Cultivos Tropicales, 2017, vol. 38, no. 3, pp. 142-147 july-september

ISSN impreso: 0258-5936
ISSN digital: 1819-4087

Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas
http://ediciones.inca.edu.cu

EFFECT OF FOLIAR APPLICATION OF OLIGOGALACTURONIDES TO TOMATO PLANT (*Solanum Lycopersicum* L.) IN THE PHYTOEXTRACTION OF COPPER FROM CONTAMINATED SOIL

Efecto de la aplicación foliar de oligogalacturónidos a plántulas de tomate (*Solanum Lycopersicum* L.) en la fitoextracción de cobre de suelo contaminado

Omar Cartaya[1]✉, Fernando Guridi[2], Adriano Cabrera[1], Ana M. Moreno[1] and Yenisei Hernández[1]

ABSTRACT. The oligogalacturonides mixture (Ogal), is a biostimulant obtained at National Institute of Agricultural Siences (INCA). This product has a high proportion of ionizable functional groups, which allows the formation of bonds with heavy metals. In this work, tomato seedlings with Ogal were sprinkled with 5, 19, 20, 30 mg L^{-1} concentrations, cultivated in an artificially contaminated soil, red ferralitic leachate type with 700 mg kg^{-1} copper (Cu). At 35 days of germination, were evaluations the total pseudo copper content in soil (mg kg^{-1}), pH, as well as height (cm), root length (cm) and content of Cu (mg kg^{-1}) in the different organs of the plant. The results showed that the height and length root of the plants grew in a contaminated environment without the application of product suffered a significant decrease in relation to the control; however, in which the product was applied, this decrease was not as marked as the previous ones, obtaining the best results when doses of 20 mg L^{-1} (dose 3 mL $plant^{-1}$) were applied. On the other hand, the extraction of Cu ions by tomato plant increased with the presence of the Ogal, mixture being more phytoextraction when 20 mg L^{-1} (doses 3 mL $plant^{-1}$) were applied.

Key words: bioremediation, heavy metal, oligogalacturonides, chelates

RESUMEN. La mezcla de oligogalacturónidos (Ogal), es un bioestimulante que se obtiene en el Instituto Nacional de Ciencias Agrícolas (INCA). Este producto presenta una alta proporción de grupos funcionales ácidos ionizables, lo cual les permite la formación de enlaces con los metales pesados. En este trabajo se asperjaron plántulas de tomate con Ogal en concentraciones de 5, 19, 20, 30 mg L^{-1}, cultivadas en un suelo Ferralítico Rojo Lixiviado Agrogénico contaminado artificialmente con 700 mg kg^{-1} cobre (Cu). A los 35 días de la germinación se evaluó el contenido de cobre pseudo total en el suelo (mg kg^{-1}), el pH, así como la altura (cm), la longitud de la raíz (cm) y el contenido de Cu (mg kg^{-1}) en los diferentes órganos de la planta. Los resultados muestran que la altura y la longitud de la raíz de las plantas que crecieron en un medio contaminado sin la aplicación de producto sufrieron un descenso con relación al control; sin embargo, en las que se aplicó el producto, esta disminución no fue tan marcada como las anteriores, obteniéndose los mejores resultados cuando se aplican dosis de 20 mg L^{-1} (dosis 3 mL $planta^{-1}$). Por otro lado, la extracción de iones Cu por las plántulas de tomate aumentó con la presencia de la mezcla de Ogal, siendo mayor su fitoextracción cuando se aplican dosis de 20 mg L^{-1} (dosis 3 mL $planta^{-1}$).

Palabras clave: biorremediación, metales pesados, oligogalacturónidos, quelatos

INTRODUCTION

The contamination by heavy metals is mainly due to anthropogenic actions, since the increased industrialization in the planet, without the previous assessment of sustainability criteria has resulted in the dumping towards agricultural soils and waters, of high amounts of residuals that exceed the limit tolerable by living beings and have become toxic bioaccumulables that are already part of the natural trophic chain (1).

In this sense, Cuba has not been exempt from this problem, since there are several areas where for various reasons the environmental security that

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[1] Instituto Nacional de Ciencias Agrícolas, gaveta postal 1, San José de las Lajas, Mayabeque, Cuba, CP 32 700
[2] Departamento de Química, Universidad Agraria de La Habana (UNAH), San José de las Lajas, Mayabeque, Cuba
✉ ocartaya@inca.edu.cu

is needed is not achieved. Due to its economic contribution, the industrial sector has important industries that despite being necessary from the economic point of view are sources of environmental pollution and of possible repercussions on the country's food security (2,3).

