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EFFECTS OF THE APPLICATION AN OLIGOGALACTURONIDE MIXTURE ON A POLLUTED SOIL CULTIVATED WITH TOMATO SEEDLINGS

Efectos de la aplicación de una mezcla de oligogalacturónidos sobre un suelo contaminado cultivado con plántulas de tomate

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ABSTRACT. It was carried out a rehearsal with a contaminated soil by copper (Cu), in order to evaluate the risk associated to the application of technical of phytorremediation with chelates (Ogal mixture) in these cases. They thought about different situations: normal soil without addition of Ogal (control), polluted soil without addition of Ogal and polluted soil and Ogal concentrations of 20, 40, 60 80 and 100 kg ha^{-1} respectively. The pseudototal copper content was measured in the soil, pH and organic matter of the soil and longitude (air and underground) of the tomato plants. The results show that the lenght of the plants that grew in a polluted soil without the product application suffered a significant descent with relationship to the control; however, to those that was applied the product, they diminished their lenght, but not so markedly as the previous ones. On the other hand, the cation of the system experiented an increase of their mobility with the presence of the Ogal mixture, being bigger their phytoextration when dose of 60 kg is applied.

Key words: quelatos, phytorremediation, heavy metal, tomato, mobility of elements, oligogalacturonides

RESUMEN. Se realizó un ensayo con un suelo contaminado por cobre (Cu), a fin de evaluar el riesgo asociado a la aplicación de técnicas de fitorremediación con quelatos (mezcla de Ogal) en estos casos. Se plantearon diferentes situaciones: suelo normal sin adición de Ogal (control), suelo contaminado sin adición de Ogal y suelo contaminado y concentraciones de Ogal de 20, 40, 60 80 y 100 kg ha^{-1} respectivamente. Se midió el contenido de cobre pseudo total en el suelo, pH y materia orgánica del suelo y longitud (aérea y subterránea) de las plantas de tomates. Los resultados muestran que la longitud de las plantas que crecieron en un medio contaminado sin la aplicación de producto sufrieron un descenso significativo con relación al control; sin embargo, a las que se les aplicó el producto, disminuyeron su longitud, pero no tan marcadamente como las anteriores. Por otro lado, los cationes del sistema experimentaron un aumento de su movilidad con la presencia de la mezcla de Ogal, siendo mayor su fitoextracción cuando se aplican dosis de 60 kg ha^{-1}.

Palabras clave: quelatos, fitorremediación, metales pesados, tomate, movilidad de elementos, oligogalacturónidos

INTRODUCTION

Among the serious problems facing humanity today, it is the rapid contamination of soil and air. Pollution brings the deterioration of the environment and therefore the impossibility of natural resources with the required safety. Heavy metal pollution is today one of the main sources of detriment to the environment (1).

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Agriculture is not immune to this problem, due to the intensive use of pesticides and fertilizers as well as improper practices drainage or irrigation. Keep in mind also hit by the rise of urban agriculture in recent times, in which materials are used as substrates, which can increase pollution areas (2).

Strenuous are attempts to find ways to recover resources that are contaminated by heavy metals, mainly soils, inexhaustible source of food. But the ways for it becomes important if not the materials are used for decontamination thereof and in food production, remains aggressive environment.

During these years, it has developed bioremediation as a possible future solution to many problems of pollution, because it is considered throughout the world as an innovative technology for the treatment of toxic waste (3); within this, it is phytoremediation, which is based on the use of plants to clean polluted environments, being phytoextraction and phytostabilisation, the most applied techniques (4).

It is known that one of the most important mechanisms used by plants tolerance to reduce heavy metal toxicity is the formation of complexes with organic ligands. Here, the mixture of oligogalacturonides (Ogal) has a high proportion of fillers, which allows them to bond formation with heavy metals, forming complexes (5, 6). However, it is not established that the effect may exist among these molecules and metal ions, nor the role in detoxification and in the processes of growth and development of plants (7, 8).

