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Original article

Influence of chitosan on sunflower (*Helianthus annuus* L.) plants grown with high levels of metal ions

Influencia de Quitomax® en plantas de girasol (*Helianthus annuus* L.) cultivadas en suelo con altos niveles de iones metálicos

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ABSTRACT: The purpose of this research was to evaluate the influence of chitosan on sunflower plants (*Helianthus annuus* L.) in soils contaminated with high levels of metal ions and to propose an economic, simple and natural alternative. The bioaccumulation of Lead (Pb) and Cadmium (Cd) was evaluated in the cultivated plants in a period of 60 days, for this, treatments were carried out that included the contaminated medium and the foliar application of Quitomax® at different concentrations. At the end of the evaluations, it was evidenced that the sunflower plant absorbs heavy metals, confirming that the application of Quitomax® contributes to solubilizing the Pb and Cd of the soil, in addition to contributing to a greater development of the crop.

Key words: pollution, biostimulants, cations.

RESUMEN: La presente investigación tuvo como propósito evaluar la influencia de la quitosana en plantas de girasol (*Helianthus annuus* L.), crecidas en suelos contaminados con altos niveles de iones metálicos y proponer una alternativa económica, simple y natural. Se evaluó la bioacumulación de Plomo (Pb) y Cadmio (Cd) en las plantas cultivadas en un periodo de 60 días, para ello, se realizaron tratamientos que incluían el suelo contaminado y la aplicación foliar de Quitomax®, a diferentes concentraciones. Al finalizar las evaluaciones se evidenció que la planta de girasol absorbe los metales pesados, confirmando que la aplicación de Quitomax® contribuye a solubilizar el Pb y Cd del suelo, además de contribuir en un mayor desarrollo del cultivo.

Palabras clave: contaminación, bioestimulantes, cationes.

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INTRODUCTION

Soil is a non-renewable natural resource and its regeneration is very slow, being constantly subjected to processes of destruction and degradation, being vulnerable to both natural and anthropogenic disturbances, including erosion, degradation and contamination (1).

The world scientific community pays attention to problems related to the anthropogenic influence on the change of soil properties caused by inefficient agricultural use under unsustainable conditions (2).

The environmental interest in the presence of heavy metals in agricultural soils is related to their cumulative nature, their low biodegradability, their capacity for inadvertent accumulation in the soil up to toxic concentrations, and their interaction with different soil properties that determine their mobility and bioavailability (3).

Different authors mention that it has been demonstrated that plants are effective in cleaning contaminated soils and have the capacity to accumulate heavy metals naturally in small and high quantities (4), since they act by functional requirements or defense mechanism, which is known as phytoremediation.

Recently, it has been established that there are natural substances called biostimulants, which when applied to seeds and plants can act as accelerators of cell metabolism and positively influence growth, development and protection against plant diseases, which leads to a significant increase in yield and fruit health (5).

Among the best known biostimulants is chitosan, derived from chitin, a very abundant natural polymer extracted from the exoskeleton of crustaceans. In the Department of Plant Physiology and Biochemistry of the National Institute of Agricultural Sciences (INCA), Cuba, the formulation known as Quitomax® (RFC No. 010/17) (6), whose active ingredient is chitosan, is obtained. It has been successfully used in the stimulation of yield and its components in beans and potatoes (7,8), being a compound of natural origin, biodegradable and environmentally friendly.

Taking into account the Quitomax® potential on plant growth and development, the following work was carried out to evaluate the effect of the Quitomax® application on the response of sunflower seedlings (*Helianthus annuus* L.) grown in a medium with high levels of metal ions.

MATERIALS AND METHODS

The soil, classified as Ferrallitic Red (9), was collected in areas of the National Institute of Agricultural Sciences, San José de las Lajas, Mayabeque, Cuba, and was contaminated with lead and cadmium at a concentration of 300 mg kg^{-1} and 15 mg kg^{-1}, respectively.