Copper (Cu) is an essential metal for the normal growth and development of plants. It is a micronutrient that participates in numerous physiological processes and an essential cofactor for many metalloproteins. However, it is also potentially toxic and problems arise when it is found in excess in the cells (4). The Cu poses serious problems due to its wide industrial use (manufacture of many products such as amalgams, enamels and pigments, reagents for tannery, algaecides, medical-surgical prostheses) and agricultural, since some applications of Cu as a fungicide, may involve exposures at high concentrations (5).

During these years, a wide range of cleaning technologies has been developed to eliminate toxic metals from water and soil. These included bioremediation as a possible future solution to many pollution problems, because they are considered an innovative technology worldwide for the treatment of toxic waste (6,7). Among its variants, is the phytoremediation (8,9), which is based on the use of plants to clean contaminated environments, phytoextraction and phytostabilisation being the most applied techniques.

In addition, growth regulators have been used in phytoremediation with plants, such as auxins and cytokinins to reduce their contamination by heavy metals; because these regulators potentially increase the growth rate and the biomass in hyperaccumulative plants (10). As well as chelating agents such as EDTA and humic and fulvic acids.

In this sense, the laboratory of Bioactive Products of the National Institute of Agricultural Sciences (INCA) obtained the mixture of oligogalacturonides (Ogal), biostimulant that has a high proportion of ionizable functional groups, which allows the formation of bonds with heavy metals (11,12). Therefore, the aim was to evaluate the effect of foliar application of a mixture of Ogal on the absorption of copper (Cu) from the soil by tomato seedlings.

MATERIALS AND METHODS

SOIL POLLUTION

Soil classified as Red Ferralitic Leachate (13) was collected in the lands belonging to the National Institute of Agricultural Sciences (INCA) and a $CuSO_4.5H_2O$ solution was prepared in an amount equivalent to 700 mg kg^{-1} (soil) of Cu, adding it to the soil seven days before planting (14,15). The contaminated and uncontaminated soil was characterized.

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

The mixture of Ogal was obtained in the INCA, according to the methodology proposed by Cabrera[A] and as a hyperaccumulative plant tomato seeds of the Amalia variety were used (16), coming from the Genetic Improvement Program of the institute, for its tolerance and hyperaccumulation of metals and generation of biomass over time which were germinated in pots (9 cm of internal diameter x 7 cm of height), with a capacity of 0, 2 kg of soil. In Table I the description of each of the treatments is shown.

Table I. Description of the treatments studied in the experiment

Treatments	Description of treatments	
	Soil	Ogal (mg L^{-1} (dose 3 mL $plant^{-1}$))
1	Normal	-
2	contaminated	-
3	contaminated	5
4	contaminated	10
5	contaminated	20
6	contaminated	30

Foliar spraying was carried out 12 days after the emergence of the plants, using a micro-sprinkler of 100 mL capacity. Ten plastic containers were used for each treatment, three seeds were planted in pots and seven days after germination the seeds were left with a plant in each one. Three repetitions of the experiment were performed, with a completely randomized design and irrigation was performed at a dose of 70 mL of water every two days.

[A] Cabrera JC. Obtención de una mezcla de oligogalacturónidos a partir de corteza de cítrico [Tesis de Doctorado]. [Mayabeque]: INCA; 2000. 100 p.

After 35 days of germination, the plants were removed and the roots were washed with running water and placed in $CaCl_2$ solution for 10 minutes, trying to remove the metals adsorbed on the walls and then washed with abundant deionized water.

The root length, the height of the plants and the content of Cu in the root and aerial part of the plant were evaluated.

MEASUREMENT OF ROOT LENGTH AND HEIGHT OF PLANTS

Height of the plant (cm): the measurements were made from the base of the stem to the bud of the terminal leaf, with the help of a graduated rule of 1 mm of precision.

Root length (cm): measurements were made from the base of the neck to the main root cap with the help of a 1 mm precision ruler.

METHODS FOR THE QUANTIFICATION OF METALLIC ELEMENTS

Characterization of the soils used

The pH was determined by the potentiometric method with a soil: water ratio of 1: 2,5.

For the determination of the pseudo total Cu content, 0,5 g of the samples were digested with 4 mL of HCl/HNO_3 (3: 1) (v/v) in a microwave oven, and the Cu concentration was determined by atomic absorption.