To evaluate the effect of applying a mixture of Ogal in absorbing metal ions from the soil tomato seedlings was our objective.

MATERIALS AND METHODS

It was collected soil classified as Ferralitic Red Leachate (9), on land belonging to the National Institute of Agricultural Sciences (INCA) and a $CuSO_4.5H_2O$ solution was prepared in a quantity equivalent to 700 mg kg^{-1} (ground) of Cu, adding it to ground seven days before planting tomato seeds of Amalia variety.

All experiments were carried out in the Department of Plant Physiology and Biochemistry of INCA, in a room of lights, keeping in conditions of 24 ± 2 °C temperature, 12 h light photoperiod and a relative humidity of 40 %, for 35 days.

The mixture of Ogal was obtained at INCA, from the enzymatic hydrolysis of pectin from citrus rind, according to the methodology proposed by Cabrera[A], with degree of polymerization (DP) between 6 and 16 and as hyperaccumulator plant, tomato seeds of the Amalia variety were used, germinated in pots, each with a capacity of 0,2 kg of soil. In Table I the detailed description of each of the treatments is shown.

Table I. Description of the treatments used in the experiment

Treatments	Description of the treatments	
	Soil	Ogal (kg ha^{-1})
1	normal	-
2	polluted	-
3	polluted	20
4	polluted	40
5	polluted	60
6	polluted	80
7	polluted	100

Soil application was performed at 10-15 days of emerged plants. Ten plastic containers for each treatment, with one plant each, the experiment was performed in triplicate, with a completely randomized design and irrigation was performed according to the technical standards of the culture were used. On the 40th day after emergence the plants, the roots were washed with tap water and were placed in $CaCl_2$ solution for 10 minutes, trying to remove the metals adsorbed on the walls of the roots and then washed with abundant deionized water. The root length, plant height and heavy metal content in the different organs of the plant were evaluated.

Characterization of soils used

A normal and contaminated soils were determined oregánica matter content (MO), according to the method of Walkley and Black (10) and the pH by the potentiometric method with a ratio soil: water of 1: 2.5.

[A] Cabrera, J. C. *Obtención de una mezcla de oligogalacturónidos a partir de corteza de cítrico*. Tesis de Doctorado, INCA, 2000, La Habana, Cuba, 100 p.

Methods for quantification of metallic elements

For determining the content of total copper in pseudo ground, 0,5 g samples were subjected to extraction with a mixture of HCl / HNO_3 (3: 1) (v/v) in a microwave oven.

For the determination of heavy metals in plants, 0,5 g of dry sample were taken and pulverized the aerial part and root, respectively, and added 4 mL of HNO_3 6 mol L^{-1}, extraction was performed in a microwave oven. Subsequently, the resulting extracts were analyzed by atomic absorption spectrophotometry with flame on a computer NovAA 350 with LD (mg kg^{-1}) of 0,01 for Cu.

Measuring root length and height plant

Plant height: plant length measurements were made from the root to the leaf terminal bud with the help of a graduated scale of 1 mm approach.

Root length: root length measurements from the base of the neck to the coping, with the help of a graduated scale of 1 mm approach.

The results were analyzed by analysis of variance (ANOVA) simple classification, in case of significant differences, the means were compared by Tukey test ($p < 0.05$) (11). For processing data STATGRAPHICS Plus statistical package version 5.1 for Windows (12) was used previously normal distribution (Kolmogorov-Smirnov) (13) and homogeneity of variance (14) is checked.

RESULTS AND DISCUSSION

Table II corresponding to the main agronomic parameters that influence decisively in the availability and distribution of heavy metals in the soil (15) results are shown.

By analyzing the characteristics of normal soil and artificially polluted, bioavailable copper content in the contaminated soil is 650 mg kg^{-1}, which represents a level of contamination for Cu. These values are above the ranges set by national and international literature. Furthermore, the change is remarkable presented in the magnitude of the pH when comparing normal and contaminated soil as to contaminate soil pH values change from neutral to acidic values (16).

