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Silver Nanoparticles Synthetized with *Cinnamomum camphora* to Reduces Total Coliforms in Soil Agricultural, Lima Perú

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The objective of this work was to Determine the percentage of total coliform reduction by applying the silver nanoparticles synthesized with the *Cinnamomum camphora* substrate in the agricultural soil. First, the silver nanoparticles were synthesized being the reducing agent vegetal extract of *Cinnamomum camphora*, this also have antiseptic properties. The synthesis gave rise to silver nanoparticles with an average size of 37 nm.
Second, it was the application of silver nanoparticles in four soil samples and in four different doses, which were 0.5 ml, 1.5 ml, 7 ml tond 10 ml and evaluated at different times. After the treatment and analysis of the total coliform concentration of the soil samples, to progressive decrease occurred ace time passed. After 72 hours, an average of 60% of the total coliform content in the agricultural soil was reduced, concluding that it is to viable way to improve the quality of the soils. However, other positive or negative impacts derived from the use of silver nanoparticles have not been yet evaluated; highlighting that the way in which nanoparticles have been synthesized is already an environmentally friendly method when not using chemical reagents.
Keywords: Silver nanoparticles silver, synthesis whit green chemistry, reduction of coliforms in soils.

1. Introduction

The problem of the pollution of the soil has done notorious in the last decade, since it sees affected by different types of contaminants, that are the product of the human activity, entering harmful substances like volatile organic compounds, toxic wastes of industry, domestic effluents, pesticides and fertilizers Like this, these substances affect gravely to the human health, vegetal and animal. A form to find a solution to the problems of pollution in the organisms receptors in the last times is the use of the nanotechnology. With the use of silver nanoparticles in an investigation obtained the reduction of 90% of bacteria in water, (Perez, 2011), in another case has allowed the control of the growth of bacteria lactic and acetic in the industry oenological, (García-Ruiz To., et al., 2015). Also it has treated soil contaminated with TPHs (Fenantreno) using the nanoparticles metallic of elementary iron reducing between 22 to 50% (Valera, 2017). It has tested synthesis of suspensions of nanoparticles of copper and chitosan and tested his antimicrobic power in front of the *Streptococcus mutans* (Trepiana, 2015). On the other hand the obtaining of nanoparticles by means of vegetal extracts has come investigating by the environmental advantage that means, for example, for the application in paintings like additives in his function antifungal (Deyá and Bellotti, 2015). In other cases has tested the nanoparticles of silver to inhibit the training of biofilms of Pseudomona aeruginosa and Staphylococcus aureus, indicates that the bacterial capacity of the silver nanoparticles is high producing a mortality of until 99.9% (Flores C., 2014).
The pollution of soil urge attention for being the natural reactor where develop multiple activities from processes of filtration, regulation of the cycle of water and biogeochemical, in addition to being universal habitat of living beings (Volke et al, 2004, pp.11-12). In a lot of countries does not exist rule related pathogens in soil, Peru is one of them, for this study took to theNo rma Mexican NOM-112-SSA1-1994, that establishes the permissible limits of microbiological characteristics in soil. The National Authority of the Water (ANA, 2016) has regulated the use of domestic residual waters treated in irrigation to avoid pollution of agricultural soil, since the pathogenic microorganisms are causers of damages to the health (Brennan J.,

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2017). It has tested the use of copper, silver and zinc nanoparticles like nano fungicides in the treatment of pathogens (funguses) arrived from the soil to foliar plants obtaining that the copper nanoparticles were more efficient (Malandrakis A.A., 2019); however, it does not have still specific studies on the impacts of the nanoparticles in the soil for example in the biogeochemical cycle in the nitrogen cycle with the simultaneous application of fertilizers (Parada, J and et al, 2019).
It has improved the quality of agricultural soil by means of the addition of seaweeds Litohothamnion calcareum nanoparticles for the improvement of production of fruit trees (Negreiros A.M.P., et al., 2019), in other cases has tested the improvement of mechanical stabilisation of soil with carbonate of calcium nanoparticles (Choobbasti J, et al, 2019). In this research, as in other previous works, Cinnamomum camphora extract polyphenols were used as reducing agents for synthetize silver nanoparticles. Then, these nanoparticles reduce the load of total coliforms in the soil.

2. Experimentation

The following stages were followed:

2.1 Characterization of soil contaminated

It determined the state of the physical chemistries and microbiological properties of the soil, specially the presence of total coliforms. The sample was collected following the Protocol of soils sampling (2014) of the Guide for Sampling of Soil, in concordance with the Supreme Decree N° 002-2017-MINAM (Standard of Environmental Quality - ECA for Soil) at Ministry of Environment (MINAM).

