12, 944. <https://doi.org/10.3390/coatings12070944>.
13. Bora, A. P., Gupta, D. P., Durbha, K. S. 2020. Sewage sludge to bio-fuel: A review on the sustainable approach of transforming sewage waste to alternative fuel. *Fuel*, 259, 116262. <https://doi.org/10.1016/j.fuel.2019.116262>.
14. Bon, D.C., Okada, D.Y., Coneglian, C.M.R. 2022. Benefits of inoculation of biological additives in the treatment of paper recycling industry effluents. *J. Water process engineer.* 50, <https://doi.org/103269.10.1016/j.jwpe.2022.103269>.
15. Arjun, V. G., Lakshan Raaj, V., Shravya, M., Laksha, V., Eunice, J. 2025. Bio-Fuel Generation from Sewage Sludge. *International Research Journal on Advanced Engineering Hub (IRJAEH)* 03: 1868-1872. <https://irjaeh.com> <https://doi.org/10.47392/IR-JAEH.2025.0271>
16. Baha, N., Bekki, A. 2015. An approach to improving salt tolerance in alfalfa (*Medicago sativa*) plants grown under salt stress: use of Bio-inoculants. *J. Plant Growth Regul.* 34, 169-182. <https://doi.org/10.1007/s00344-014-9455-8>.
17. Defo, C., Nguema, P. F.N., Tenkam, A. F. 2021. Diagnosis of urban sewage sludge management in a tropical environment and analysis of improvement strategies: Case of the city of Ebolowa, Cameroon (Central Africa). *Int. J. Eng. Res. Dev.* 17(2): 37-55.
18. Martin, E., Matocha, J. E. 1997. Plant analysis as an aid in the fertilization of forage crops. In *Sol Testing and Plant Analysis*. L.M. V/alsh and J.D. Beaton (eds). Soil Science Society of America, Inc. Madison, Wisconsin USA, 393-426.
19. Kalloum, S., Bouabdessalem, H., Touzi, A., Iddou, A., Ouali, M.S. 2011. Biogas production from the sludge of the municipal wastewater treatment plant of Adrar city (southwest of Algeria), *Biomass Bioenergy* 35 (7): 2554-2560. <https://doi.org/10.1016/j.biombioe.2011.02.012>.
20. Miguel, N., Sarasa, J., López, A., Gómez, J., Mosteo, R., Ormad, M. P. 2020. Study of Evolution of Microbiological Properties in Sewage Sludge-Amended Soils: *Int. J. Environ. Res. Public Health*. 17(18): 6696. <https://doi.org/10.3390/ijerph17186696>.
21. Kone, M., Service, E., Ouattara, Y., Ouattara, P., Bonou, L., Joly, P. 2016. Characterization of sewage sludge deposited on the drying beds of Zagtouli. *Int. J. Biol. Chem. Sci.* 10(6): 2781-2795. <https://doi.org/10.4314/ijbcs.v10i6.30>.
22. Altmeyer, N., Abadia, G., Schmitt, S., Leprince, A. 1990. Microbiological risks and work in wastewater treatment plants. *Documents for the occupational physician*, 44.
23. Douaer, N., Douaoui, A., Mehaiguene, M., Zouidi, M., Hamza, W. 2021. The effect of municipal sewage sludge on physico-chemical and microbial agricultural soil properties. *Notulae Sci. Biol.* 13(1): 10804. <https://doi.org/10.15835/nsb13110804>.
24. Adonadaga, M.G., Martienssen, M. 2015. In situ Identification of Filamentous Bacteria from Activated Sludge Wastewater Treatment Plants in Ghana. *J. Appl. Environ. Microbiol.* 3: 75-81. <https://doi.org/10.12691/jaem-3-3-3>.
25. Krzyzanowski, J. F., de Souza Lauretto, M., Nardocci, A. C., Sato, M. I. Z., Razzolini, M.P. 2016. Assessing the probability of

- infection by Salmonella due to sewage sludge use in agriculture under several exposure scenarios for crops and soil ingestion. *Sci. Total Environ.* 568: 66-74. <https://doi.org/doi.org/10.1016/j.scitotenv.2016.05.129>.
26. Din, G., Farooqi, A., Sajjad, W., Irfan, M., Gul, S, Ali Shah. A. 2021. Cadmium and antibiotic-resistant *Acinetobacter calcoaceticus* reported from sewage treatment plant. *J. Basic Microbiol.* 61(3): 230-240. <https://doi.org/10.1002/jobm.202000538>.