Table II. Chemical and physical properties of soils

Indicators	Treatments	
	Natural soil	Polluted soil
Cu (mg kg^{-1})	45,54±0,02	650,28±0,05
MO (%)	19,6± 0,03	2,22± 0,04
pH	7,4± 0,1	3,6± 0,1

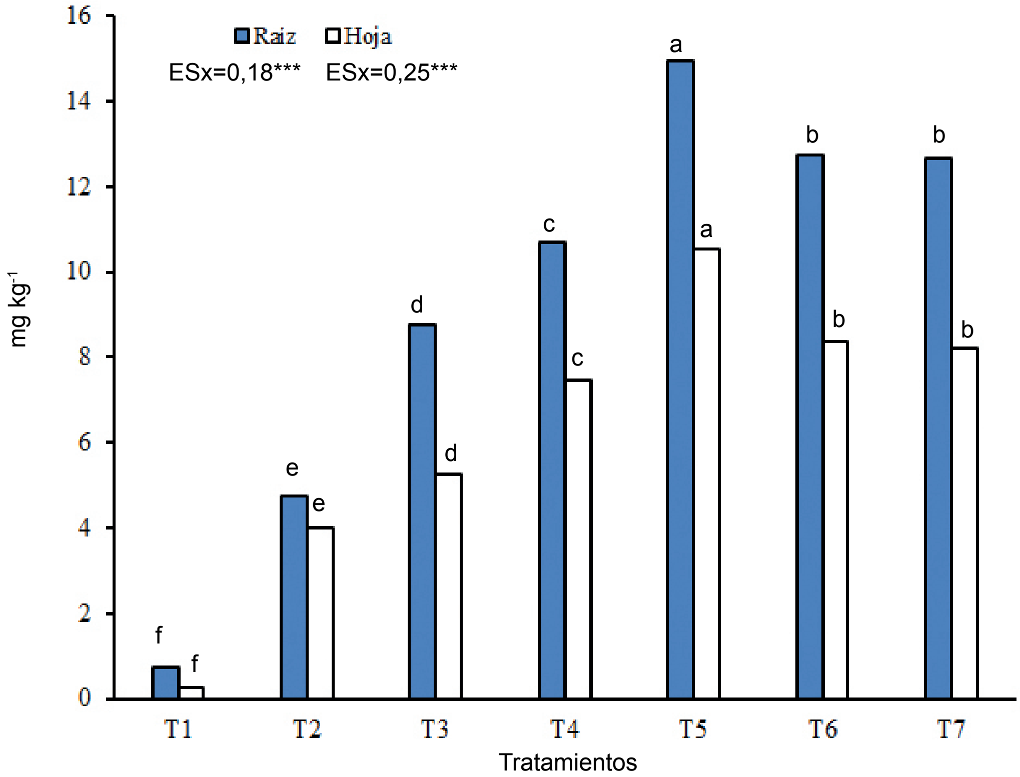
The pH of the contaminated soil is acidic values, which can significantly affect soil fertility. This aspect is very important because at pH values close to 7, macronutrients have a high mobility in soil and greater assimilation rate for plants; whereas, the adsorption of heavy by the same metals is limited, so that the low pH values of contaminated soil, favor the absorption of extremely excessive or toxic levels of these elements, a phenomenon that usually occurs in plants developed substrates with acid pH (17).

Also low values of organic matter in the soil can ensure high availability of metal ions, since the organic matter regulating the bioavailability of heavy metals by binding to the same (18).

In Figure 1 the content of copper ions in the roots and the aerial parts of the tomato plants is observed when the mixture is applied to soil Ogal. The content of copper ions in plants grown in the contaminated medium is greater than the control treatment (T1), with significant differences among them; however plants grown in the contaminated environment and they were administered the mixture of Ogal, have a higher content than non-product was applied (T2).

When analyzing the behavior of seedlings grown in the contaminated medium, with different doses of the Ogal mixture, where higher content of copper ions presented in the roots and aerial part was the treatment with a dose of 60 kg was applied has 1, showing a significant difference with other treatments.

