LEAD BIOACCUMULATION BY THREE TROPICAL PLANT SPECIES

by

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ABSTRACT

Previous studies indicate that fast growing plants with high biomass could be employed for the remediation of lead (Pb) contaminated sites. Phytoextraction of Pb in a contaminated substrate was evaluated. To evaluate a possible phytotoxicity; physiological indicators such as chlorophyll content and electrolyte leakage were studied. Also, the growth, tolerance index and translocation index of *Cucurbita moschata*, *Capsicum chinense* and *Cajanus cajan* were evaluated. Finally, the effect of the chelating agent (EDTA) on Pb availability and potential translocation was examined. The metal content in the substrate was analyzed using a Flame Atomic Absorption Spectrophotometer (F-AAS) while the Pb content in vegetal tissues (root, stem, leaf and fruit) was estimated using a Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS). To the eighth week of exposure, the highest absorption of lead was observed in the treatment of 60 mg/kg of Pb with 200 mg/kg of EDTA. Root tissue samples of *C. moschata* and *C. cajan* leaves samples accumulated 24.82 and 28.94 mg of Pb /kg of dry weight, respectively. The chlorophyll content, electrolyte leakage, length of roots and stems, and biomass content were unaffected by the exposure to lead or EDTA.

RESUMEN

Estudios previos indican que plantas de crecimiento rápido y con gran biomasa pueden ser empleadas en la remediación de ambientes contaminados con plomo. La fitoextracción de Pb en un sustrato contaminado fue evaluada. Para examinar una posible fitotoxicidad; indicadores fisiológicos como contenido de clorofila y salida de electrólitos fueron estudiados. Asimismo, el crecimiento, índice de tolerancia e índice de translocación de *Cucurbita moschata*, *Capsicum chinense* y *Cajanus cajan* fueron evaluados. Finalmente, el efecto del agente quelante EDTA sobre la biodisponibilidad de plomo y su potencial translocación fue estudiado. El contenido de metal en el sustrato fue analizado usando un Espectrofotómetro de Absorción Atómica de Flama (F-AAS, por sus siglas en inglés), mientras que, el contenido de plomo en tejido vegetal (raíces, tallos, hojas y frutos) fue estimado usando un Espectrofotómetro de Absorción Atómica acoplado a un horno de grafito (GF-AAS, por sus siglas en inglés). A la octava semana de exposición la mayor absorción de plomo fue observada en el tratamiento con 60 mg/kg de Pb con 200 mg/kg de EDTA. Raíces de *C. moschata* y hojas de *C. cajan* acumularon 24.82 y 28.94 mg de plomo/kg peso seco, respectivamente. El contenido de clorofila, salida de electrólitos, longitud de raíces y tallos y el contenido de biomasa no fueron afectados por la exposición a plomo o EDTA.

Esta tesis se la dedico a:

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Por todas sus enseñanzas y apoyo incondicional
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1 INTRODUCTION

Heavy metal contamination of soils occurs due to industrial activities such as mining and smelting processes and; military, urban and agricultural activities (application of insecticides or soil amendments such as municipal sewage sludges) (Zhen-Guo et al., 2002). One common soil pollutant is lead, highly toxic to living organisms and difficult to remediate. Previous research has evaluated remediation of Pb-contaminated soils by employing chemical, physical, or biological treatments (Mulligan et al., 2001). Recently, the use of plants as bioremediators (phytoremediation) of metal-impacted soils has gained increasing importance for its low cost, avoiding harm to soil properties and producing a low bioavailability of this widespread contaminants in the soils (Klassen et al., 2000). Phytoremediation does not have the destructive impact on soil fertility and structure that some more vigorous conventional technologies have such as acid extraction and soil washing. This technology can be applied "in situ" to remediate shallow soil, ground water and surface water bodies. Also, phytoremediation has been perceived to be a more environmentally-friendly "green" and low-tech alternative to more active and intrusive remedial methods (EPA, 2001).

1.1 Statement of the Problem

Widespread heavy metal pollution in Puerto Rico has resulted in the designation of several locations in the National Priority List. For example, between 1991 and 1993, the Environmental Protection Agency (EPA) analyzed several soil and water samples in Toa Baja (Puerto Rico). Analyses showed high levels of soil contamination with barium, lead and

vanadium. In this study, EPA found concentrations of lead of 9,530 mg/kg well above the maximum permissible levels of 420 mg/kg. In 1999, EPA collected additional samples and found an average lead content of 18,735 mg/kg. In the municipality of Vega Baja, EPA found in 1998 high levels of lead, cadmium, copper, antimony and silver in soils at a depth of 2 feet (EPA, 1999).

The presence of metals in several environments of Puerto Rico, like Vieques Island and agricultural areas, demostrate the necessity to find better technologies to clean up and/or for contained contaminants. Since certain plants have a strong capacity to tolerate, accumulate and stabilize high concentrations of metals, phytoremediation has emerged as an alternative solution.

The Caribbean has been included as one of the 'hotspots' for biodiversity (Myers et al., 2000) and ranks among the top eight in the world. This region inherently possesses a promising opportunity for studying the ecology and physiology of plants to metal exposure. The results of this investigation could increase the use of plants as bioindicators in remediation processes and provide new options for cleanup strategies in Puerto Rico and other metal-contaminated sites.

1.2 Contamination with heavy metals

Geological and anthropogenic activities are sources of heavy metal contamination (Dembitsky, 2003). Volcanic activities, industrial effluents, fuel production, mining, smelting processes, military operations, utilization of agricultural chemicals, and manufacturing products release enormous amount of heavy metals into the soil, water and air (Mulligan and

Yong, 2001). Severe contamination of heavy metals in soils may cause a variety of environmental problems, including groundwater contamination and toxicity to plants, animals, and humans (Jianwei et al., 1997).

Toxic metals interfer with biochemical and homeostatic processes in the cell through the production of free radicals. The biological consequence of each metal depends on the target accumulation organ, the particular chemical pathway that disrupts the chemical form of the metal, and its oxidation state (McIntyre, 2003). Kidney dysfunction is a common effect of exposure to As, Cd, Pb, Hg and U (Schnoor, 1995) while chromosomal damage and cancer have been shown in humans exposed to Cd and Pb (Tsao, 2003). Neurological, hematological, immune effects have also been shown in humans exposed to lead (Cunningham et al., 1995).

Significant increases in the heavy metal content of cultivated soils has been observed near industrial areas. Heavy metals such as lead are easily taken up by plants from the soil and accumulated in diferent organs. Pb is considered a general protoplasmic poison, which is cumulative, slow acting and subtle. Soils contaminated with Pb cause a sharp decrease in crop productivity (Sharma and Shanker, 2005).

1.3 Lead

The natural content of Pb in soil, the least mobile among heavy metals in soil, is strongly related to the composition of the bedrock. Although, the Pb species can vary from one soil type to another, this element is associated mainly with clay minerals, Mn oxides, Fe or Al hydroxides, organic matter, and calcium carbonate or phosphate particles. The level of

Pb in soils exposed to various pollution sources can reach as high as 2% of dry soil material, which represents a dangerous source of exposure to humans through the food chain or dust inhalation (Kabata-Pendias, 2001).

On the contrary, the lethal dose of lead to plants is difficult to define; nonetheless several authors have suggested that toxic concentrations in plant tissue range between 30 and 300 mg/kg. In addition, the solubility of lead in soil can be affected by high pH values (forming precipitates and complexes) and lower temperatures (Kabata-Pendias, 2001; Sahi et al., 2002).

1.4 Phytoremediation

Among the several remediation technologies, phytoremediation is an economical alternative (Cunningham et al., 1995). Selected plants can be used to extract metals from soil, water and sediments, including the removal of radioactive elements, and the possible mineralization of toxic organic compounds (Tsao, 2003). Phytoremediation has been succesfully employed to restore soil contaminated with metals, chlorinated solvents, hydrocarbons, polycyclic aromatic hydrocarbons, polychlorinated biphenyl, chlorinated pesticides, explosives and surfactants (Macek et al., 2000). There are different phytoremediation techniques such as phytodegradation, phytovolatilization, rhizodegradation, phytostabilization, rhizofiltration and phytoextraction.

Phytodegradation is the uptake, metabolism and degradation of contaminants within the plant, or the degradation of contaminants in the soil, sediments, sludges, groundwater by enzymes produced and released by the plant. Some compounds subjected to

phytodegradation are: organic compounds such as trinitrotoluene, trichloroethylene, and herbicides such as atrazine and bentazon (EPA, 2001).

Phytovolatilization is the uptake of contaminants (organic contaminants such as trichloroethylene, toluene, ethylbenzene, and inorganic contaminants such as mercury, selenium) by the plant, and subsequent release of volatile contaminants, volatile degradation products, or volatile forms of initially non-volatile contaminants. This technology can be applied in the removal of contaminants in ground water, soils, sediments and sludges (EPA, 2001).

In rhizodegradation, indigenous microbiota is estimulated by the plant root system to degrade organics directly in the soil. This technology is effective for compounds such as petroleum hydrocarbons, chlorinated solvents, pesticides, polychlorinated biphenyls (PCBs) and surfactants (EPA, 2001).

Phytostabilization is the use of plants to stabilize contaminants through chemical, biological, and physical modification directly (“in situ”) in the soil. In addition, plants can reduce wind and water erosion of the soil, thus preventing dispersal of the contaminant in runoff or fugitive dust emissions, and may reduce or prevent leachate generation. The mobility of contaminants (e.g. lead, chromium, mercury, copper or zinc) is reduced by the accumulation of contaminants by plant roots, absorption onto roots, or precipitation within the root zone (EPA, 2001).

Rhizofiltration is the removal by plant roots of contaminants in surface water, waste waters or extracted groundwater, through adsorption or precipitation onto the roots, or absorption into the roots. This technology is applied to metals such as Pb, Cd, Cu, Fe, Ni, Mn, Zn, Cr and radionuclides (EPA, 2001).

A different phytoremediation approach is known as phytoextraction. Phytoextraction, also known as phytoacumulation, phytoabsortion or phytosequestration, was developed specifically for inorganics pollutants such as metals (Ag, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Zn), metalloids (As, Se), radionuclides (^{90}Sr, ^{137}Cs, ^{243}U, ^{238}U) and nonmetals (Schnoor, 1995). In this process, the removal of contaminants is achieved through the root network and the accumulation potential into the plant biomass. After sequestration, the biomass is harvested to complete the extraction of contaminants from the environment (McIntyre, 2003).

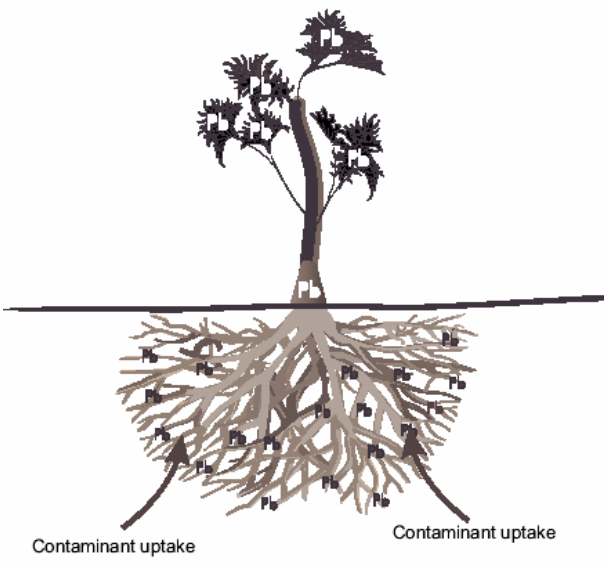


Figure 1.4 Schematic representation of the phytoextraction process (EPA, 2000)

The efficiency of phytoremediation is dependent on the morphology and depth of the roots, growth rate, evapotranspiration rate, assimilation and metabolism of contaminant, production of exudates and detoxifying enzymes (Karthikeyan and Kulakow 2003; Schnoor, 1995).

The uptake of metals by plants is regulated by specific abilities of the plant and soil factors such as the pH, water regime, organic matter content, cation exchange capacity, nutrient balance, clay content and concentration of other trace metals. Climatic conditions will also influence the metal uptake rate. For example, high uptake of metals has been found in environments with temperatures that promote higher growth and evapotranspiration rates. Mechanisms of uptake differ among plants and metal type. Elements such as Pb and Ni are preferably absorbed passively (transport of metals from regions of greater concentrations to regions of lesser concentration), while Cu, Mo and Zn are preferably absorbed actively (transport of metals against a concentrations gradient) (Kabata-Pendias, 2001; Sahi et al., 2002; Schnoor and McCutcheon, 2003).

1.5 Plants accumulate lead

The success of phytoremediation depends upon the identification of plant species that tolerate stress, accumulate heavy metals and produce large amounts of biomass. In general, plants which accumulate more than 1000 mg/kg Pb, are called hyperaccumulators. An example of such plants is *Thlaspi rotundifolium*, which can accumulate Pb in shoots upto 8,200 mg/kg. However, this species is not suited for phytoremediation of Pb in contaminated soils due to its slow growth rate and small biomass production (Hong-Yun et al., 2005).