 27. Malcheva, B. Z., Petrov., P. G, Stefanova, V. V. 2022. Microbiological Control in Decontamination of Sludge from Wastewater Treatment Plant. *Processes.* 10 (2): 406. <https://doi.org/10.3390/pr10020406>.
 28. Sharma, P., Singh, SP. 2022. Identification and profiling of the industrial sludge microbial community. *Arch Microbiol.* 204, 234. <https://doi.org/10.1007/s00203-022-02831-y>.
 29. Li, M., Song, G., Liu, R. 2022. Inactivation and risk control of pathogenic microorganisms in municipal sludge treatment. *Sci. Ing.* 16, 70. <https://doi.org/10.1007/s11783-021-1504-5>
 30. Stiborova, H., Kracmarova, M., Vesela, T., Biesiekierska, M., Cerny, J., Balik, J., Demnerova, K. 2021. Impact of Long-Term Manure and Sewage Sludge Application to Soil as Organic Fertilizer on the Incidence of Pathogenic Microorganisms and Antibiotic Resistance Genes. *Agronomy*, 11(7), 1423. <https://doi.org/10.3390/agronomy11071423>
 31. Ongun, A. R., Tepecik, M., KAYIKÇIOĞLU, H. H., Delibacak, S., Birişçi, T., Aktaş, E., BALIK, G. 2023. Effects of sewage sludge treatments on plant nutrients, heavy metals and tall fescue (*Festuca arundinacea* Schreb.). *Turkish Journal of Agriculture and Forestry*, 47(6), 828-841.
 32. Ben, Rebah F., Tyagi, R. D., Prevost, D. 2002. Production of ' *S. meliloti* using wastewater sludge as a raw material: effect of nutrient addition and pH control. *Environ. Technol.* 23 (6): 623-629. <https://doi.org/10.1080/09593332308618378>.
 33. Dufresne, G. 2004. Valorization of residual materials for the production of Rhizobium-based biofertilizers: optimization of the fermentation process with *Sinorhizobium meliloti* (Doctoral dissertation, University of Quebec, National Institute of Scientific Research).
 34. García, A., Peinado, R. A., & Medina, M. (2018). Effect of yeast extract on growth of Rhizobium species. *Microbial Ecology*, 75(4), 992-1003. <https://doi.org/10.1007/s00248-017-1066-8>.
 35. DE Neve, P. (2020). Impact of organic amendments on soil and plant health. *Soil Biology and Biochemistry*. <https://doi.org/10.1016/j.soilbio.2020.107869>.
 36. Thilakarathna, M. S., Raizada, M. N. 2017. A meta-analysis of the effectiveness of diverse rhizobia inoculants on soybean traits under field conditions. *Soil Biol. Biochem.* 105: 177-196. <https://doi.org/10.1016/j.soilbio.2016.11.022>.
 37. Ben Rebah, F. 2001. Use of sewage sludge as a culture medium for the production of Rhizobium-based inoculum. D. thesis, INRS-ETE, Sainte-Foy, QC, Canada, 114.
 38. Mesbah, N., Rezki Bekki, M. A., Aouad, L., Rouane Hacene, O., Durand, G., & Bekki, A. (2024). Analysis of the fertilizing potential of sewage sludge and its impact on agriculture and the environment. *International Journal of Recycling of Organic Waste in Agriculture*, 14(2). <https://doi.org/10.57647/ijrowa-sfzz-1f78>.
 39. Smith, R. S. 1996. Legume inoculant formulation and application. *Can. J. Microbiol.* 38: 485-492. <https://doi.org/10.1139/m92-080>.
 40. Feng, L., Roughley, R., Copeland, L. 2002. Morphology changes of Rhizobium in peat cultures. *Appl. Environ. Microbiol.* 68(3):1064-1070. <https://doi.org/10.1128/AEM.68.3.1064-1070.2002>.
 41. Hungria, M., Loureiro., M F., Mendes, I C., Campo, R J., Graham, P. H. 2005. Inoculant Preparation, Production and Application. *Nitrogen Fixation in Agric., For. Ecol. Environ.* 223-253. <https://doi.org/10.1007/1-4020-3544-6-11>.