The results showed that metals can form stable complexes with the mixture of Ogal, becoming part, over time, of an available chemical form and, if not retained by another fraction of the soil, can remain in the soil solution and it is absorbed by the roots of plants. Another crucial factor is the acid soil pH, which facilitates the exchange of protons of the carboxylic groups which are completely soluble with ions absorbed metals, which also facilitates the formation of the metal complexes (19, 20).



T1. Imbibed seeds for 4 hours in water, uncontaminated soil (Control)
T2. Imbibed seeds in water for 4 hours, contaminated soil
T3. Imbibed seeds for 4 hours in water, contaminated soil + Ogal (20 kg ha^{-1})
T4. Imbibed seeds in water for 4 hours, contaminated + Ogal (40 kg ha^{-1}) soil
T5. Imbibed seeds in water for 4 hours, contaminated + Ogal (60 kg ha^{-1}) soil
T6. Imbibed seeds in water for 4 hours, contaminated + Ogal (80 kg ha^{-1}) soil
T7. Imbibed seeds for 4 hours in water, contaminated soil + Ogal (100 kg ha^{-1})
*Different letters indicate significant differences according to Duncan p≤0,05

Figure 1. Content of copper ions in the roots and aerial parts of tomato seedlings when the Ogal mixture is applied to the soil

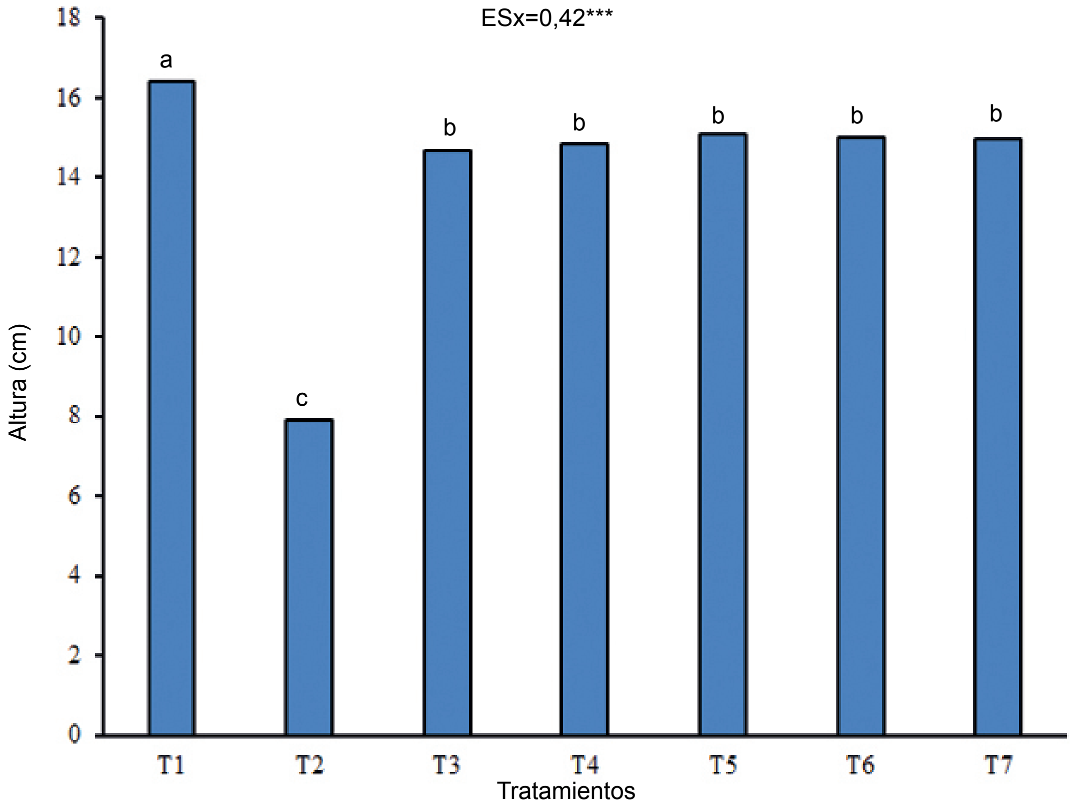
Hence the decrease in the content of these elements in the bioavailable fraction of the soil, and this is reflected in the increased content of metals in plants treated with the mixture of Ogal.