The Environmental Biotechnology Application Division of Environment of Canada has compiled a database of terrestrial and aquatic plants with potential value for phytoremediating metal contaminated sites. The most important aquatic plants that accumulate lead are: *Azolla filiculoides*, *Bacopa monnieri*, *Eichhornia crassipes*, *Lemna minor*, *Salvinia molesta*, *Spirodela polyrrhiza* and *Vallisneria americana*. On the other hand, the terrestrial plants are represented by: *Brassica juncea*, *Helianthus annuus*, *Agrostis castellana*, *Thlaspi caerulescens* and *Athyrium yokoscense* (McIntyre, 2003).

There are few studies on the use of plants to remediate heavy metals in tropical environments. Unfortunately, most of the known metal-accumulating plants have a slow growth rate while weedy plants produce low biomass. Some species have undefined growth requirements and characteristics that make their field application difficult. Therefore, it is necessary to identify crop and crop-related species that can tolerate, accumulate heavy metals and produce high biomass in short periods of time. Particular emphasis was placed on the crop-related members of the *Cucurbitaceae*, *Fabaceae* and *Solanaceae*.

Fast growing plants were chosen for this project: (i) *Cucurbita moschata*, commonly used as food and medicine (frequently called as butternut squash, calabaza pumpkin, cheese pumpkin, golden cushaw, pumpkin and squash), (ii) *Cajanus cajan*, used as food, soil improver and medicine (commonly called Congo-pea, pigeon-pea, guandúl and guisante-de-Angola), and (iii) *Capsicum chinense*, used as food condiment and medicine (called bonnet pepper, datil pepper, habanero pepper and squash pepper).

1.6 Objectives

The present investigation was focused on *Capsicum chinense, Cucurbita moschata* and *Cajanus cajan as* potential plants to phytoextract lead from an artificial Pb amended substrate.

The objectives of this investigation were:

- To evaluate the chlorophyll content in leaves, as an indicator of inhibition of photosynthesis.
- To evaluate Pb-induced stress to the cell membrane using the percentage of electrolyte leakage method.
- To quantify metal removal from Pb-amended substrates.
- To evaluate the lead accumulation capacity of roots, stems and leaves in *Cucurbita moschata* and *Cajanus cajan* and also in fruits of *Capsicum chinense*.
- To evaluate the benefits of chelanting agents such as EDTA on the removal of lead.

2 PREVIOUS WORKS

Many studies have used plants to remove Pb in soil and water including the use of chelate agents to improve the metal bioavailability to the plants (Wu et al., 1999). However, there is little information about using *Cucurbita moschata*, *Cajanus cajan* and *Capsicum chinense* for potential phytoremediation processes.

Recent studies with *Sesbania drummondii* in a hydroponic medium containing 1,000 ppm of lead nitrate demonstrated a higher accumulation of lead at pH 3.7 rather than at pH 7.7 (Sahi et al., 2002).

Axtell et al. (2003) studied the ability of *Microspora* (a macro-alga) to remove soluble lead and *Lemna minor* (an aquatic plant) to remove soluble lead and nickel under various laboratory conditions. *Microspora* was tested in a batch and semi-batch process and *Lemna minor* was only tested in a batch process. *Microspora* was exposed to 39.4 mg/ml of lead over 10 days. Results indicate that 97 and 95% of total lead was removed by *Microspora* in the batch and semi-batch process, respectively. *Lemna minor* was exposed to lead concentrations of 0.0, 5.0, and 10.0 mg/ml and nickel concentrations of 0.0, 2.5, and 5.0 mg/ml in the experiment. *Lemna minor* removed only 76% of the lead and 82% of the nickel. No synergistic/antagonistic effects were noted in terms of metal removal.

Qu et al., (2003) evaluated root uptake of lead in four turfgrasses. The plants were: *Eremochloa ophiuroides*, *Buchlöe dactyloides*, *Festuca arundinacea* and *Spartina patens*.

Grasses were grown hydroponically in solutions containing from 0 to 450 mg/L total Pb. The maximum Pb accumulation in roots of the four species was in the range of 20 mg/g dry root weight. *Festuca arundinacea* and *Spartina patens* survived at 450 mg/L Pb solution without showing obvious damage while *Eremochloa ophiuroides* and *Buchlöe dactyloides* deteriorated or died at this concentration.

Previous studies indicate that plants of the *Cucurbitaceae* family can uptake persistent organic soil contaminant, such as DDE (*p,p*-dichlorodiphenyldichloroethylene). The uptake of DDE depends on particular characteristics of the plant species and subspecies. In an environment contaminated with DDE, *Cucurbita pepo* var. *pepo* extracted 0.31% of the DDE present and *C. pepo* var. *texana* extracted 0.065%. The increased uptake of DDE in *Cucurbita pepo* var. *pepo* was the result of low molecular weight organic acid exudation (White et al., 2003)

Wang and coworkers (2004) investigated the uptake and translocation of DDE between two members of the *Cucurbitaceae* family: *Cucurbita pepo* and *Cucumis sativus*. Each species was grown under three cultivation regimes: dense (five plants in 5 kg of soil); nondense (one plant in 80 kg soil); and field conditions (two to three plants in approximately 789 kg soil). Under field conditions, *Cucurbita pepo* phytoextracted 1.3% of the *p,p´*-DDE with 98% of the contaminant in the aerial tissues while *Cucumis sativus* phytoextracted 0.09% with 83% of the contaminant in the aerial tissues. Under dense cultivation, *Cucurbita pepo* removed only 0.59% with 48% in the aerial tissues. *Cucumis sativus* removed 0.78% with 5.7% in aerial plant biomass.

Sarret et al. (2001) studied the internalized speciation of Zn and Pb in roots and leaves of *Phaseolus vulgaris* (*Fabaceae*). The plants were grown in zinc sulfate, zinc EDTA (ethylenediaminetetraacetic acid), lead nitrate, and lead EDTA solutions. Zn was predominantly present as Zn phosphate dihydrate in the roots and leaves when plants were grown in zinc sulfate and zinc EDTA. Pb was predominantly found in the leaves as cerussite (lead carbonate) when the plant was grown in Pb nitrate solution and as a mixture of Pb-EDTA and an undetermined species in contact with Pb-EDTA solution. *Phaseolus vulgaris* dissociated metal ions totally (Zn) or partly (Pb) from the two metal-EDTA complexes in nutrient solution and changed these metals into other forms.

Steinborn and Breen (1999) examined heavy metal levels in soils, higher plant species (*Teucrium scorodonia*, *Primula vulgaris*, and *Succisa pratensis*) and moss species (*Hylocomium splendens* and *Rhytidiadelphus loreus*) sampled from the area surrounding the Shallee mine (Ireland). The samples were analysed for lead, copper and zinc, using flame atomic absorption spectrophotometry. Results indicated high lead levels in all soil and moss samples, but copper and zinc levels were similar to levels found in uncontaminated areas.

Youngman et al. (1998) evaluated the plant potential to remediate soils contaminated with zinc, lead and cadmium. Two soil types were vegetated by means of a grass/legume seeding mixture. Herbaceous shoot and root material was analyzed for zinc, lead and cadmium by ICP spectrometry. Their results demonstrated for both substrate types significant accumulation of all three metals in the root system with relatively low concentrations being

partitioned to the shoots. Partitioning between shoots and roots for the three metals at both soil sites averaged 1:5 (shoot: root).

The influence of growth conditions in manipulated floating row covers for Zn, Cu, Cd and Pb removal by *Brassica pekinensis* was examined by Moreno and coworkers (2002). The treatments were: (i) an uncovered cultivation, (ii) a perforated polyethylene [50 *u*m thick] and (iii) a 17g m^{-2} polypropylene nonwoven fleece. The influence of environmental factors manipulated by floating row covers (particularly under uncovered cultivation) increased total heavy metal accumulation in aboveground plant components with respect to the open-air crop. The total content of Zn, Cd, Cu, and Pb was 30, 50, 90, and 40% higher in perforated polyethylene than uncovered cultivation.

Differences in root-zone temperatures and accumulation of metals were examined by Baghour et al., (2001). Plastic mulches affected the concentrations and phytoaccumulation capacity of Cd and Pb in different organs of *Solanum tuberosum*. Physiological and biochemical indicators were altered during these conditions. The treatments were: T_0 (an uncovered cultivation), T_1 (transparent polyethylene), T_2 (white polyethylene), T_3 (white and black polyethylene) and T_4 (black polyethylene). The different treatments had a significant effect on the mean root-zone temperatures (T_0 = 16°C, T_1 = 20°C, T_2 = 23°C, T_3 = 27°C, T_4 = 30°C), and different responses in the accumulation of Cd and Pb. A high accumulation of Cd was observed in the roots and lower levels in other organs, in the treatments T_2 and T_3; and, higher levels in the tubers and lower levels in roots, stems and leaves were observed in T_2 and T_3 for Pb. On the other hand, high levels of peroxidase and catalase activity were

obtained in the white and black polyethylene treatment. Lower concentrations of chlorophyll were found in treatments with higher levels of Cd and Pb.

Begonia et al. (2005) studied the effectiveness of *Festuca arundinacea* (tall fescue) as a phytoextraction species. This experiment was conducted to determine whether pre-harvest amendments of EDTA alone or in combination with acetic acid could further enhance the shoot accumulation (translocation index) of Pb by tall fescue grown on a Pb-contaminated soil. Results revealed relative tolerance of tall fescue to moderate levels of Pb as determined by non-significant differences of root and shoot biomass. Translocation index, which is a measurement of metal partitioning to the shoots, was significantly enhanced with chelating agent addition, especially when EDTA and acetic acid were used in combination.

In order to increase bioavailability and accumulation potencial of plants at Pb-contaminated sites, Huang et al. (1997) examined amendments with chelating agents. The order of effectiveness defined as increased Pb-desorption from soil was EDTA > HEDTA (hydroxyethylenediaminetriacetic acid) > DTPA (diethylenetriaminepentaacetic acid) > EGTA (ethylene glycol tetraacetic acid) > EDDHA (ethylenediaminedi (*o*-hydroxyphenylacetic) acid). The addition of chelating agents in Pb-contaminated soil (total soil Pb concentration of 2,500 mg/kg) increased twenty-fold the translocation of lead from roots to shoots in *Zea mays* and *Pisum sativum*.

Zhen-Guo et al. (2002) conducted studies to evaluate various chelating agents in Pb removal by *Brassica rapa*. The chelate agents were: EDTA, DTPA, HEDTA, NTA (nitrilotriacetic acid) and citric acid. EDTA was the best to solubilize soil-bound Pb and

enhance Pb accumulation in the shoots and roots. Lead concentrations in the shoots reached 5,010 mg/kg dry matter after 7 days of exposure and 4,620 mg/kg 14 after application of EDTA.

In 2003, Barocsi and colleagues conducted several tests with multiple doses of EDTA to increase plant tolerance and allowing phytoextraction of high levels of Pb and Cd in *Brassica juncea*. Instead of a single dose treatment, the chelate agent was applied in several small increments, thus providing time for plants to initiate their adaptation mechanisms. The final accumulated Pb amount was doubled with multiple dose treatments, which translates to a 50% reduction in cleanup time.

Preliminary studies with *Capsicum chinense*, *Cucurbita moschata* and *Cajanus cajan* in hydroponic medium contaminated with lead (1 mg/kg and 10 mg/kg) showed metal uptake in roots, shoots and leaves. Massol and Díaz (2001) surveyed vegetation from civilian areas on the Island of Vieques when live impact practices by the US Navy were taking place. They found lead and cadmium levels above European Standards in all three species. Their findings, however, suggest primary uptake of metals from dust deposition (Massol et al., 2005).

3 EXPERIMENTAL SETUP

3.1 Biological material

Three plant species of agricultural value were chosen for this project: *Cucurbita moschata*, C*ajanus cajan* and *Capsicum chinense* (Figure 3.3). Seeds for *Cucurbita moschata*, *Cajanus cajan*, and small plants of *Capsicum chinense* (approximately two months old) grown in commercial sustrate JIFFY-MIX were provided by the UPR Experiment Station in Lajas, the Horticulture Department of the UPRM and a commercial location (Jardines Eneida, Cabo Rojo), respectively.

(a)

(b)

(c)

Figure 3.1 Photograph of one-week old of *Cucurbita moschata* (a) *Cajanus cajan* (b), and 8-week old of *Capsicum chinense* (c) grown at the UPR Experiment Station facilities in Lajas.

3.2 Techniques and procedures

3.2.1 Germination and transplanting

Seeds of *Cucurbita moschata* and *Cajanus cajan* were germinated in a commercial sustrate called JIFFY-MIX. This substrate was composed of sphagnum peat moss, horticultural perlite and lime. Seedlings of *Capsicum chinense* were obtained from a commercial nursery of approximately two months old. After seven weeks, a total of 36 seedlings with similar characteristics of height and development within each species were selected and transplanted to one-gallon pots.