2.2 Syntheses of silver nanoparticles

It used 20 g of leaves *of Cinnamomum camphora* previously wash, dried to acclimatise temperature. Next, it proceeded to make an extraction solid-liquid of the polyphenols of the *Cinnamomum camphora* using 3 g of this and crushing them, to a temperature of 60°C, in agitation to 900 rpm by a time of 20 minutes. Afterwards it filters, this extract was used for the synthesis later (Figure 1a). On the other hand, it prepared a solution of silver nitrate in deionized water taking 0.34 g of $AgNO_3$ dissolving in 20 mL of water.
For the synthesis stage, the AgNO3 solution is taken and with stirring of 900 rpm and at 60 ° C 4.4 mL of Cinnamomum camphora extract is added. When it presents a change of a brown colour it means will culminate the process of nanoparticles synthesis (Figure 1b), by means of the Tyndall effect (that it consists in observing the reflection of the light in the colloidal particles) verifies the nanoparticles presence since to simple sight are not visible. They were sent to a laboratory to verify their characterization.

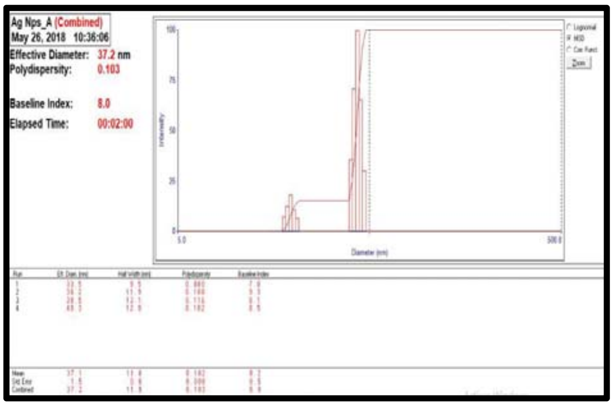

a


b

Figure 1a: Cinnamomum camphora Extract *Figure 1b: Cinnamomum camphora Nanoparticles*

2.3 Characterization of the nanoparticles

The characterization of the nanoparticles did in the laboratory of sciences of the National University of Engineering, by means of dispersion of dynamic light (DLS), with the result that presents in the *Graphic 1*. It found nanoparticles of diameter of 37.2 nm.



Source: Laboratory of sciences-UNI

Graphic 1: Size of nanoparticles of silver measured by DLS

2.4 Treatment of the soil with silver nanoparticles

Silver nanoparticles synthesized in solution and stabilized at a pH around 7 using a buffer solution, were injected into the soil sample for a certain time. These were placed in four tanks where different amounts of silver nanoparticles were injected according to Figure 2.

Sample of soil	M1	M2	M3	M4
Dose of Ag nanoparticles	0.5 mL	1.5 mL	7 mL	10 mL
pH	6.8	7.23	7.45	7.54

Source: Own

Figure 2: Design of the samples of soil and dose of the nanoparticles used

2.5 Characterization of the soil afterwards of the treatment

At this stage, the analysis was performed on soil samples treated with nanoparticles silver to determine the impact of the method related to the level total coliforms in the soil. These results are shown in the following section below.

3. Results and Discussion

3.1 State and characteristic initials of the soil

The soil presented the physicochemical properties detailed in Table 1, highlighting that the soil was textural class, clay loam soil type and pH nearly to neutral. Analysis made in the Laboratory ANOBA LAB SAC.

Initial Coliforms in the soil: The amount of total coliforms in the soil was 1,100 NMP / g on average, in the five soil monitoring points studied (Report of Laboratory ALAB Analytical E.I.R.L).

Table 1: Physicochemical initial properties of the soil

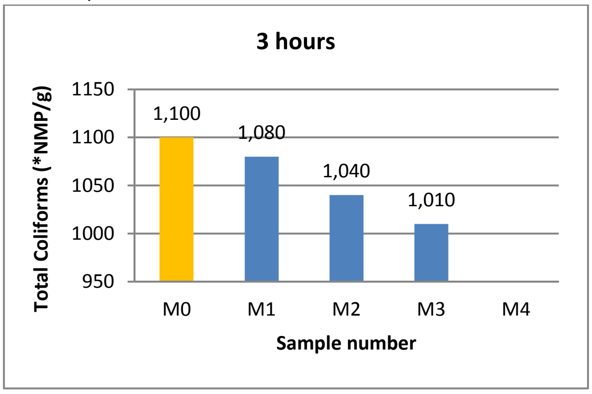
Physicochemical parameters	Units	Results
Sand	%	36
Silt	%	30
Clay	%	34
Class textural	-	clay loam
PH (1/1)	Und. pH	7.87
CE (1/1)	dS/m	0.57
Carbonates	%$CaCO_3$	6.22
Organic matter oxidizable	%	2.15
Interchangeable acidity	meq/100g	<0.2