This experiment was developed under semi-controlled conditions in polyethylene bags of 2 kg capacity and sunflower seeds were used as a model of hyperaccumulator plant. Initially, the evaluation of the percentage of germination power of sunflower was carried out by treatment with water.

Quitomax® was obtained from the National Institute of Agricultural Sciences (INCA), according to the proposed methodology (6) and was foliar applied 10 days after the plants emerged. The treatments carried out are shown in Table 1.

In all the experiments, ten plastic containers were used for each treatment, three sunflower seeds were sown per bag, to ensure their growth, as well as to reduce the probability of losing an individual by death, during the experimental trial, with a completely randomized design, and irrigation was carried out applying 500 mL of water every two days.

The contaminated and uncontaminated soil was determined the content of exchangeable cations, from an extraction with NH_4OAc 1 mol L^{-1} at pH 7 and determination by titration with EDTA (Ca^{2+} and Mg^{2+}), flame photometry (K^+), the organic matter content was determined according to the colorimetric method of Walkley and Black and the pH by the potentiometric method with a soil:water ratio of 1:2.5 (10).

For the determination of the pseudo-total contents of heavy metals, 0.5 g of the sieved samples were used; these were digested with 4 mL of HCl/HNO_3 (3:1) (v:v), for the content of bioavailable metals, 0.25 g of sample were agitated with EDTA (0.02 mol L^{-1}) during 24 hours (11) and the concentrations in all cases were determined in an atomic absorption spectrophotometer.

Sixty days after the plants emerged, root length, plant height (from the edge of the soil, from the stem to the apex of the main stem), the dry mass of the aerial part and root (dried at 70 °C, until a constant weight was achieved), the content of heavy metals in the root and aerial part of the

Tabla 1. Aplicación de Quitomax® a plantas de girasol, a diferentes concentraciones, en un suelo contaminado

Treatments	Treatment description	
	Soil	Quitomax®
1	Control	-
2	Pb 300 mg kg^{-1}	-
3	Pb 300 mg kg^{-1}	Foliar spraying (1 g L^{-1}) (dose 10 mL x plant)
4	Pb 300 mg kg^{-1}	Foliar spraying (1.5 g L^{-1}) (dose 10 mL x plant)
5	Cd 15 mg kg^{-1}	-
6	Cd 15 mg kg^{-1}	Foliar spraying (1 g L^{-1}) (dose 10 mL x plant)
7	Cd 15 mg kg^{-1}	Foliar spraying (1.5 g L^{-1}) (dose 10 mL x plant)

plant were evaluated, as described (12), under the atomic absorption spectroscopy technique.

The results were processed using the Statistical Package for Social Sciences (SPSS), version 25.0. A simple rank analysis of variance (ANOVA) was performed to show significance differences for p≤0.05 and Duncan *post hoc* statistical test to compare means.

RESULTS AND DISCUSSION

The results in the evaluation of physicochemical parameters that influence the availability and distribution of heavy metals in the soils used are presented in Table 2.

It was evident that the main modification caused by the artificial contamination of the soil was a considerable decrease in pH, although all the magnitudes evaluated (without considering the cation contents of the heavy metals under study), had slightly higher values than those found in the uncontaminated soil, except for organic matter.

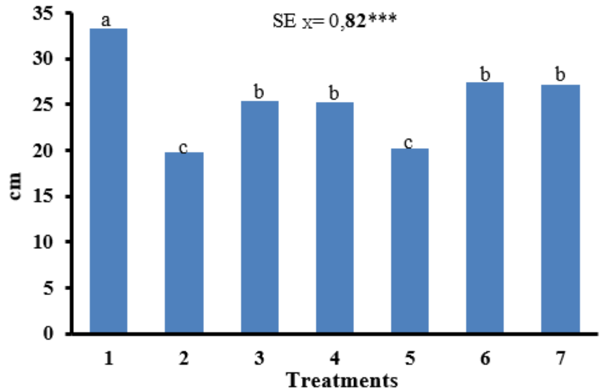
Low organic matter contents, such as those found in the soils under study, can lead to a high availability of metallic cations, given the regulatory function it has in the bioavailability of heavy metals by binding to them (13).