Each pot contained sphagnum peat moss, horticultural perlite and vermiculite in the proportion 1:1:1, respectively (50 grams of each ingredient per pot). The macronutrients were added in a fertilizer mix Maxigro 14-14-14. While micronutrients were supplied as Nutrileaf fertilizer every two weeks.

3.2.2 Experimental conditions

All species were cultivated in a shade house covered with polyethylene and watered three times per day using a drip irrigation system (spaghetti-type). The irrigation time was two minutes with a flow of approximately 2.0 L per hr in each pot (Figure 3.4). These conditions were established considering the water pressure of the area, and to avoid leaching of metals and nutrients from the pots.

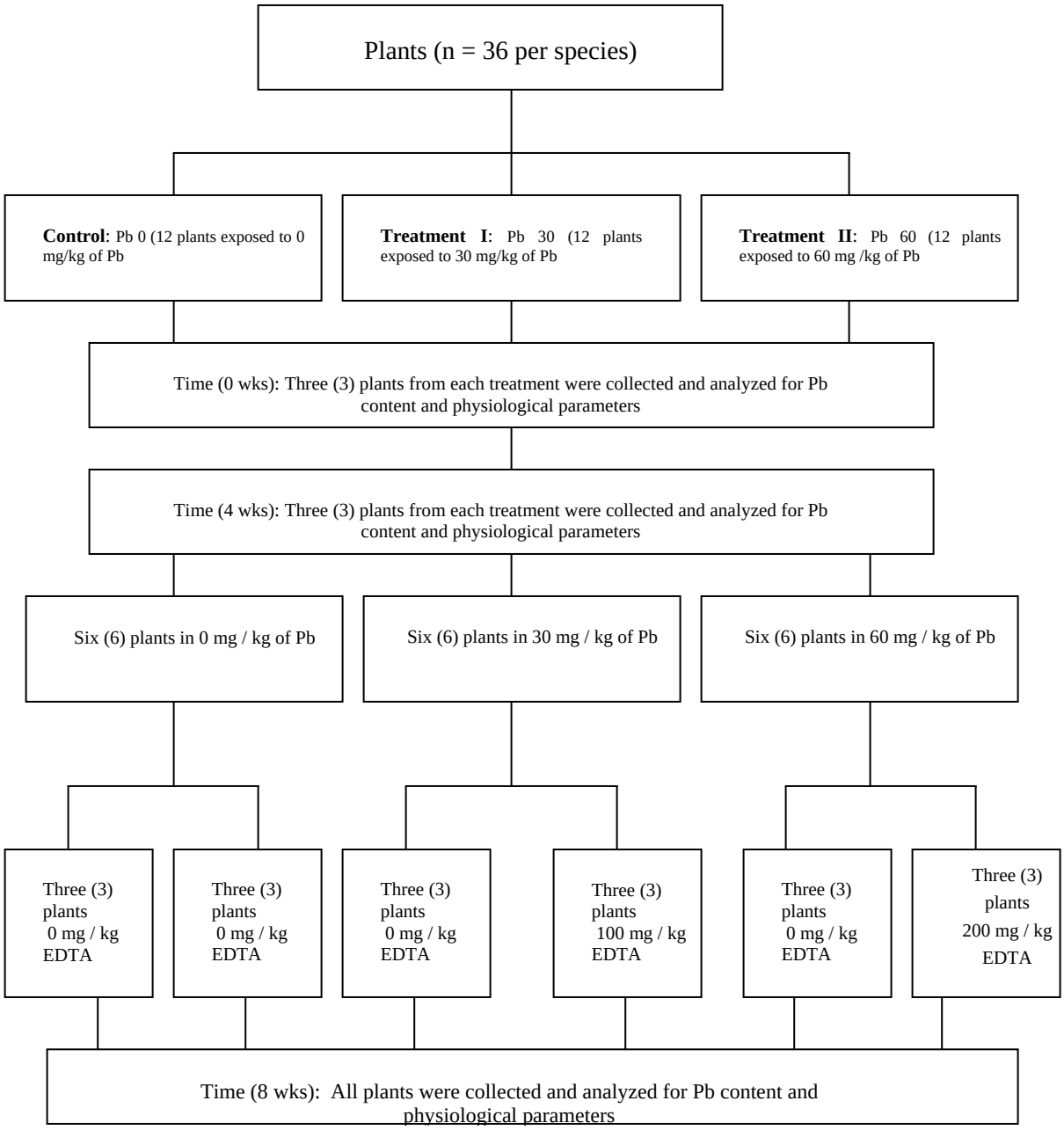
(a)

(b)

Figure 3.2 Emitter irrigation system used in this project working at a flow rate of 2.0 L per hr (a) and irrigation system (b).

3.2.3 Substrate treatments

The influence of Pb and EDTA on *Capsicum chinense*, *Cucurbita moschata* and *Cajanus cajan* was examined using the following substrate amendment and control treatments:



Two aliquots of 15 ml of lead nitrate solution were added to the pots in order to obtain contaminated substrate with 30 mg/kg and 60 mg/kg of lead, respectively. In the treatments with EDTA, two aliquots of 15 mL of EDTA were added to the pots in order to obtain contaminated substrate with 100 mg/kg and 200 mg/kg of EDTA, respectively.

3.3 Measure of biomass, length of root and stem

The plants collected were rinsed with tap water, air dried and weighed individually (grams of fresh weight). The length of roots and stems were measured in centimeters after 0, 4 and 8 weeks of treatment.

3.4 Tolerance test and metal translocation

The metal tolerance index of the plants was calculated as the quotient between the mean relative growth rates (R_1) with lead and without lead (R_2). The means relative growth rates were calculated using the fresh weight of the plant (grams).

The following equation was used to calculate the relative growth rates:

$$R = \frac{(\ln W_2 - \ln W_1)}{t_2 - t_1}$$

Where,

W = fresh weight of whole plant (g), W_1 at start and W_2 at the end of treatment period (4 and 8 weeks).

t = time (4 or 8 weeks of exposure)

R = growth rate

The translocation index was calculated using the following equation:

$$\frac{(Stem + leaf\ Pb\ accumulation)}{(Stem + leaf + root\ Pb\ accumulation)} * 100$$

3.5 Quantification of chlorophyll

3.5.1 Preparation of standard solutions and calibration curve

Standard solutions of 0.1, 0.5, 1.0, 3.0, 5.0 and 10.0 µg/ml of chlorophyll a and b (Sigma-Aldrich Cat.# C5753 and Cat.# C5878, respectively) were prepared using spectrophotometric grade acetone in a dark environment to avoid chorophyll damage by light exposure. All standard curves showed a correlation coefficient of $R^2 > 0.99$.

3.5.2 Spectrophotometry UV-VIS measurement

The chlorophyll content was determinated at 0, 4 and 8 weeks of treatment. Fresh leaves (0.5 g) were extracted with 10 ml of 80% acetone as described by Alan (1994). The absorbance of the extract was measurement at 663 nm and 645 nm in the UV- VIS light spectrophotometer model DR/4000U.

Concentrations of chlorophyll a and b (mg/L) were calculated using a linear regression curve from the calibration standards. Concentrations of chlorophyll a and b were expressed in mg/g fresh weight of plant.

$$y = mx + b$$

Where,

y = absorbance values

m = slope

b = intercept

x = concentration of chlorophyll (mg / L) calculated.

$$Chlorophyll\ concentration\ (mg/g) = \frac{reading\ of\ \ Spectrophotometer\,(mg/L)*0.010L}{fresh\ weight\ of\ \ the\ sample\,(g)}$$

Reading of Spectrophotometer (mg/L) = reading of chlorophyll a or b in the sample

0.010 L = 10 mL = total volume of the sample

3.6 Measurement of electrolyte leakage

The level of electrolyte leakage was estimated according to Sullivan and Ross (1979). Fresh leaves (0.1 g) were cut into disks (1 cm of diameter) and placed in a test tube containing 10 ml of deionized water. Immediately the electrical conductivity (ECa) was measured using a conductimeter (YSI model 30). The tubes were covered with plastic caps and placed in a water bath at a temperature of 45 to 55 °C for 30 minutes. After incubation, a second electrical conductivity measurement was obtained (ECb). Finally, the tubes were incubated at 100 °C for 10 minutes before the final electrical conductivity (ECc) measurement was taken. The electrolyte leakage was expressed using the following formula:

$$Electrolyte\ leakage\ (\%) = \frac{ECb - ECa}{ECc} * 100$$

Where,

ECa = electrical conductivity when the leaves were treated to ambient temperature.

ECb = electrical conductivity when the leaves were treated at 45–55 °C.

ECc = electrical conductivity when the leaves were treated at 100 °C.

3.7 Total Pb content in the growing medium

Atomic absortion-Flame (F-AA) was used to quantify the lead content in the substrate. Atomic absortion-Graphite furnace (GF-AA) with lower detection limits than F-AA was used to quantify lead levels in plant tissue samples (roots, stems, leaves and fruits).

Figure 3.3 Flame Atomic Absorption Spectrophotometer.

3.7.1 Preparation of standard solutions

Standard solutions of 0.2, 0.5, 1.0, 5.0 and 10.0 ppm of lead and blanks in 10% HCl were prepared using ACS grade hydrochloric acid and stored in plastic bottles at room temperature.

3.7.2 Detection and quantification limits

The detection (D.L) and quantification (Q.L) limits for all samples were determinated in a Flame Atomic Absorption Spectrophotometer, using the standard solutions and the following equation:

(1) $D.L. = \frac{3\ Sb}{m}$

(2) $Q.L. = \frac{10\ Sb}{m}$

Sb = Standard deviation of intercept

m = average of slope

3.7.3 Metal extraction protocol

Growing medium samples were collected (soil from each pot was manually homogeneized and one central portion was taken) and pulverized in a ceramic mortar. Approximately 0.5 g of wet material was weighed in a porcelain dish and oven-dried at 105°C for 24 hrs. Dry weight was calculated. The dried samples were placed in a muffle at 650°C for 2 hrs. Then, the samples were dissolved in 3 ml of concentrated HCl in a 600 ml beaker. The porcelain dishes were rinsed with 1 ml of concentrated HNO_3 and three times with 15 ml of 10% HCl. Each sample was covered with a watch glass and heated for a few

minutes at a moderate temperature on a hot plate. Hydrochloric acid (10%) was added as needed to avoid sample drying. Samples were cooled at room temperature. Immediately, 1 ml of HNO_3 and 3 ml of HCl were added. Samples were heated for a few minutes and finally cooled and filtered into a 100 ml volumetric flask. Final volume was adjusted with 10% HCl.

3.7.4 F-AAS measurement and quality control

Lead was determinated using Flame Atomic Absorption Spectrophotometry. Acetylene was used as fuel with a hollow cathode lamp as a radiation source. The following parameters were used: wavelength 283.3 nm, lamp current 8mA, slit 0.7nm, fuel C_2H_2 2.5 $L.min^{-1}$, oxidant air 9.4 $L.min^{-1}$, equation type linear-intercept calculated. The standard curve was determinated using five standards with observed correlation coefficients not less than 0.99. A test of interference was performed once every ten samples using spikes (Appendix H). Analyses were conducted in triplicate.

3.7.5 Estimation of Pb content

Soil Pb concentration was calculated using the following the equation:

$$Pb\ concentration\ (mg/kg) = \frac{reading\ of\ \ F\ (mg/L) * 0.10L}{dry\ weight\ of\ \ the\ sample\ (g)}$$

Where,

Reading of F (mg/L) = reading of Pb in the substrate

0.10 L = total volume of the sample

Dry weight of the sample (g) = (weight of dried total sample at 105°C) – (weight of porcelain dish).

3.8 Quantification of lead in plants

(a)

(b)

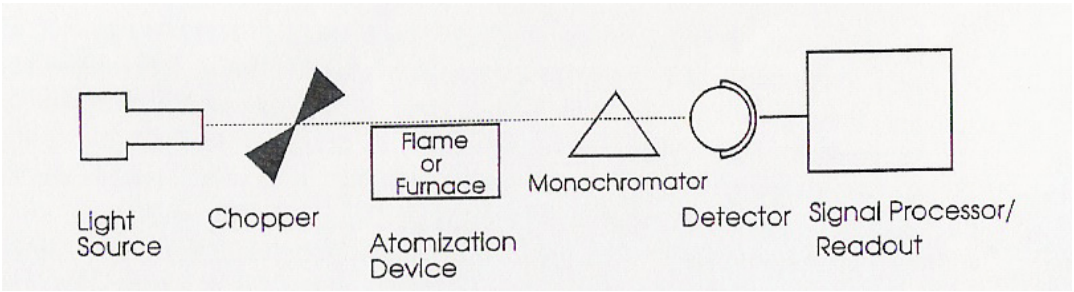


Figure 3.4 Graphite Furnace Atomic Absorption Spectrophotometer (a). Basic diagram of GF-AAS (b).