Cu content in plants

Both the aerial part and the root of each plant were dried in a forced air circulation oven at 70 ºC for 72 hours and weighed on an analytical balance until constant weight was obtained. Subsequently, 0,5 g of dry and milled sample was taken from the aerial part and from the root respectively with a size of 60 Mesh and 4 mL of concentrated HNO_3 were added. Digestion was done in a microwave oven. They were transferred to glass containers and diluted with 20 mL of distilled water, filtered and the amount of Cu in the supernatant was determined by means of an atomic absorption spectrophotometer in a NovAA 350 device with LD (mg kg^{-1}) of 0,01 for Cu (17).

The results were subjected to analysis of variance (ANOVA) of simple classification, in case of significant differences, the means were compared according to the Tukey test ($p < 0,05$) (18). For the processing of the data, the statistical package STATGRAPHICS Plus version 5.0 for Windows (19) was used.

RESULTS AND DISCUSSION

When analyzing the characteristics of normal soil and that which was artificially contaminated (Table II), the content of bioavailable Cu in contaminated soil was 650 mg kg^{-1}, which represents a high level of contamination for Cu according to the results obtained in another investigation when determining the content of metals in Ferralitic soils with low anthropic activity (20).

Table II. Chemical-physical properties of soils

Indicators	Treatments	
	Natural soil	Contaminated soil
Cu (mg Kg^{-1})	45,54±0,02	650,28±0,05
pH	7,4±0,1	3,6±0,1

The change presented in the magnitude of the pH is remarkable when comparing the normal and contaminated soil, since when contaminating it, the pH values change from neutral to acidic values, which can significantly influence the fertility of the soil (21).

This aspect is very important, since at pH values close to seven the macronutrients have a high mobility in the soil and a higher rate of assimilation by the plants; while, the adsorption of heavy metals by them is limited.

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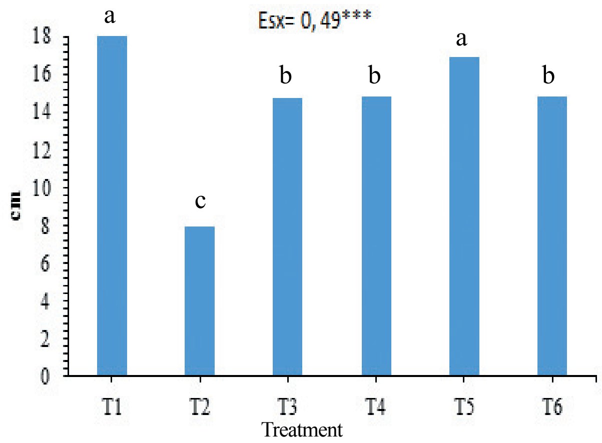
In other studies it is stated that the low pH values of contaminated soil (22) favor the absorption of extremely excessive or toxic levels of metals, a phenomenon that has been proven in teak plants (*Tectona grandis*) developed on substrates with acidic pH.

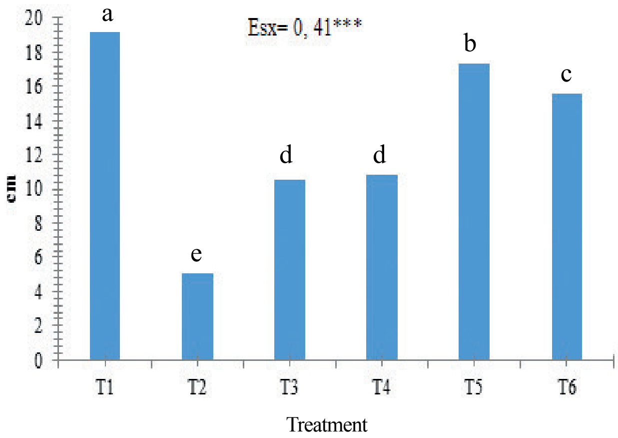
These pH values are of vital importance, because at pH values higher than 3.8 the protons of the carboxylic groups of the Ogal mixture are completely dissociated and facilitates the bond with the metal ions present in the soil, as demonstrated when studying the influence of pH on the sorption of metal ions (23).

When analyzing the height of tomato plants grown in a contaminated natural soil (Figure 1), significant differences were observed between the control plants (T1) and those that grew in the contaminated medium without the foliar application of the Ogal mixture. However, when analyzing the behavior of the plants that grew in a contaminated medium without the application of the Ogal (T2) mixture with those that grew in the contaminated medium with the application of this, they showed greater growth, which demonstrates the effect of the contamination of metallic ions on the growth in height of the plants and the attenuation of this effect as a consequence of the foliar spray of the mixture of Ogal.