Source: it Inform laboratory ANOBA LAB SAC

3.2 Total Coliforms in the soil after the treatment with silver nanoparticles (NPsAg)

Considering the design shown in Figure 1, the soil treatment was made out with their respective repetitions to determine the level of total coliforms, in the times of 3, 48 and 72 hours after the start of the process. The analyses made in the Laboratory ALAB Analytical Laboratory E.I.R.L. using the norm of reference FDA/BAM, On-line 8th.ed.Rev.To, 3 1998. September 2002-Chapter 4, A, B, C, D, and F (Reviewed the 2013), con the Technician: Proofs of identification of coliforms organism: IMViC

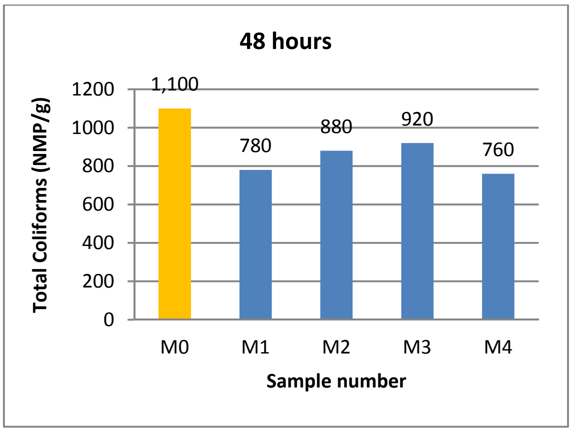
In three hours of treatment:
In the M4 sample, the greatest level reduction of the total coliforms in the soil was obtained, in comparison to the other samples. It was reduced from 1,100 to 990 NMP/g equivalent to 10%, after treatment with NPsAg. See *Graphic* 2.



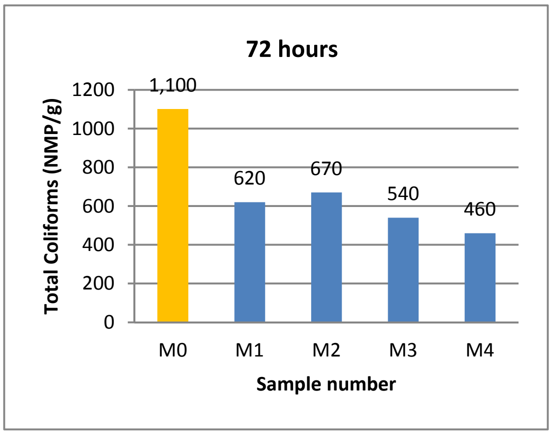
Source: Own
**NMP (most likely number)*

Graphic 2: Level of reduction of the total coliforms in the soil treated during 3 hours with solution containing silver nanoparticles.

In 48 hours of treatment:
Continuing the treatment with silver nanoparticles (NPsAg) in the same soil samples, at the end of 48 hours, it was found that in the M4 sample there was a greater reduction of total coliforms, coinciding that it is in this sample where also added greater amount of NPsAg, see *Graphic* 3a. In this sample it was reduced from 1,100 to 760 NMP/g, equivalent to 30% reduction of coliforms.
In 72 hours of treatment:
At 72 hours of treatment, in the M4 soil sample, was obtained the greatest reduction of total coliforms, 10 mL of NPsAg was used for the treatment and at pH of 7.54, (See *Graphic* 3b). The reduction was from 1,100 (initial) to 460 NMP/g, equivalent to 58.19%.



a



b

Source: Own

Graphic 3a: Level of reduction of the total coliforms in the soil treated during 48 hours with solution containing silver nanoparticles.

Source: Own

Graphic 3b: Level of reduction of the total coliforms in the soil treated during 72 hours with solution containing silver nanoparticles.

This confirms that silver nanoparticles allow the reduction of total coliforms, as was investigated by Flores (2014), who reduced up to 99.9% of *Pseudomona aeruginosa* and *Staphylococus aureus*. Also for other pollutants, nanotechnology allows them to be reduced, such as Valera's research (2017) which, with elemental iron nanoparticles, achieved up to 50% reduction of phenanthrene. Therefore, the use of nanotechnology in the remediation of contaminated soil is an alternative, and there are already several projects carried out such as the European project NANOREM (2015), which after their tests confirm that it is possible to decontaminate soil and water effectively and safely. An important aspect of this topic is the synthesis of nanoparticles, the ecological form existing through the use of plant extracts as a reducing agent, for example, the research of Ledesma et al. (2014), among others.

4. Conclusion

It was established that through the use of silver nanoparticles synthesized with *Cinnamomum camphora* extract of the size of 37 nm, it was able to reduce the total coliforms of a soil from the initial amount of 1,100 to 460 NMP/g. Therefore, it can be said that nanotechnology is the alternative way of using this practical and reliable method for the treatment of contaminated soils with a high coliform load.

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