The sensitivity of F-ASS is limited by the small fraction of sample getting into the flame. The most commonly used method to achieve greater sensitivity in atomic absortion is electrothermal atomization. A type of electrothermal atomizer is the graphite tube furnace which includes an atomizer, a power supply and a programmer. The atomizer assembly consists of a graphite tube placed horizontally in the light path of the spectrometer. An autosampler piston pipette is used to dispense the sample onto a graphite platform within the

tube. The tube is heated applying an electrical potential to graphite contacts which hold the tube in place. Upon heating, an atomic vapor of the element to be analyzed is created in a confined space. The low flow of inert gas through the graphite tube increase the residence time for absortion of light. Thus graphite furnace atomization results in greater sensitivity and lower detection limits than atomization using flame (Sparks et al., 1996).

3.8.1 Detection and quantification limits

The detection and quantification limits for all samples were determinated in a Graphite Furnace Atomic Absorption Spectrophotometer. This procedure was similar to detection and quantification limits for total soil Pb content.

3.8.2 Metal extraction of roots, stems, leaves and fruits

Plant tissue samples were collected at 0, 4 and 8 weeks of exposure. Plants were rinsed with tap water, distilled water and finally deionized water. Plants were separated into leaves, stems, roots and fruits (only for *Capsicum chinense*), sliced into small fragments, placed into Ziploc bags and stored in the refrigerator at 5°C until analysis. Samples of 3.0 g of leaves, stems, roots and fruits were oven-dried at 70°C for 24 hours and the dry weight of the samples was calculated. The dried samples were placed in a furnace muffle at 700° C for 48 hrs. The ash was dissolved in 5 ml of 20 % HCl and the solution was transferred to a 600 ml beaker. The porcelain dishes were rinsed twice with 5 ml of 20% HCl and the solution was added to the same beaker. Samples were covered with watch glasses and heated on a hot

plate until boiling. Hydrochloric acid (20%) was added as needed to avoid sample drying. Samples were filtered into a 50 volumetric flask. Final volume was adjusted with 10% HCl.

3.8.3 GF-AAS measurement and quality control

The Pb content was determinated using Graphite furnace - Atomic Absorption Spectrophotometry. Argon gas provided an inert atmosphere inside the furnace. Graphite tubes with L'vov platforms were employed. A hollow cathode lamp of lead was used as a radiation source. Chemicals modifiers (0.06 % $MgNO_3$ + 1.0 % $NH_4H_2PO_4$) and a standard addition procedure were used to eliminate matrix effects.

3.8.4 Calculations

Concentrations of lead in plants were calculated using the following the equation:

$$Pb\ concentration\ (mg/kg) = \frac{reading\ of\ GF\ (ug/L) * 0.050\,L}{dry\ weight\ of\ the\ sample\ (g)}$$

Where,

Reading of GF (ug/L) = reading of Pb in the sample

0.050 L = total volume of the sample

Dry weight of the sample = (weight of dried total sample at 105°C) – (weight of porcelain dish).

3.9 Soil bioavailability estimates

The bioavailable fraction of lead was determined using Pb-contaminated substrate (100 mg/kg of $Pb(NO_3)_2$ with 100 mg/kg EDTA and without EDTA, and 200 mg/kg of $Pb(NO_3)_2$ with 200 mg/kg EDTA and without EDTA). The bioavailable lead was measured

after 0, 24, 72 and 144 hours. To evaluate temporal variations dried samples of approximately 4 g were placed in 50 ml plastic centrifuge tubes. Hydrochloric acid 0.1 M (20 ml) was added to the centrifuge tubes and shaken for 30 min on a Rotamix at 85 rpm/min (Sparks et al., 1996). Samples were filtered and analyzed using Flame Atomic Absortion Spectrophotometry. All samples were analyzed inmediately after collection.

Concentrations of bioavailable lead were calculated using the following the equation:

$$Bioavailable\ Pb\ \ (mg/kg) = \frac{reading\ of\ \ F\,(mg/L) * 0.40\,L}{dry\ weight\ of\ \ the\ sample\ (g)}$$

Where,

Reading of F (mg/L) = reading of Pb in the substrate

0.40 L = total volume of the sample

Sample dry weight (g) = (weight of dried total sample at 105°C) – (weight of porcelain dish)

3.10 Statistical analysis

All data were evaluated as average values of three replicates using the Infostat statistical software package. One-way ANOVA was employed to determine whether the average of lead in stems, leaves and roots was significantly different at $p < 0.05$. The comparison between treatments was evaluated using the Tukey Test. In some cases, data were transformed to achieve homogeneity of variances and normality of data. A Student T-test was used when the transformed data did not present variance homogeneity. In cases with values below of detection limits (N.D. not detected), half of the detection limit was used for the statistical analysis.

4 RESULTS AND DISCUSSION

4.1 Biomass, growth and shoots and Tolerance Index

The influence of Pb on plant growth was examined (Table 4.1). Biomass production was not inhibited by any of the Pb treatments with or without EDTA. On the contrary, the studied plants exhibited increases of biomass with time, but at different rates depending on the species. These results were consistent with the absence of any phytotoxic symptoms such as: dark green leaves, chlorosis, wilting of older leaves, and slow growth of foliage or necrosis (Begonia et al., 2005). In general, *Capsicum chinense* and *Cucurbita moschata* showed robust growth in Pb amended soils and displayed higher content of biomass than *Cajanus cajan* after 8 weeks of exposure. However, the increase of biomass for *Capsicum chinense* and *Cucurbita. moschata* was slightly higher in the control non-exposed treatment. *Capsicum chinense* increased the biomass from 37.1 mg/kg to 177.9 mg/kg in the control (approximately five-fold); from 69.7 mg/kg to 155.1 mg/kg in the Pb-30 treatment (approximately double); and from 65.6 mg/kg to 196.3 mg/kg in the Pb-60 treatment (approximately three-fold). *Cucurbita moschata* increased the biomass from 60.7 mg/kg to 171.1 mg/kg in the control (approximately three-fold); from 105.9 mg/kg to 193.9 mg/kg in the Pb-30 treatment (approximately double); and from 100.1 mg/kg to 173.1 mg/kg in the Pb-60 treatment (approximately double) after 8 weeks of exposure. On the other hand, *Cajanus cajan* showed a increase of biomass in two-fold in all treatments (with lead and without lead). *Cajanus cajan* increased the biomass from 54.0 mg/kg to 109.5 mg/kg in the control; from

52.0 mg/kg to 95.4 mg/kg in the Pb-30 treatment; and from 49.1 mg/kg to 106.2 mg/kg in the Pb-60 treatment after 8 weeks of exposure. Pang et al. (2003) found that the chlorophyll content or photosynthesis rate is not affected in plants exposed to Pb, and consequently, the production of biomass was not inhibited.

Length of roots and stems of exposed and control plants are shown in Tables 4.2 and 4.3, respectively. The length of roots in *Capsicum chinense*, *Cucurbita moschata* and *Cajanus cajan* were unaffected by the experimental variables. Control values were similar to treatment Pb values. Roots of *Capsicum chinense* reached values of 28.0 cm ± 4.6 cm in the controls, 30.3 cm ± 6.8 cm in the Pb-30 treatment, and 31.0 ± 12.3 cm in the Pb-60 treatment. Roots of *Cucurbita moschata* reached values of 32.0 cm ± 7.9 cm in the controls, 30.0 cm ± 1.0 cm in the Pb-30 treatment, and 37.0 ± 4.6 cm in the Pb-60 treatment. Roots of *Cajanus cajan* reached values of 32.3 cm ± 8.1 cm in the controls, 28.3 cm ± 0.6 cm in the Pb-30 treatment, and 26.7 ± 3.8 cm in the Pb-60 treatment after 8 weeks of exposure. The length of roots was not affected by the time of treatment. One limiting factor in the growth of the roots could be the small space in the pots (1 gallon), which limited the expansion of the roots. Sudhakar et al. (1992) documented the role of Pb in inhibiting the elongation process of root growth in radishes. In this study, the lack of observed inhibition suggests that alterations in root elongation due to Pb exposure could be species specific.

Similarly to patterns of root growth, the length of stems was not affected in Pb-amended treatments. In general, control values were similar to the Pb treatment values after 4 and 8 weeks of growth. *Capsicum chinense* did not show an increase in the length of stems

among treatments including the unexposed reference plants. Lengths of stems for *Capsicum chinense* were 66.7 cm ± 14.2 cm in the control, 61.3 cm ± 2.1 cm in the Pb-30 treatment, and 72.7 cm ± 20.2 cm in the Pb-60 treatment after of 8 weeks of exposure. *Cajanus cajan* showed a slight increase in all treatments at 4 weeks. Control values were 117.0 cm ± 20.4 cm, Pb-30 values were 105.7 cm ± 48.2 cm, and Pb-60 values were 112.7 cm ± 33.7 cm for *Cajanus cajan* after of 4 weeks of exposure. Values for length of roots for *Cajanus cajan* were similar at 4 weeks and 8 weeks of exposure. *Cucurbita moschata* presented a strong increase in root length at 4 and 8 weeks in comparison to 0 weeks. Control values of 99.3 cm ± 11.0, 126.3 cm ± 23.8 cm and, 174.0 cm ± 22.5 cm were found at 0, 4 and 8 weeks of exposure, respectively. Pb-30 values of 100.3 cm ± 11.2 cm, 190.0 cm ± 20.0 cm, and 233.3 cm ± 66.1 cm were found at 0, 4 and 8 weeks of exposure, respectively. Pb-60 values of 99.3 cm ± 10.0 cm, 134.7 cm ± 11.0 cm, 212.3 cm ± 51.4 cm were found at 0, 4 and 8 weeks of exposure, respectively. The Pb added did not affect the development of stems; this may indicate the ability of the species to adapt to an environment contaminated with Pb.

Metal tolerance is one of the most important criteria to select an appropriate plant to remediate sites contaminated with heavy metals (Hakmaoui et al., 2006). In our study, a tolerance index was estimated considering the biomass content (Table 4.4). *Cajanus cajan* showed high values, whereas, *Capsicum chinense* and *Cucurbita moschata* showed lesser values. Values of 1.81 and 1.61 in the treatment with 60 mg/kg of lead at 4 and 8 weeks, respectively, were found for *Cajanus cajan*. Values of 0.63 and 0.66 in the Pb-60 treatment at 4 and 8 weeks, respectively, were obtained for *Capsicum chinense*. Values of 0.62 and 0.51

in the Pb-60 treatment at 4 and 8 weeks, respectively, were found for *C. moschata*. The results indicate that the plants studied can be considered tolerant to Pb contaminated soils.

Baker (1981) suggested two basic strategies of tolerance in plants: (i) the "excluder" strategy in which the concentrations of heavy metals are maintained at constant level in the soil and (ii) the "accumulator" strategy in which metals are concentrated within the plant tissues, implying a highly specialized physiology. Sharma and Shanker (2005) described tolerance strategies in plants exposed to Pb. These strategies include: exclusion, detoxification mechanisms and non-specific defense systems. Capacity of exclusion in plants is related to radial oxygen loss from roots, efficiency to immobilize metals in the rhizosphere, localization in the cell wall, binding to –COOH groups of mucilage uronic acid and its precipitation by oxalate. Detoxification mechanisms include sequestration of Pb in the vacuoles by the formation of complexes, binding of Pb to glutathione (GSH), amino acids and phytochelatins (PCs). Non - specific defense mechanisms include accumulation of osmolytes and changes in the chemical composition of the cell wall.

Souza et al. (2003) mentioned that plant and algal exposure to heavy metals produces phytochelatins, whose function is protecting the cellular metabolism by forming complexes with the heavy metals. Milone et al. (2003) studied the antioxidative responses of two cultivars of *Triticum durum* exposed to cadmium. They found that one cultivar *Ofanto'* showed a high Cd concentration in roots and high translocation of Cd from roots to shoots. A possible mechanism mentioned was that the *Ofanto'* had an efficient Cd detoxification by

vacuolar compartmentalization, which prevents the free circulation of Cd ions and forces them into a limited area.

4.2 Chlorophyll content

Reduction in the chlorophyll a, b and total content with time was observed in *C. chinense*, *C. moschata* and *C. cajan* (0 to 8 weeks of treatment) with lack of variation among the treatments (Tables 4.5 to 4.7).

Chlorophyll a, b and total content ranged from 0.0133 mg/g to 0.0877 mg/g; 0.0095 mg /g to 0.0584 mg /g; and 0.0227 mg /g to 0.1458 mg/g, respectively. In general, the chlorophyll content in the controls was similar to other treatments. Apparently, the different crop species were tolerant to the Pb concentration used and, therefore, chlorophyll production was not affected.