T1 Seeds soaked 4 hours in water, uncontaminated soil (Control)
T2 Seeds soaked 4 hours in water, contaminated soil
T3 Seeds soaked 4 hours in water, contaminated soil foliar spray Ogal 5 mg L^{-1}
T4 Seeds soaked 4 hours in water, contaminated soil foliar spray Ogal 10 mg L^{-1}
T5 Seeds soaked 4 hours in water, contaminated soil foliar spray Ogal 20 mg L^{-1}
T6 Seeds soaked 4 hours in water, contaminated soil foliar spray Ogal 30 mg L^{-1}
* Different letters indicate significant differences according to Tukey for p≤0,05

Figure 1. Height of tomato plants grown in a contaminated soil and treated with different concentrations of the Ogal mixture



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T6 Seeds soaked 4 hours in water, contaminated soil foliar spray Ogal 30 mg L^{-1}
* Different letters indicate significant differences according to Tukey for p≤0,05

Figure 2. Root length of tomato seedlings grown in a contaminated soil and treated with different doses of the Ogal mixture

In addition, when the Ogal mixture is applied, a tendency to a higher height is observed in the plants where it was applied at a concentration of 20 mg L^{-1}, reaching significantly the same as the plants that grew in a natural soil without artificial pollution.

Figure 2 shows the effect of Cu on the length of the root of tomato seedlings treated with the Ogal mixture. In general, differences are observed in the root length of the plants that grew in a medium without contamination (T1) in relation to those that grew in contact with the Cu ion.

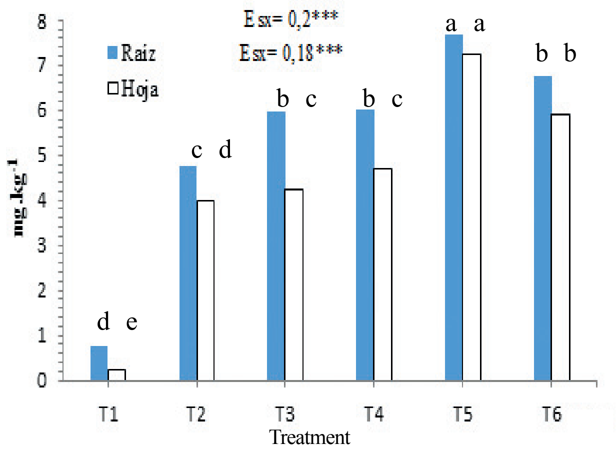
However, among them it can be seen that there was a tendency to decrease the root length in the plants that grew in contact with the Cu without previous application of these compounds (T2), presenting differences in relation to those that received treatment with the product.

The results obtained corroborate the attenuating effect of the Ogal mixture, since with all the treatments used at least the root length was doubled with respect to T2. In the case of the spray with the mixture of Ogal at a concentration of Ogal 20 mg L^{-1} (dose 3 mL plant^{-1}) the plants achieved a significantly lower root length than those that grew in the soil without contamination.

Figure 3 shows the results of the Cu ions content evaluations in the roots and leaves of the tomato plants due to the application of the foliar Ogal mixture. The application of the mixture of Ogal positively influenced the increase of the concentration of Cu in the root and in the aerial part, reaching the plants developed in the contaminated soil and treated with the product, superior contents of metals in their organs to the found in the seedlings developed in the normal soil (T1) and in the contaminated soil without the application of the product (T2).

When analyzing the content of metals in the different organs in the seedlings sprinkled with the mixture of Ogal, the highest levels were found when applied at a concentration of 20 mg L^{-1} both in the roots and in the aerial part.

These results are similar to those obtained by other researchers (24), who argue that metals are mainly stored in the root system compared to aerial parts, since it is first absorbed by the roots and then translocated to the aerial part. In another study (25), it was shown that the content of metals in various organs of a plant decreases in the following order: root> leaves> stem> inflorescences> seeds. However, this order may vary according to the plant species.