Results confirming the increase in the content of Cu, Mn and Zn in plant organs caused by the application of EDTA soil were found by Peu (21), who they reported that the application of this chelator increased the content of these metals tissues native plants grown in arid ecosystems.

When analyzing the effect of the mixture of Ogal when applied to the soil at the height of tomato plants (Figure 2) it shows that plants grown under normal conditions (T1) have a greater height relative to those grown in the medium contaminated with significant differences between them, showing the phytotoxic effect of this ion in tomato plants.

In the case of plants grown in contaminated environment, which were in the presence of a product on the floor, they have a higher growth from which grew without the application of the same, with significant differences between them. On the other hand, they plants are grown with different doses of the product in soil no significant differences between them.

These results seem to indicate that the mixture stimulates growth Ogal height also when plants are subjected to stress by a heavy metal (22).



T1. Seeds soaked for 4 hours in water, unpolluted soil (Control)
T2. Imbibed seeds in water for 4 hours, contaminated soil
T3. Imbibed seeds for 4 hours in water, contaminated soil + Ogal (20 kg ha^{-1})
T4. Imbibed seeds in water for 4 hours, contaminated + Ogal (40 kg ha^{-1}) soil
T5. Imbibed seeds in water for 4 hours, contaminated + Ogal (60 kg ha^{-1}) soil
T6. Imbibed seeds in water for 4 hours, contaminated + Ogal (80 kg ha^{-1}) soil
T7. Imbibed seeds for 4 hours in water, contaminated soil + Ogal (100 kg ha^{-1})
* Different letters indicate significant differences according to Duncan $p \leq 0{,}05$

Figura 2. Height of tomato seedlings when the Ogal mixture is applied on the ground in a polluted environment

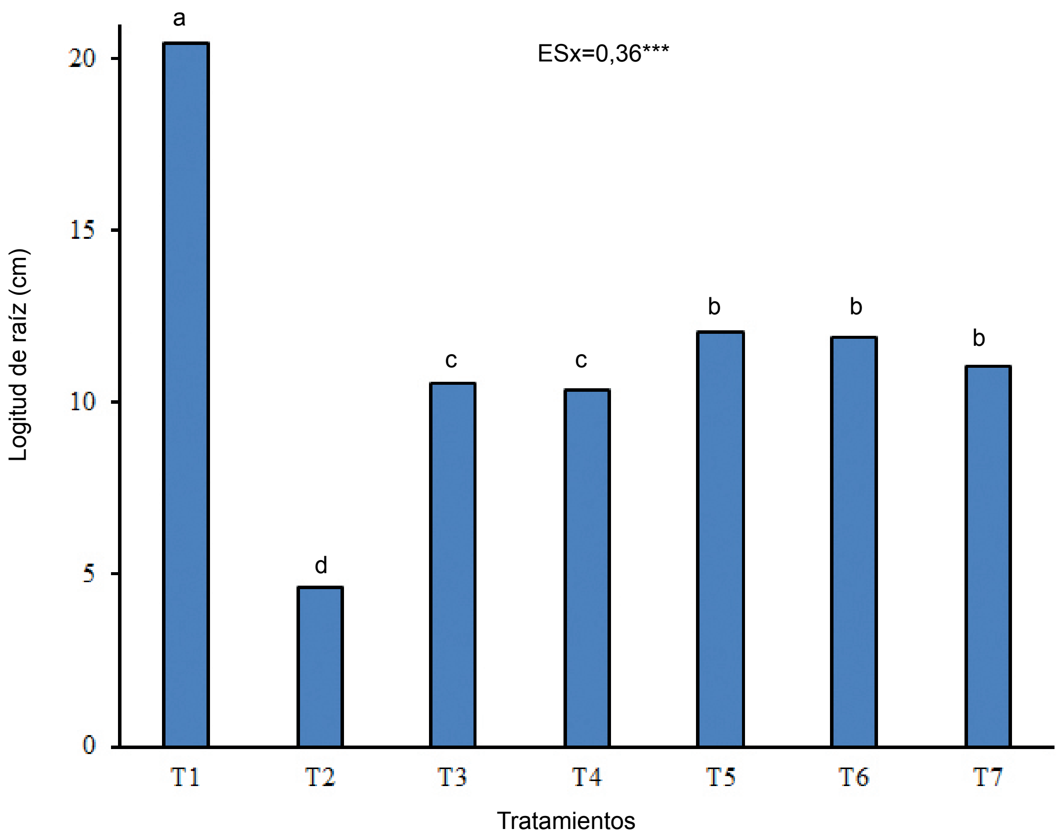
Heavy metals not only affect the growth of plants, by a significant decrease in osmotic potential of the substrate, but by its own toxicity. Excess heavy metals or their soluble chelates can induce a series of biochemical and physiological changes, including inhibition of root growth (23, 24) is. It is known that for most plant species, the root is the organ most affected by high levels of metals, with the inhibition of root growth, symptom of toxicity observed (25).