Pang et al. (2003) found a decrease in chlorophyll content and photosynthetic rate in plants of *Vetiver zizanioides* exposed to 100 mg/kg of Pb. Liu et al. (2004) found lower chlorophyll levels in populations of *Rumex dentatus* exposed to high levels of copper. Moreover, Pb inhibits chlorophyll synthesis by causing impaired uptake of essential elements such as Mg and Fe by plants. It damages the photosynthetic apparatus due to its affinity for N- and S-ligands in proteins. In addition, an enhancement of chlorophyll degradation occurs in Pb-treated plants due to increased chlorophyllase activity (Sharma and Shanker, 2005). The decrease of chlorophyll could impact normal growth and eventually cause plant death. If

this effect ocurrs in plants, they are considered sensitive plants. In this research the chlorophyll content data strongly support the description of all three tested species as resistant to Pb.

On the other hand, the species studied showed slightly higher content of chlorophyll a, b and total in juvenile plants than adult plants, in all treatments (with and without lead). Although, leaves of Pb-exposed plants did not show signs of senescence, is possible that the plants were entering the aging phase.

4.3 Electrolyte leakage

Damage to cell membranes was studied by calculating the percentage of electrolyte leakage (Table 4.8). The percentage of electrolyte leakage increased in Pb-exposed plants after 4 weeks of treatment in all species. These differences disappeared after 8 weeks of exposure, when lack of significant differences of electrolyte leakage was found among treatments. Overall, the percentage of electrolyte leakage increased with time of exposure. *Capsicum chinense*, *Cucurbita moschata* and *Cajanus cajan* presented an increase of electrolyte leakage at 4 and 8 weeks of treatment in comparison to week 0, previous to Pb exposure. The addition of EDTA, however, resulted in slightly lower levels of electrolyte leakage when compared to control values. These observations suggest a potential protective mechanism by the EDTA in the integrity of cell membrane. It is possible that mature plants increase the electrolyte leakage as a mechanism of detoxification, eliminating some excessive minerals

inside the cell. The addition of EDTA probably could avoid the increase of electrolyte leakage through the formation of a metal – EDTA complex inside the cell.

One of the most important effects of heavy metals at the cellular level is the alteration of membrane integrity by the formation of reactive oxygen species (ROS). Redox metals such as Cu, Fe, and Hg may directly produce ROS while non-redox metals such as Cd and Pb could indirectly form ROS by the mediation of lipoxygenase (LOX) (Milone et al., 2003). Pb ions induce lipid peroxidation, reduction in the level of saturated fatty acids and increases in the content of membrane unsaturated fatty acids in several plant species (Sharma and Shanker, 2005). Verma and Dubey (2003) reported increases of lipid peroxidation in seeds of *Oryza sativa* grown in sand culture with 500 and 1000 uM Pb $(NO_3)_2$.

A wide range of protective mechanisms has been documented for plants to remove ROS before damage to sensitive compartments of the cellular machinery occurs. These mechanisms are divided in two groups: non-enzymatic antioxidants (such as glutathione, ascorbate, tocopherols and carotenoids) and enzymatic antioxidants (such as catalase, peroxidases, superoxide dismutases (SOD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR)) (Verma and Dubey, 2003).

The three plant species used in this research could have employed the antioxidant strategy, which avoided damage to cell membranes. Ramachandra et al., (2004) described stimulation of antioxidant enzymes such as superoxide dismutase, catalase, peroxidase, glutathione reductase and monodehydroascorbate reductase in stressed plants environment

(e.g. exposure to heavy metals). Verma and Dubey (2003) reported an increase of peroxidase activity in soybean and rice seeds, increases of superoxide dismutase in *Lupinus luteus* and *Oryza sativa* plants and an increase of ascorbate peroxidase and glutathione reductase in rice seeds in Pb stressed plants.

4.4 Metal uptake and translocation index

The concentrations of Pb in roots, stems, leaves and fruits for *Capsicum chinense*, *Cucurbita moschata* and *Cajanus cajan* are showed in tables 4.10, 4.11 and 4.12, respectively. Roots, stems, leaves and fruits of *Capsicum chinense* presented relatively low Pb accumulation levels. The highest concentration found in roots was 6.35 mg/kg of Pb in the Pb-60 treatment after 8 weeks of exposure. The addition of EDTA (100 y 200 mg/kg) did not improve the metal accumulation in this species. Fruits of *Capsicum chinense* showed values between 0.54 mg/kg to 1.60 mg/kg of lead. The level of Pb found in fruit tissue samples surpassed the maximum levels recommended by Codex Alimentarius de la FAO (0.1 mg/kg of lead), and therefore, the fruits of *Capsicum chinens*e should not be used for human consumption (FAO, 2001).

The Pb content in roots, stems and leaves of *Cucurbita moschata* significantly increased in plants exposed to 60 mg/kg of lead when compared to the Pb-30 treatment after week 8 of exposure. Roots showed 13.98 mg/kg of lead in the Pb-60 treatment whereas 4.74 mg/kg in the Pb-30 treatment. Stems contained 11.54 mg/kg of lead in the Pb-60 treatment and 2.63 mg/kg of lead in the Pb-30 treatment after 8 weeks. The content of Pb in leaves was

twice as high in Pb – 60 exposed plants (8.01 mg/kg of lead) than Pb - 30 treated plants (4.32 mg/kg of lead) at the end of the experiment.

The addition of EDTA significantly increased the uptake of lead in roots of *Cucurbita moschata* especially in those treatments with higher concentration of lead. Roots increased the concentration of Pb by two-fold when plants were exposed to Pb-60/200 EDTA compared with Pb-60 treatment after 8 weeks of exposure.

Large Pb ions cannot cross easily the Casparian strip of the roots due to their size and charge characteristics, but once they form complexes with chelating agents such as EDTA, their solubility increases and they become partially "invisible" to those processes that would normally prevent their movement, such as precipitation with phosphates and carbonates or binding to the cell wall through mechanisms such as cation exchange (Sharma & Shanker, 2005). This could be considered a mechanism of uptake of lead in roots of *Cucurbita moschata*.

On the contrary, accumulation of lead in stems of *Cucurbita moschata* was not enhanced by the addition of EDTA. Surprisingly, the uptake of Pb in leaves appears to increase by the chelating agent only in the Pb-30 treatment. In this treatment, leaves accumulated 9.46 and 4.32 mg/kg of Pb with and without EDTA, respectively.

A slight increase of lead was observed in roots of *Cajanus cajan* growing at 60 mg/kg. Roots accumulated 5.02 mg/kg of lead at the 60 mg/kg level in comparison to 2.82 mg/ kg of lead at the 30 mg/kg level. The increase of the Pb concentration in the substrate did not affect the accumulation level of Pb in stems or leaves in *Cajanus cajan*.

The addition of EDTA did not affect the net uptake of lead in roots and stems of *Cajanus cajan*; however, accumulation of lead was significantly higher in leaves. *Cajanus cajan* increased the accumulation of lead by two-fold in the Pb-30/100 EDTA compared with the Pb-30 treatment, and ten-fold in the Pb-60/200 EDTA compared with the Pb-60 treatment. Results of past studies indicate that amendments with synthetic chelating agents in contaminated soil increased accumulation levels in shoots (stems and leaves). The synthetic chelate EDTA forms a soluble complex with many metals, including Pb, and can solubilize Pb from soil particles (Sharma and Shanker, 2005). Huang and Cunningham (1996) found that application of EDTA to Pb - contaminated soils induces higher uptake of Pb by plants, causing the accumulation of Pb more than 1% (w/w) in shoot dry biomass.

One of the attributes of an effective phytoextraction species is its ability to maximize the amount of metal that is partitioned to the above-ground, harvestable biomass (e.g., stems and leaves). This phenomenon can be described by the translocation index. In the absence of EDTA, translocation indices of *Capsicum chinense, Cucurbita moschata* and *Cajanus cajan* grown at 60 mg/kg of Pb were: 38.2%, 58.3% and 41.1%, respectively (table 4.13). Translocation indices increased strongly to 83.2% for *Cajanus cajan* when 200 mg/kg of EDTA was added. The role of EDTA in enhancing metal availability and translocation to aerial compartments of the plant appears to be plant-specific. For example, the translocation index in *Cucurbita moschata* did not increase when plants were growing in EDTA amended soils, while *Capsicum chinense* showed a slight increase in metal accumulation.

Zhen-Guo et al. (2002) confirmed the role of synthetic chelates in inducing Pb accumulation in cabbage. These scientists demonstrated a strong correlation of Pb accumulation in plant shoots with the formation of Pb-EDTA complexes. The Pb-EDTA complex was the major form of Pb absorbed and translocated by the plant. The metal chelate complexes may enter to roots and break the root endodermis and Casparian strip, and be rapidly transported to the shoots.

The uptake of metals by the plants depends mostly on the bioavailable form of the element in the soil. Although the total Pb concentrations in many contaminated soils may be high, the phytoavailable Pb fraction (water soluble and exchangeable) is usually very low due to the strong association of Pb with organic matter, Fe – Mn oxides, and clays, and precipitation as carbonates, hydroxides, and phosphates. Complexing agents such as EDTA, HEDTA, and NTA, can desorb heavy metals from soil matrix to form water-soluble metal complexes and to increase metal uptake by plants (Zhen–Guo et al., 2002). In their research, the bioavailability of Pb was examined in substrate contaminated with Pb and substrate contaminated with Pb and EDTA adjustment. Our results indicated that the bioavalability of Pb is not a limiting factor since higher than 80% of total lead was available. However, this finding should not underestimate the potential and beneficial role of EDTA in plant metal uptake.

In general, the studied plants showed a great tolerance to contaminated environments. The treatments with Pb and EDTA and without EDTA did not inhibit the normal development of the species. The integrity of the cell membranes was not affected with the

treatments. In addition, the chlorophyll content was similar in the treatments and in the controls. On the other hand, the addition of EDTA only had a positive effect in roots of *Cucurbita moschata* and leaves of *Cajanus cajan*.

Phytoextraction is a strategy of phytoremediation particularly applicable to metals, metalloids and radionuclides. In this technology, plants remove metals from soils and concentrate the metal fraction in the harvestable parts. A gross mass balance analaysis of total soil Pb suggests that this technology is economically feasible only if plants can accumulate greater than 1% in their shoots (stems and leaves) (Huang et al., 1997). Although phytoextraction is a technology regularly studied to remove several heavy metals and, frequently, enhanced with the addition of chelating agents; our results suggest major limitations of phytoextraction in the removal of Pb. The Table 4.9 summarizes benefits and limitations of phytoextraction of lead from contaminated soils.

TABLE 4.1 Total biomass content (g, FW)

Plant sp.	Time (wks)	Treatment[1,2]				
		Control	Pb - 30	Pb-30/100 EDTA	Pb - 60	Pb-60/200 EDTA
C. chinense	16	37.1 ± 16.8	69.7 ± 13.8	-	65.6 ± 13.8	-
	20	130.3 ± 56.9	130.2 ± 38.6	-	147.4 ± 46.5	-
	24	177.9 ± 36.1	155.1 ± 46.2	152.6 ± 65.8	196.3 ± 52.5	177.1 ± 50.1
C. moschata	8	60.7 ± 1.6	105.9 ± 11.4	-	100.1 ± 38.1	-
	12	126.5 ± 25.3	171.6 ± 62.3	-	150.7 ± 27.2	-
	16	171.1 ± 47.4	193.9 ± 60.0	178.6 ± 125.9	173.1 ± 14.3	146.2 ± 44.0
C. cajan	8	54.0 ± 19.5	52.0 ± 20.0	-	49.1 ± 27.9	-
	12	79.0 ± 8.8	78.2 ± 20.0	-	95.7 ± 6.5	-
	16	109.5 ± 30.6	95.4 ± 2.5	85.2 ± 12.8	106.2 ± 27.2	126.6 ± 2.4

[1]Control (0 mg/kg of Pb), Pb - 30 (30 ± 8 mg/kg of Pb), Pb – 30 with 100 EDTA (30 ± 8 mg/kg of Pb with 100 mg/kg of EDTA), Pb - 60 (60 ± 18 mg/kg of Pb), Pb - 60/200 EDTA (60 ± 18 mg/kg of Pb with 200 mg/kg of EDTA)
[2]Average ± Standard Deviation; n = 3

⁻, not determined

TABLE 4.2 Summary of root length in exposed and unexposed plants (cm)

Plant sp.	Time (wks)	Treatment[1,2]				
		Control	Pb - 30	Pb-30/100 EDTA	Pb - 60	Pb-60/200 EDTA
C. chinense	16	29.0 ± 3.6	30.0 ± 2.0	-	29.7 ± 4.5	-
	20	46.0 ± 5.3	33.7 ± 1.5	-	28.7 ± 5.7	-
	24	28.0 ± 4.6	30.3 ± 6.8	37.3 ± 15.3	31.0 ± 12.3	34.0 ± 8.7
C. moschata	8	30.3 ± 0.6	30.0 ± 1.0	-	30.3 ± 0.6	-
	12	31.0 ± 1.7	30.7 ± 3.1	-	31.3 ± 4.2	-
	16	32.0 ± 7.9	30.0 ± 1.0	31.7 ± 13.6	37.0 ± 4.6	27.7 ± 2.5
C. cajan	8	30.3 ± 0.6	30.3 ± 0.6	-	30.3 ± 0.6	-
	12	26.3 ± 1.5	29.3 ± 4.0	-	29.0 ± 4.0	-
	16	32.3 ± 8.1	28.3 ± 0.6	29.3 ± 3.1	26.7 ± 3.8	32.7 ± 7.0