T1 Seeds soaked 4 hours in water, uncontaminated soil (Control)
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T6 Seeds soaked 4 hours in water, contaminated soil foliar spray Ogal 30 mg L^{-1}
* Different letters indicate significant differences according to Tukey for p≤0,05

Figure 3. Levels of Cu (II) ions in the roots and leaves of the tomato plants treated with different concentrations of the Ogal mixture

According to the results obtained, the application of the mixture of Ogal at a concentration of 20 mg L^{-1} (T5) has provided beneficial effects, because it favors the foliar and radicular growth of the tomato plants grown in a polluted medium due to the promotion of plant growth, and among other causes to cell lengthening and stimulation of cell division, as one of the multiple physiological effects produced in the plants by the mixture of Ogal (26). On the other hand, another study has explained that this increase in growth is due to an increase in carbon skeletons (27) which can be used for the synthesis of new compounds, so that this greater vegetative development of plants makes possible a greater removal of Cu ions, increasing their content in leaves and roots.

Obviously, the results indicate that the concentrations of heavy metals present in the soil, even when they exceed the general permissible limits, do not prove to be lethal for the plants, since no visible phytotoxic effects were evidenced, reason why it can be expressed that they have developed mechanisms of internal accumulation and detoxification, which means the neutralization of excess metal at the intra-cellular level thanks to the induction of antioxidant defenses that allow it to survive in these abiotic stress conditions, as suggested by other researchers (28).

CONCLUSION

When applying the mixture of Ogal to 20 mg L^{-1} (dose 3 mL plant^{-1}) levels of extraction of Cu ions are achieved by the complete tomato seedlings of 14,93 mg kg^{-1}, which favors the process of phytoextraction of this metallic ion by hyper accumulating plants.

RECOMMENDATIONS

The foliar application of the Ogal mixture modifies the bioavailability of metal ions in the soil, in addition tomato plants can participate in the process of phytoextraction in contaminated soils due to their tolerance and hyperaccumulation of metals and biomass formation over time; provided that an environmentally appropriate final provision is made and consumption is prevented.

BIBLIOGRAPHY

1. Chrastný V, Komárek M, Procházka J, Pechar L, Vank A, Peníek V, Farka J. 50 years of different landscape management influencing retention of metals in soils. Journal of Geochemical Exploration. 2012;115:59-68.
2. García D, Olivares S, Santana J, Lima L, Ruiz L, Calderón P, Ávila I. Evaluación de riesgos a la salud por exposición a metales pesados en cercanías de sitios potencialmente peligrosos con actividad agrícola. Revista Cubana de Salud y Trabajo. 2012;13(1):10-8.
3. Díaz O, Lima L, García D, D´Alessandro K, Torres O, Olivares S, Blanco Y. Assessment of heavy metal con¬tent in urban agricultural soils from the surrounding of steel-smelter plant using X-ray fluorescence. Nucleus. 2015;57:38-43.
4. Yruela I. Copper in plants. Brazilian Journal of Plant Physiology. 2005;17:145-56.
5. Riedel GF. Copper. In: Jørgensen SE, Fath BD, editors. Encyclopedia of Ecology [Internet]. Oxford: Academic Press; 2008 [cited 2017 May 7]. p. 778–83. Available from: http://www.sciencedirect.com/science/article/pii/B9780080454054003803
6. Barcelo J. Perpectivas actuales de la fitorremediación. Anuari Reail. Academia de Catalunya. 2003;47:13- 45.
7. Balderas L, Sánchez Y. Biorremediation of soil polluted by 75000 ppm of waste motor oil applying biostimulation and phytoremediation with Sorghum vulgare and Bacillus cereus or Burkholderia cepacia. J. Selva Andina Res. Soc. 2015;6(1):23-32.
8. Delgadillo A, González C, Prieto F, Villagómez J, Acevedo O. Phytoremediation: an alternative to eliminate pollution. Trop. subtrop. Agroecosyt. 2011;14(2):56- 62.
9. García E, García E, Juárez LF, Juárez L, Montiel J, Gómez M. La respuesta de haba (Vicia faba, L.) cul-tivada en un suelo contaminado con diferentes con¬centraciones de cadmio. Rev. Int. Contam. Ambient. 2012;28(2):119-26.
10. Navarro JP, Aquilar IA, López-Moya JR. Aspectos bioquímicos y genéticos de la tolerancia y acumu¬lación de metales pesados en plantas. Ecosistemas. 2007;4(2):1- 17.