The change in pH can have a significant influence on soil fertility. It is known that at pH values close to 7, macronutrients have a high mobility in the soil and a higher rate of assimilation by plants, while the absorption of metallic cations is limited, especially heavy metals (14).

Other authors suggest that low pH values in contaminated soils favor the absorption of extremely excessive or toxic levels of metals, a phenomenon that has been proven in plants developed in substrates with acid pH (15).

The values found for the metal cations Pb and Cd (208.24 and 8.26 mg kg^{-1} respectively), in the soil that was artificially contaminated in this experiment, represent a considerable level of contamination, since they are much higher than those reported previously (16), when determining the available content of heavy metals in ferrallitic soils with low anthropic activity.

When evaluating the effect of foliar spraying of different concentrations of Quitomax® on the height of sunflower plants grown in contaminated soil (Figure 1), differences were recorded between all of them and the control treatment (T1). Among the plants grown in contaminated soil, the fact that the plants contaminated with different metals, where the product was applied at different concentrations, showed no differences between them and significantly outperformed the plants that did not have the product applied to them, stood out.



T1-Normal soil (Control), T2-Contaminated soil (Pb 300 mg kg^{-1}), T3-Contaminated soil (Pb 300 mg kg^{-1}) and Quitomax® foliar spray (1 g L^{-1}), T4-Contaminated soil (Pb 300 mg kg^{-1}) and Quitomax® foliar spray (1, 5 g L^{-1}), T5- Contaminated soil (Cd 15 mg kg^{-1}), T6-Contaminated soil (Cd 15 mg kg^{-1}) and Quitomax® foliar spray (1 g L^{-1}), T7- Contaminated soil (Cd 15 mg kg^{-1}) and Quitomax® foliar spray (1.5 g L^{-1}).

*Different letters indicate significant differences according to Tuckey for p≤0.05

Figure 1. Height of sunflower plants grown in a soil contaminated with Pb and Cd and treated with Quitomax®

The evaluation results of root length are shown in Figure 2. It was observed that the plants grown in the contaminated medium, where Quitomax® was applied at different concentrations, showed lower values than those of the control treatment (T1), but higher values than the plants grown in the contaminated medium without the application of Quitomax®.

It is noteworthy that with the different concentrations of Quitomax® studied, with the treatment in soil contaminated with Pb and foliar spraying of Quitomax® at 1.5 g L^{-1} (T4) plants achieved a root length that exceeded the root length of the contaminated plants, to which Quitomax® was not applied (T2, 5), but did not match the other treatments with applications of Quitomax®.

Plants grown in a medium with high levels of heavy metals reduce their growth, which has been attributed to a decrease in the biosynthesis of gibberellins and tryptophan, metabolites that influence cell division.

Table 2. Chemical and physicochemical characteristics of the soils studied

Indicators	Uncontaminated soil	Contaminated soil Pb	Contaminated soil Cd
Organic matter	3.07± 0.02	2.03± 0.03	2.03± 0.03
P (ppm)	158.4±2.2	265.6±0.2	243.8±0.2
K (cmol kg^{-1})	5.78±0.01	6.44±0.01	5.78±0.01
Ca (cmol kg^{-1})	35.63±0.12	38.43±0.12	35.63±0.12
Mg (cmol kg^{-1})	9.8±0.3	13.73±0.3	9.8±0.3
pH (H_2O)	7.2	6.3	5.3
Methal (mg kg^{-1})	85.6/4.3	208.24	8.26