[1]Control (0 mg/kg of Pb), Pb - 30 (30 ± 8 mg/kg of Pb), Pb – 30 with 100 EDTA (30 ± 8 mg/kg of Pb with 100 mg/kg of EDTA), Pb - 60 (60 ± 18 mg/kg of Pb), Pb - 60/200 EDTA (60 ± 18 mg/kg of Pb with 200 mg/kg of EDTA)
[2]Average ± Standard Deviation; n = 3

–, not determined

TABLE 4.3 Summary of stem length in exposed and unexposed plants (cm)

Plants sp.	Time (wks)	Treatment[1,2]				
		Control	Pb - 30	Pb-30/100 EDTA	Pb - 60	Pb-60/200 EDTA
C. chinense	16	53.3 ± 11.0	45.3 ± 3.8	-	51.3 ± 9.1	-
	20	65.7 ± 11.7	45.7 ± 6.8	-	53.7 ± 13.7	-
	24	66.7 ± 14.2	61.3 ± 2.1	68.7 ± 18.7	72.7 ± 20.2	82.0 ± 8.2
C. moschata	8	99.3 ± 11.0	100.3 ± 11.2	-	99.3 ± 10.0	-
	12	126.3 ± 23.8	190.0 ± 20.0	-	134.7 ± 11.0	-
	16	174.0 ± 22.5	233.3 ± 66.1	167.0 ± 111.0	212.3 ± 51.4	187.3 ± 34.5
C. cajan	8	69.3 ± 14.5	68.7 ± 13.3	-	65.7 ± 8.1	-
	12	117.0 ± 20.4	105.7 ± 48.2	-	112.7 ± 33.7	-
	16	127.3 ± 3.1	112.3 ± 18.6	112.3 ± 8.6	114.0 ± 20.3	136.7 ± 14.7

[1]Control (0 mg/kg of Pb), Pb - 30 (30 ± 8 mg/kg of Pb), Pb – 30 with 100 EDTA (30 ± 8 mg/kg of Pb with 100 mg/kg of EDTA), Pb - 60 (60 ± 18 mg/kg of Pb), Pb - 60/200 EDTA (60 ± 18 mg/kg of Pb with 200 mg/kg of EDTA)
[2]Average ± Standard Deviation; n = 3

–, not determined

TABLE 4.4 Tolerance Index (TI) based on biomass data

Plant sp.	Time (wks)	Treatment[1]		
		Control	Pb - 30	Pb - 60
C. chinense	4	1.00	0.48	0.63
	8	1.00	0.48	0.66
C. moschata	4	1.00	0.60	0.62
	8	1.00	0.49	0.51
C. cajan	4	1.00	1.04	1.81
	8	1.00	0.91	1.16

[1]Control (0 mg/kg of Pb), Pb-30 (30 ± 8 mg/kg of Pb), Pb-60 (60 ± 18 mg/kg of Pb)

TABLE 4.5 Content of chlorophyll a, b and total (mg/g fresh weight) of *C. chinense*

	Time (wks)	Treatment[1,2]				
		Control	**Pb - 30**	**Pb-30/100 EDTA**	**Pb - 60**	**Pb-60/200 EDTA**
Chlorophyll a	**16**	0.0431 ± 0.0004	0.0399 ± 0.0106	-	0.0343 ± 0.0149	-
	20	0.0245 ± 0.0155	0.0133 ± 0.0025	-	0.0194 ± 0.0039	-
	24	0.0180 ± 0.0052	0.0256 ± 0.0166	0.0195 ± 0.0079	0.0143 ± 0.0150	0.0138 ± 0.0008
Chlorophyll b	**16**	0.0346 ± 0.0185	0.0304 ± 0.0117	-	0.0250 ± 0.0148	-
	20	0.0242 ± 0.0152	0.0095 ± 0.0023	-	0.0185 ± 0.0083	-
	24	0.0202 ± 0.0085	0.0297 ± 0.0211	0.0231 ± 0.0148	0.0158 ± 0.0232	0.0118 ± 0.0005
Total chlorophyll	**16**	0.0861 ± 0.0310	0.0703 ± 0.0223	-	0.0593 ± 0.0295	-
	20	0.0487 ± 0.0304	0.0227 ±0.0044	-	0.0380 ± 0.0121	-
	24	0.0382 ± 0.0137	0.0553 ± 0.0375	0.0425 ± 0.0227	0.0301 ± 0.0382	0.0256 ± 0.0012

[1]Control (0 mg/kg of Pb), Pb - 30 (30 ± 8 mg/kg of Pb), Pb – 30 with 100 EDTA (30 ± 8 mg/kg of Pb with 100 mg/kg of EDTA), Pb - 60 (60 ± 18 mg/kg of Pb), Pb - 60/200 EDTA (60 ± 18 mg/kg of Pb with 200 mg/kg of EDTA)
[2]Average ± Standard Deviation; n = 3

–, not determined

TABLE 4.6 Content of chlorophyll a, b and total (mg/g fresh weight) of *C. moschata*

	Time (wks)	Treatment[1,2]				
		Control	**Pb - 30**	**Pb-30/100 EDTA**	**Pb - 60**	**Pb-60/200 EDTA**
Chlorophyll a	**8**	0.0573 ± 0.0219	0.0554 ± 0.0113	-	0.0568 ± 0.0245	-
	12	0.0447 ± 0.0125	0.0455 ± 0.0152	-	0.0303 ± 0.0072	-
	16	0.0160 ± 0.0090	0.0215 ± 0.0094	0.0208 ± 0.0152	0.0274 ± 0.0072	0.0182 ± 0.0069
Chlorophyll b	**8**	0.0441 ± 0.0160	0.0431 ± 0.0148	-	0.0353 ± 0.0173	-
	12	0.0313 ± 0.0082	0.0353 ± 0.0047	-	0.0191 ± 0.0075	-
	16	0.0146 ± 0.0159	0.0194 ± 0.0093	0.0214 ± 0.0154	0.0285 ± 0.0141	0.0149 ± 0.0047
Total chlorophyll	**8**	0.1015 ± 0.0380	0.0985 ± 0.0261	-	0.0921 ± 0.0418	-
	12	0.0760 ± 0.0207	0.0809 ± 0.0172	-	0.0494 ± 0.0123	-
	16	0.0305 ± 0.0248	0.0409 ± 0.0185	0.0422 ± 0.0307	0.0559 ± 0.0213	0.0332 ± 0.0116

[1]Control (0 mg/kg of Pb), Pb - 30 (30 ± 8 mg/kg of Pb), Pb – 30 with 100 EDTA (30 ± 8 mg/kg of Pb with 100 mg/kg of EDTA), Pb - 60 (60 ± 18 mg/kg of Pb), Pb - 60/200 EDTA (60 ± 18 mg/kg of Pb with 200 mg/kg of EDTA)
[2]Average ± Standard Deviation; n = 3

–, not determined

TABLE 4.7 Chlorophyll content (mg/g fresh weight) in *C. cajan*

	Time (wks)	Treatment[1,2]				
		Control	**Pb - 30**	**Pb-30/100 EDTA**	**Pb - 60**	**Pb-60/200 EDTA**
Chlorophyll a	**8**	0.0633 ± 0.0101	0.0742 ± 0.0102	-	0.0877 ± 0.0053	-
	12	0.0438 ± 0.0141	0.0523 ± 0.0126	-	0.0589 ± 0.0119	-
	16	0.0433 ± 0.0050	0.0354 ± 0.0029	0.0390 ± 0.0062	0.0427 ± 0.0033	0.0311 ± 0.0116
Chlorophyll b	**8**	0.0452 ± 0.0100	0.0584 ± 0.0149	-	0.0582 ± 0.0079	-
	12	0.0308 ± 0.0068	0.0354 ± 0.0116	-	0.0412 ± 0.0084	-
	16	0.0366 ± 0.0050	0.0479 ± 0.0148	0.0276 ± 0.0087	0.0472 ± 0.0337	0.0276 ± 0.0149
Total chlorophyll	**8**	0.1086 ± 0.0184	0.1326 ± 0.0248	-	0.1458 ± 0.0131	-
	12	0.0745 ± 0.0209	0.0877 ± 0.0242	-	0.1002 ± 0.0195	-
	16	0.0800 ± 0.0082	0.0833 ± 0.0119	0.0666 ± 0.0139	0.0899 ± 0.0369	0.0587 ± 0.0265

[1]Control (0 mg/kg of Pb), Pb - 30 (30 ± 8 mg/kg of Pb), Pb – 30 with 100 EDTA (30 ± 8 mg/kg of Pb with 100 mg/kg of EDTA), Pb - 60 (60 ± 18 mg/kg of Pb), Pb - 60/200 EDTA (60 ± 18 mg/kg of Pb with 200 mg/kg of EDTA)
[2]Average ± Standard Deviation; n = 3

–, not determined

TABLE 4.8 Percentage (%) of electrolyte leakage

Plant sp.	Time (wks)	Treatment[1,2]				
		Control	Pb - 30	Pb-30/100 EDTA	Pb - 60	Pb-60/200 EDTA
C. chinense	16	8.5 ± 1.1	9.9 ± 0.9	-	10.8 ± 1.3	-
	20	11.5 ± 4.8	41.2 ± 8.2	-	37.9 ± 12.3	-
	24	40.6 ± 5.0	28.1 ± 9.9	14.6 ± 2.9	48.1 ± 1.7	18.1 ± 6.0
C. moschata	8	32.5 ± 2.2	32.1 ± 1.4	-	33.7 ± 3.1	-
	12	37.7 ± 33.3	69.5 ± 21.6	-	80.4 ± 4.1	-
	16	54.3 ± 3.0	58.5 ± 6.0	40.1 ± 4.2	60.0 ± 14.9	28.4 ± 11.2
C. cajan	8	12.0 ± 0.9	13.7 ± 2.4	-	13.3 ± 2.8	-
	12	10.8 ± 3.5	53.9 ± 1.4	-	50.0 ± 10.1	-
	16	35.1 ± 2.1	35.0 ± 4.9	12.2 ± 1.4	45.7 ± 8.8	15.2 ± 5.2

[1]Control (0 mg/kg of Pb), Pb - 30 (30 ± 8 mg/kg of Pb), Pb – 30 with 100 EDTA (30 ± 8 mg/kg of Pb with 100 mg/kg of EDTA), Pb - 60 (60 ± 18 mg/kg of Pb), Pb - 60/200 EDTA (60 ± 18 mg/kg of Pb with 200 mg/kg of EDTA)
[2]Average ± Standard Deviation; n = 3

–, not determined

TABLE 4.9 Benefits and limitations of the phytoextraction of lead

Benefits	Limitations
- Some species such as *Cajanus cajan, Cucurbita moschata and Capsicum chinense* can tolerate concentrations of Pb commonly toxic to plants.	- The percentage of lead in plants is smaller than in the subtrates. Pb was diluted 6 times within the plants (*Cajanus cajan*) in comparison to Pb in the substrates.
-Plants can uptake lead from substrate with a minimal environmental disturbance.	- Plants accumulate a small percentage in the aerial tissues. A maximum of 0.001% of Pb was accumulated in shoots of *Cucurbita moschat*a.
- Plants can extract a significant amount of lead from subtrate. *Cajanus cajan* extracted 14% of Pb with 83% of the contaminant in the aerial tissues while *Cucurbita moschata* extracted 20% of Pb with 46% of the contaminant in the aerial tissues.	- Phytoextraction of lead can transfer the metal to the fruits (fruits of *Capsicum chinense* accumulated concentrations of Pb greater than the maximum toxic level (0.1 mg/kg), which demonstrates the mobilization of Pb into the food chain and potential negative effect on humans and the environment.
- The root system of plants provides an enormous surface area for absorption and accumulation	
- Phytoextraction of lead could be an inexpensive technology in comparison to traditional technologies.	