11. Elmachliy S, Chefetz B, Tel-Or E, Vidal L, Canals A, Gedanken A. Removal of silver and lead ions from water wastes using Azolla filiculoides, an aquatic plant, which adsorbs and reduces the ions into the corresponding metallic nanoparticles under microwave radiation in 5 min. Water, Air, and Soil Pollution. 2011;218(4):365-70.
12. Ninan N, Muthiah M, Park IK, Elain A, Thomas S, Grohens Y. Pectin/ carboxymethyl cellulose/ microfibrillated cellulose composite scaffolds for tissue engineering. Carbohydrate Polymers. 2013;98:877-85.
13. Hernández JA, Pérez JJM, Bosch ID, Castro SN. Clasificación de los suelos de Cuba 2015. Mayabeque, Cuba: Ediciones INCA; 2015. 93 p.
14. Lei S, Han-qiao L, Guo-xia W, Zhen-hua W, Wei Y. Removal of Heavy Metals from Contaminated Soils by Washing with Citric Acid and Subsequent Treatment of Soil-washing Solutions. Advanced Materials Research. 2014;937:646-51.
15. Fánor P, Poveda J. La toxicidad por exceso de Mn y Zn disminuye la producción de materia seca, los pigmentos foliares y la calidad del fruto en fresa (Fragaria sp. cv. Camarosa). Agronomía Colombiana. 2005;23(2):283-9.
16. Frederick A, Ching JA. Phytoremediation Potential of Tomato (Lycopersicon Esculentum Mill). In: Artificially Contaminated Soils DLSU Research Congress. Manila, Philippines: de La Salle University; 2014.
17. Hernández HJM, Olivares SE, Villanueva FI, Ro¬dríguez FH, Vázquez AR, Pisan ZJF. Aplicación de lodos residua¬les, estiércol bovino y fertilizante químico en el cultivo de sorgo forrajero. Rev. Int. Contam. Ambie. 2005;21:31-6.
18. Tukey JW. Bias and confidence in not quite large samples. The Annals of Mathematical Statistics. 1958;29(2):614–23.
19. Statistical Graphics Crop. STATGRAPHICS® Plus [Internet]. 2000. (Profesional). Available from: http://www.statgraphics.com/statgraphics/statgraphics.nsf/pd/pdpricing.
20. Reyes R, Pierre G, Guridi F, Valdés R. Disponibilidad de metales pesados en suelos Ferralíticos con baja actividad antrópica en San José de las Lajas, Mayabeque. Revista Ciencias Técnicas Agropecuarias. 2014;23(3):37-40.
21. Muñiz O, Arozarena N, Grun M. Contenido de Cd, Pb, Cu, Zn, Ni y Cr en los principales suelos cubanos. In: I Congreso de la Sociedad Cubana de la Ciencia del Suelo. La Habana, Cuba. 1988. p. 224.
22. Moya R, Arce V, González E, Olivares C, Rios V. Efecto de las propiedades fisicas y quìmicas del suelo en algu¬nas propiedades de la madera de teca (Tectona grandis). Rev. Árvore. 2010;34(6):153-61.
23. Cartaya O, Inés R, Peniche C, Garrido ML. Empleo de polímeros naturales como alternativa para la remediación de suelos contaminados por metales pesados. Rev. Int. Contam. Ambient. 2011;27(1):41-6.
24. Sharma RK, Agrawal M, Marshall FM. Heavy metals (Cu, Cd, Zn and Pb) contamination of vegetables in Urban India: a case study in Varanasi. Environment Pollution. 2008;154:254-63.
25. Nielsen M, Bruhn A, Rasmussen M, Olesen B, Larsen M, Møller H. Cultivation of Ulva lactuca with manure for simultaneous bioremediation and biomass production. Journal Applicate Phycology. 2012;24:449-58.
26. Falcón A, Costales D, González-Peña D, Nápoles M. Nuevos productos naturales para la agricultura: Las oligo¬sacarinas. Cultivos Tropicales. 2015;36(Suppl.):111-29.
27. Izquierdo H. Evaluación de un oligogalacturónido de origen natural y ecológico en la micropropagación y pro¬ducción sostenible de Plátanos y Bananos. La Habana, Cuba: Informe Final del Proyecto del programa del MINAG: producciones ecológicas; 2008.
28. Rodríguez PH, Gincchio R, Badilla-Ohlbaum R, Allen HE, Lagos GE. Effect of soil copper content and pH on copper uptake of selected vegetables grown under controlled conditions. Environment Toxicology Chemistry. 2012;21(4):1736-44.

Received: September 7th, 2016
Accepted: March 14th, 2017

Revista Cultivos Tropicales
Instituto Nacional de Ciencias Agrícolas