In Figure 3 the length of the root of plants shown when the mixture of Ogal to the ground, generally applies, further growth is observed in the root length of plants grown in normal medium (T1) on which they grew in a contaminated environment, showing significant differences between them.

However, when analyzing the behavior in the root length of plants grown in contaminated environment, to which we applied the product showed less length to the control plants, showing significant differences among them; but higher than those grown in the contaminated environment without product application (T2), showing significant differences among the results.

By observing plants grown in the medium contaminated with the product application to the soil, the plants were subjected to a dose of 60 kg ha^{-1} or higher, they showed better values in the root length.

This behavior suggests that the application of the Ogal mixture to 60 kg ha^{-1} or higher can attenuate the toxicity or at least stimulate elongation of the main root of the tomato plants in Amalia cultivar, subjected to a medium with high levels of heavy metals.



T1. Imbibed seeds for 4 hours in water, unpolluted soil (Control)
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T3. Imbibed seeds for 4 hours in water, contaminated soil + Ogal (20 kg ha^{-1})
T4. Imbibed seeds in water for 4 hours, contaminated + Ogal (40 kg ha^{-1}) soil
T5. Imbibed seeds in water for 4 hours, contaminated + Ogal (60 kg ha^{-1}) soil
T6. Imbibed seeds in water for 4 hours, contaminated + Ogal (80 kg ha^{-1}) soil
T7. Imbibed seeds for 4 hours in water, contaminated soil + Ogal (100 kg ha^{-1})
* Different letters indicate significant differences according to Duncan p≤0,05

Figura 3. Effect of applying Ogal down the length of the root tomato seedlings grown in a contaminated environment

Generally, it is shown that excessive amounts of metal in the soil affect adversely the growth and development of plants. Biological processes such as seed germination and vegetative growth are affected by high concentrations of metals.

As a result of the metal stress, plants can respond with a wide range of physiological molecular, cellular and organism level responses. These include, for example, changes in the development and plant morphology (inhibition of apical growth, increased root growth and changes in the life cycle), adjustment in ion transport (concentration, removal and sequestration ions) and metabolic changes (carbon metabolism and synthesis of compatible solutes).

Results from the literature show that plant roots are shortened and thickened, because of the metal ions act directly on the metabolism, interfering with the transfer of ions through cell membranes, which subsequently affects the ability of the plant for the absorption of water and nutrients from the soil (26, 27).

CONCLUSIONS

When applying the dose 60 kg ha^{-1} at ground, extraction levels of Cu^{2+} ions are achieved by seedlings complete tomato 25, 47 mg kg^{-1}, which favors the process of phytoextraction of this metal ion by hyperaccumulator plants.

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