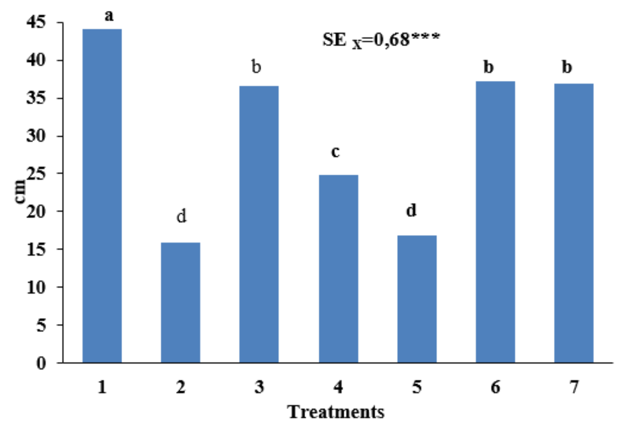


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Figure 2. Root length of sunflower plants grown in a soil contaminated with Pb and Cd and treated with Quitomax®

This behavior may be due to the growth regulating Quitomax® effect, which suggests that its application may attenuate the toxicity or at least stimulate the growth of sunflower plants subjected to a medium with high levels of heavy metals.

When analyzing the effect of the different metal cations on the dry mass of the root and aerial part of sunflower plants treated with Quitomax® (Figure 3), it became evident that the contamination of the medium affected this indicator.

It was found that none of the different concentrations of Quitomax® applied completely attenuated the effect of contamination on the dry mass of the aerial part (Figure 3 A), even though they favored this indicator in comparison with plants in contaminated medium, where it was not applied. It was also detected that the different concentrations studied did not show significant differences between them. Likewise, the best results were achieved when sprayed at a concentration of 1 g L^{-1} of Quitomax®. A similar trend was recorded for root dry mass (Figure 3 B).

Hyperaccumulator plants generally have low biomass, because they use more energy in the mechanisms necessary to adapt to the high metal concentrations in their tissues (17). This was due to the toxicity of the metals, not only from their concentration, but also from the mobility and reactivity of the compounds for metal solubility.

In summary, the positive effect caused by the application of Quitomax® on these indicators of the growth of sunflower plants, cultivated in medium with high levels of the cations Cd(II) and Pb(II), was verified, being the concentration of 1 g L^{-1}, in doses of 10 mL x plant, with which the most important results are obtained. It should also be noted that the records on sunflower development describe a period of 100 and 120 days to reach its maximum height and biomass respectively, in this experiment, the sunflower crop only lasted 60 days, to evaluate its tolerance to the accumulation of lead and cadmium in contaminated soils.

The concentrations of heavy metals used were not lethal for sunflower plants, since there were no visible phytotoxic effects, which led the plants to develop adaptation mechanisms to the high metal concentrations that allow them to survive under these conditions (18). These

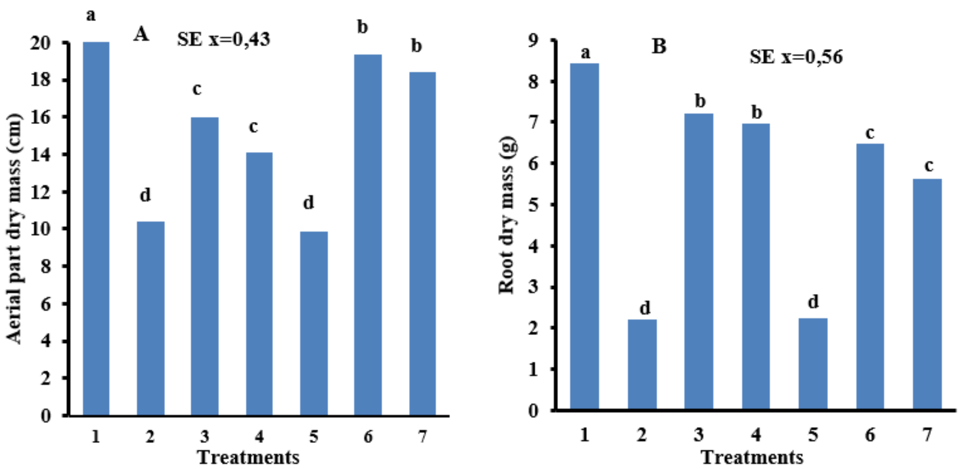

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Figure 3. Dry mass of the aerial part (A) and root (B) of sunflower plants grown in soil contaminated with Pb and Cd and treated with Quitomax®

mechanisms include: immobilization of toxic ions in the cell wall, impediment of absorption through the protoplasm layers; induction of protein stress as protection against metal toxicity; compartmentalization and formation of compounds with organic and inorganic acids (19,20).