TABLE 4.10 Bioaccumulation of Pb in *Capsicum chinense* (mg/kg)

Parts of plant	Time (wks)	Treatment[1,2]				
		Control	**Pb - 30**	**Pb-30/100 EDTA**	**Pb - 60**	**Pb-60/200 EDTA**
Roots	16	N.D.	3.29 ± 1.55	-	3.89 ± 1.44	-
	20	N.D.	1.16 ± 0.49	-	2.54 ± 1.64	-
	24	0.17 ± 0.09	1.81 ± 0.74	2.79 ± 1.21	6.35 ± 0.94	3.19 ± 2.22
Stems	16	0.34 ± 0.32	N.D.	-	2.18 ± 0.74	-
	20	N.D.	0.82 ± 0.33	-	0.72 ± 0.32	-
	24	0.67 ± 0.50	0.93 ± 0.29	0.80 ± 0.21	1.83 ± 0.89	0.87 ± 0.40
Leaves	16	0.88 ± 1.30	1.06 ± 0.92	-	3.07 ± 0.66	-
	20	N.D.	1.03 ± 0.30	-	1.27 ± 0.49	-
	24	0.47 ± 0.44	1.75 ± 1.50	1.24 ± 0.49	2.10 ± 1.11	2.45 ± 1.66
Fruits	16	-	-	-		-
	20	-	-	-	0.80 ± 0.01	-
	24	0.65 ± 0.06	1.60 ± 1.76	1.39 ± 0.06	0.54 ± 0.36	1.07 ± 0.44

[1]Control (0 mg/kg of Pb), Pb - 30 (30 ± 8 mg/kg of Pb), Pb – 30 with 100 EDTA (30 ± 8 mg/kg of Pb with 100 mg/kg of EDTA), Pb - 60 (60 ± 18 mg/kg of Pb), Pb - 60/200 EDTA (60 ± 18 mg/kg of Pb with 200 mg/kg of EDTA)

[2]Average ± Standard Deviation; n = 3

–, not determined

N.D., not detected

TABLE 4.11 Bioaccumulation of lead in *Cucurbita moschata* (mg/kg)

Parts of plant	Time (wks)	Treatment[1,2]				
		Control	Pb - 30	Pb-30/100 EDTA	Pb - 60	Pb-60/200 EDTA
Roots	8	N.D.	4.18 ± 0.55	-	2.26 ± 0.17	-
	12	N.D.	5.11 ± 1.22	-	9.48 ± 1.34	-
	16	N.D.	4.74 ± 2.35	6.73 ± 0.03	13.98 ± 1.12	24.82 ± 1.26
Stems	8	N.D.	0.93 ± 0.52	-	2.07 ± 0.44	-
	12	0.43 ± 0.37	1.09 ± 0.54	-	14.96 ± 1.08	-
	16	N.D.	2.63 ± 1.08	3.67 ± 1.51	11.54 ± 0.25	12.14 ± 1.02
Leaves	8	N.D.	1.63 ± 1.52	-	2.53 ± 0.51	-
	12	N.D.	2.29 ± 1.45	-	2.77 ± 0.67	-
	16	N.D.	4.32 ± 2.10	9.46 ± 0.64	8.01 ± 0.09	8.80 ± 4.10

[1]Control (0 mg/kg of Pb), Pb - 30 (30 ± 8 mg/kg of Pb), Pb – 30 with 100 EDTA (30 ± 8 mg/kg of Pb with 100 mg/kg of EDTA), Pb - 60 (60 ± 18 mg/kg of Pb), Pb - 60/200 EDTA (60 ± 18 mg/kg of Pb with 200 mg/kg of EDTA)
[2]Average ± Standard Deviation; n = 3

–, not determined
N.D., not detected

TABLE 4.12 Bioaccumulation of lead in *Cajanus cajan* (mg/kg)

Parts of plant	Time (wks)	Treatment[1,2]				
		Control	Pb - 30	Pb-30/100 EDTA	Pb - 60	Pb-60/200 EDTA
Roots	8	N.D.	3.62 ± 0.11	-	3.48 ± 1.60	-
	12	N.D.	3.39 ± 0.23	-	3.03 ± 1.65	-
	16	N.D.	2.82 ± 0.28	2.21 ± 1.02	5.02 ± 1.22	5.96 ± 1.36
Stems	8	0.28 ± 0.18	0.36 ± 0.41	-	0.93 ± 0.03	-
	12	0.14 ± 0.02	0.50 ± 0.08	-	0.74 ± 0.50	-
	16	0.19 ± 0.08	0.76 ± 0.49	0.70 ± 0.13	0.49 ± 0.15	0.61 ± 0.04
Leaves	8	N.D.	1.80 ± 0.14	-	3.00 ± 0.07	-
	12	N.D.	1.45 ± 0.62	-	2.04 ± 0.78	-
	16	N.D.	6.01 ± 4.02	13.65 ± 3.28	3.02 ± 0.82	28.94 ± 8.00

[1]Control (0 mg/kg of Pb), Pb - 30 (30 ± 8 mg/kg of Pb), Pb – 30 with 100 EDTA (30 ± 8 mg/kg of Pb with 100 mg/kg of EDTA), Pb - 60 (60 ± 18 mg/kg of Pb), Pb - 60/200 EDTA (60 ± 18 mg/kg of Pb with 200 mg/kg of EDTA)

[2]Average ± Standard Deviation; n = 3

–, not determined

N.D., not detected

TABLE 4.13 Metal translocation from roots to the stems and leaves (%)

Plants species	Treatments	Translocation index[1]
C. chinense	Pb-60	38.2
	Pb–60 w/ 200 EDTA	51.0
C. moschata	Pb-60	58.3
	Pb–60 w/ 200 EDTA	45.8
C. cajan	Pb-60	41.1
	Pb–60 w/ 200 EDTA	83.2

[1]Data are average stems and leaves content divided by average stems, leaves and roots content, n = 3. Pb - 60 (60 ± 18 mg/kg of Pb), Pb - 60 / 200 EDTA (60 ± 18 mg/kg of Pb + 200 mg/kg of EDTA)

CONCLUSIONS

- The chlorophyll content, percentage of electrolyte leakage and length of roots and stems in *Cucurbita moschata*, *Capsicum chinense* and *Cajanus cajan* were not affected in soils contaminated with 30 and 60 mg/kg of lead, demonstrating the phytoresistance of crop species to moderate levels of pollutants.
- In general, the biomass content increased similarly during exposure to lead at either 30 mg/kg or 60 mg/kg of lead.
- The addition of EDTA did not affect the uptake of lead in *Capsicum chinense*, but it increased the accumulation of lead in roots and leaves of *Cucurbita moschata* and *Cajanus cajan*, respectively.
- The addition of EDTA increased the translocation of lead from roots to leaves only in *Cajanus cajan*.
- In general, plants were tolerant to Pb contaminated environments. The studied species showed higher tolerance indices in the treatment with 60 mg/kg of lead.
- Plants showed a dilution effect when soil Pb levels were compared to plant tissue content. Thus, indicating a potential limitation of phytoextraction in removing efficiently heavy metals from contaminated soils.

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APPENDIX A. CALIBRATION CURVES FOR CHLOROPHYLL A AND B

Concentrations of chlorophyll a and b, reading of absorbance and average are showed in the Table A.1 and Table A.2, respectively. These data were used for calculated the equation of straight line and the Correlation coefficient (Figure A.1 and Figure A.2).

TABLE A.1 Data used to calculate the calibration curve for chlorophyll a

Concentrations (mg / L)	Reading 1	Reading 2	Reading 3	Average
0.0	0.0000	0.0000	0.0000	0.0000
0.5	0.0820	0.0820	0.0820	0.0820
1.0	0.1120	0.1110	0.1120	0.1117
3.0	0.2650	0.2650	0.2650	0.2650
5.0	0.4580	0.4590	0.4580	0.4583

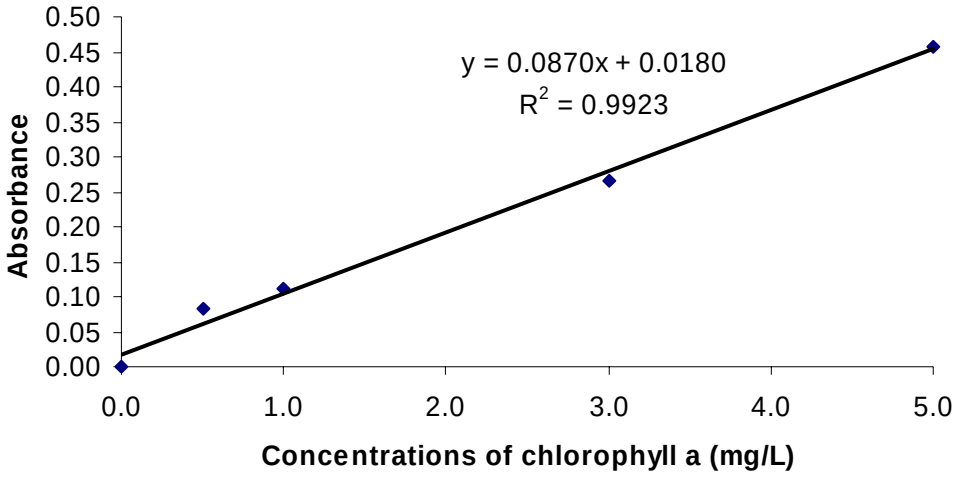

Figure A.1 Calibration curve for chlorophyll a

TABLE A.2 Data used to calculate the calibration curve for chlorophyll b

Concentrations (mg / L)	Reading 1	Reading 2	Reading 3	Average
0.0	0.0000	0.0000	0.0000	0.0000
0.5	0.0240	0.0240	0.0240	0.0240
1.0	0.0430	0.0430	0.0430	0.0430
3.0	0.1910	0.1910	0.1910	0.1910
5.0	0.2850	0.2850	0.2850	0.2850

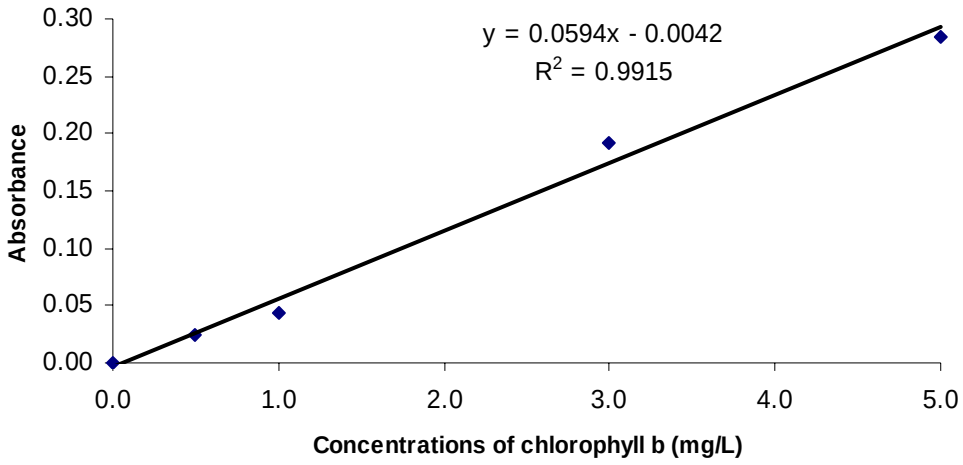

Figure A.2 Calibration curve for chlorophyll b

APPENDIX B. DETECTION AND QUANTIFICATION LIMITS IN F-AAS.

The detection and quantification limits were calculated using the standard deviation of intercept and the average of slope.

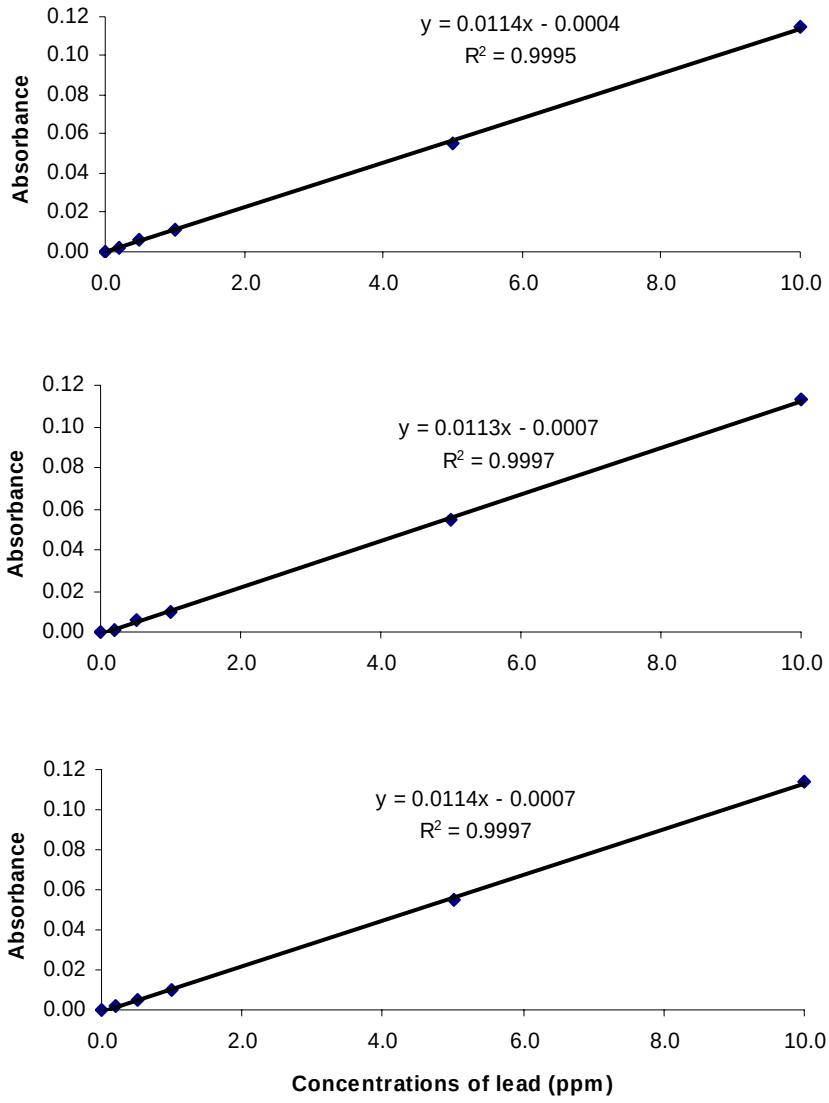

Figure B.1: Curves used for calculating the D.L and Q.L.