Table 3 shows the levels of the metal cations Cd(II) and Pb(II) in the roots, leaf and stem of sunflower plants. It was evident in all treatments that the root is the organ where the highest accumulation for the two metal cations occurs.

In general, it was verified that with the application of Quitomax®, practically in all the concentrations used, there was an increase in the concentration of the cations in all parts of the plants, which showed the stimulatory effect of the application of the product.

The accumulation of toxic metals in the different organs of sunflower plants occurred in the following order: root>leaves>stem. This has also been observed in other studies (21), behavior that is attributed to the fact that some species base their resistance to metals on the strategy of efficient exclusion of the metal, restricting its transport to the aerial part (22).

Metals, in order to accumulate in the different plant organelles, must be mobilized and absorbed from the soil, sequestered in the root, and then transported in the xylem to the aerial parts of the plant. Each step requires a complex interaction of chelating compounds and specific transporters for each metal cation, which affects their rate of accumulation in plants (23,24), hence the different content of metal cations found in the different organs of sunflower plants.

In the case of lead, this metal fixed higher contents in roots, especially in those corresponding to the treatments where Quitomax® was applied. In the case of leaves and stems, it was observed that leaves concentrated more lead than stems.

Cadmium showed a similar behavior, although Cd is more mobile throughout the plant.

Although it has been suggested that high lead contents in roots are due to the formation of lead-phosphate bonds (25), which increase tissue mass, or to the retention of lead embedded in the lignin-cellulose structure, since the plant root secretes enzymes, amino acids and sugars, which stimulate the growth of microorganisms and promote a very pronounced and branched root network; However, the results of this study showed lower weights the more lead the sunflower accumulated, similar to those reported by other authors (26). On the other hand, it has been suggested that cadmium absorption is due to the transpiration of mucilage that thickens the external layer of the root and the formation of metal complexes (27), which seemed to occur in this experiment, facilitated by the discrete amounts of this contaminant present in the soil.

CONCLUSIONS

- Sunflower has phytoremediation capacity, because it manages to bioaccumulate Cd and Pb in the leaf mass for all treatments, thus reducing the concentration of heavy metal in the soil.
- Quitomax® shows potential to be used in the phytoremediation of contaminated environments, with the use of hyperaccumulator plants, since it attenuates the toxicity of the metal, stimulates its growth and development and the extraction of heavy metals

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Table 3. Concentration of heavy metals (mg kg^{-1} dry mass) in root, leaf and stem of sunflower plants treated with Quitomax® at 60 days of development

Treatments	Root	Leaf	Stem
1	6.62	33.3	33.32
2	34.32	14.39	3.79
3	127.76	25.36	5.36
4	125.25	25.25	9.29
5	1.88	1.43	0.8
6	3.8	7.2	4.22
7	3.5	6.47	3.71

T1-Normal soil (Control), T2-Contaminated soil (Pb 300 mg kg^{-1}), T3-Contaminated soil (Pb 300 mg kg^{-1}) and Quitomax® foliar spray (1 g L^{-1}), T4-Contaminated soil (Pb 300 mg kg^{-1}) and Quitomax® foliar spray (1.5 g L^{-1}), T5- Contaminated soil (Cd 15 mg kg^{-1}), T6-Contaminated soil (Cd 15 mg kg-1) and Quitomax® foliar spray (1 g L^{-1}), T7- Contaminated soil (Cd 15 mg kg^{-1}) and Quitomax® foliar spray (1.5 g L^{-1}).

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