TABLE B.1 Intercept and slope used to calculate detection and quantification limits

Parameters	**Curve 1**	**Curve 2**	**Curve 3**
Intercept	0.0004	0.0007	0.0007
Slope	0.0114	0.0113	0.0114

Standard deviation of intercept = 0.0002

Average of slope = 0.0114

$$Detection\ Limit = \frac{3(0.0002)}{0.0114} = 0.05\ ppm$$

$$Quantification\ \ Limit = \frac{10(0.0002)}{0.0114} = 0.18\ ppm$$

APPENDIX C. CALIBRATION CURVES FOR SUBSTRATE

Concentrations of lead, reading of absorbance and average are showed in the Table C.1. These data were used for calculated the equation of straight line and the correlation coefficient (Figure C.1).

TABLE C.1 Data used to calculate the calibration curve for substrate

Concentrations (ppm)	Reading 1	Reading 2	Reading 3	Average
0.0	0.0000	0.0000	0.0000	0.0000
0.2	0.0030	0.0030	0.0030	0.0030
0.5	0.0080	0.0070	0.0070	0.0073
1.0	0.0140	0.0130	0.0140	0.0137
5.0	0.0610	0.0610	0.0610	0.0610
10.0	0.1180	0.1200	0.1220	0.1200

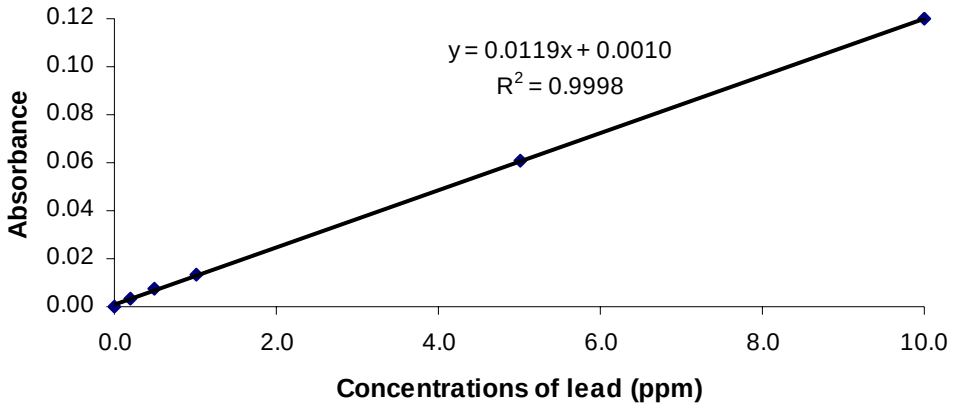

Figure C.1 Calibration curve for substrate

APPENDIX D. CONCENTRATIONS OF LEAD IN SUBSTRATE

TABLE D.1. Concentrations of total lead in substrate (mg/kg)

Time (wks)	Treatment[1,2]				
	Control	**Pb - 30**	**Pb-30/100 EDTA**	**Pb - 60**	**Pb-60/200 EDTA**
0	N.D.	32.81 ± 7.75	-	53.40 ± 17.50	-
4	N.D.	30.73 ± 11.35	-	54.61 ± 16.95	-
8	N.D.	27.92 ± 14.35	30.96 ± 12.38	55.32 ± 15.70	53.01 ± 19.00

[1]Control (0 mg/kg of Pb), Pb - 30 (30 ± 8 mg/kg of Pb), Pb – 30 with 100 EDTA (30 ± 8 mg/kg of Pb with 100 mg/kg of EDTA), Pb - 60 (60 ± 18 mg/kg of Pb), Pb - 60/200 EDTA (60 ± 18 mg/kg of Pb with 200 mg/kg of EDTA)

[2]Average ± Standard Deviation; n = 3

–, not determined

N.D., not detected

APPENDIX E. DETECTION AND QUANTIFICATION LIMITS IN GF-AAS

The detection and quantification limits were calculated using the standard deviation of intercept and the average of slope.

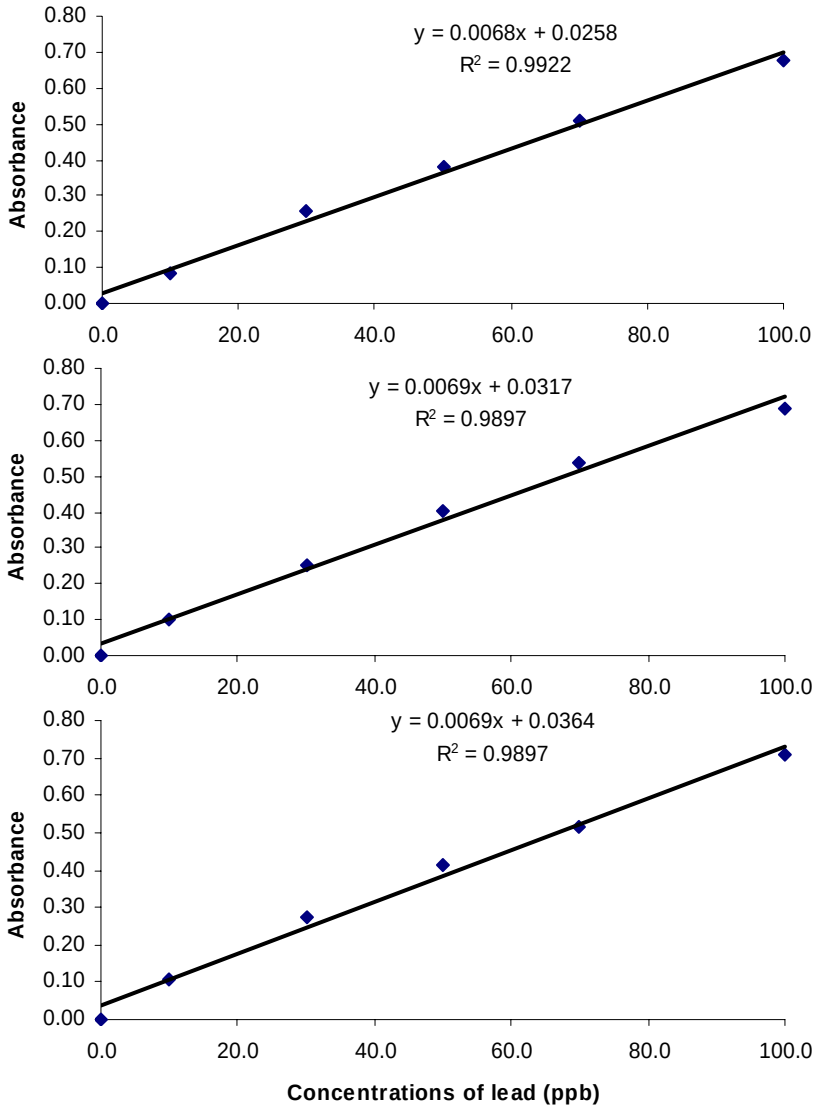

Figure E.1: Curves used for calculating the D.L and Q.L

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TABLE E.1 Intercept and slope used to calculate detection and quantification limits

Parameters	**Curve 1**	**Curve 2**	**Curve 3**
Intercept	0.0258	0.0317	0.0364
Slope	0.0068	0.0069	0.0069

Standard deviation of intercept = 0.0053

Average of slope = 0.0069

$$Detection\ Limit = \frac{3(0.0053)}{0.0068} = 2.32\ ppb$$

$$Quantification\ \ Limit = \frac{10(0.0053)}{0.0068} = 7.74\ ppb$$

APPENDIX F. STANDARD CONDITIONS FOR GF-AAS

TABLE F.1 Time / temperature program of the electrothermal atomizer (temperature of the stage / ramp time / hold time) used in the GF – AAS measurements.

Stage	Program
Drying	120 °C / 20 s / 30 s
Ashing	400 °C / 30 s / 25 s
Cooling	20 °C / 1 s / 15 s
Atomization	1700 °C / 0 s / 5 s
Cleaning	2600 °C / 1 s / 5 s

APPENDIX G. EXAMPLE OF STANDARD ADDITION

Due to matrix effects the analytical response for an analyte in a complex sample may not be the same as for the analyte in a simple sample. An alternative procedure is the standard addition method. Following this method, samples were divided into four portions, so that a know amount of the analyte (a spike) was added to one portion. These four samples, the original and the others, were analyzed. The difference in analytical response between the unspiked and spiked samples provided a calibration point to determinate the analyte concentration in the original sample.

The concentrations of lead showed in the Table F.1 were added to the same sample, with the purpose to eliminate the matrix interferences. The averages of the lectures versus the concentrations of lead are represented in the Figure F.1 the equation of straight line was used for calculated the real concentration of sample.

For each sample it was necessary to find the equation of straight line for calculated its real concentration. Samples with high concentrations of lead were diluted with hydrochloric acid 10%

TABLE G.1 Data used to calculate the real concentration of one sample

Concentrations of lead (ppb)	Reading 1	Reading 2	Average
0.0	0.098	0.099	0.099
25.0	0.143	0.132	0.138
50.0	0.180	0.176	0.178
100.0	0.237	0.256	0.247

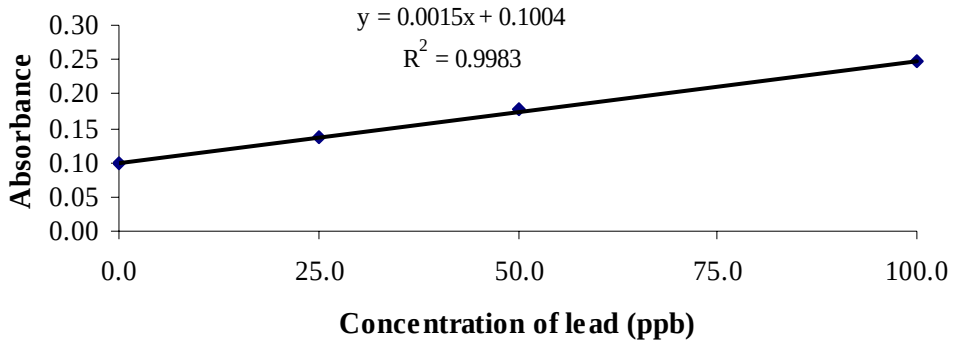

Figure G.1: Curve used for calculate the real concentration of one sample

APPENDIX H. QUALITY CONTROLS

Each ten samples, the percentage of recovery were calculated. Values between 80 -115 % were accepted. When the values were smaller or higher than the normal range, samples were read again.
The following equation was used to calculate the percentage of recovery:

$$P = 100 * \frac{(A - B)}{T}$$

Where:

P = Percentage of recovery
A = Measured concentration of analyte after spiking
B = Measured concentration of analyte before spiking
T = True concentration of the spike

One example:

$$P = 100 * \frac{(31 - 12)}{20} = 95\%$$

APPENDIX I. ONE - WAY ANOVA FOR ALL EXPERIMENTS

The following letters were used for ANOVA's analyses:
C (control), A_1 (30 mg/kg of Pb), A_2 (30 mg/kg of Pb and 100 mg /kg of EDTA), B_1 (60 mg/kg of Pb), B_2 (60 mg/kg of Pb and 200 mg /kg of EDTA).

Stems of *Capsicum chinense* (A_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,74	0,65	40,73

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	1,11	2	0,55	8,50	0,0178
Tiempo	1,11	2	0,55	8,50	0,0178
Error	0,39	6	0,07		
Total	1,50	8			

Test:Tukey Alfa:=0,05 DMS:=0,63958

Error: 0,0652 gl: 6

Tiempo	Medias	n	
0,00	0,13	3	A
4,00	0,82	3	B
8,00	0,93	3	B

Letras distintas indican diferencias significativas(p<= 0,05)

Stems of *Capsicum chinense* (B_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,55	0,40	44,10

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	3,49	2	1,75	3,63	0,0927
Tiempo	3,49	2	1,75	3,63	0,0927
Error	2,89	6	0,48		
Total	6,38	8			

Test:Tukey Alfa:=0,05 DMS:=1,73781

Error: 0,4812 gl: 6

Tiempo	Medias	n	
4,00	0,72	3	A
8,00	1,83	3	A
0,00	2,18	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Stems of *Cucurbita moschata* (A_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,60	0,47	48,88

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	5,25	2	2,62	4,56	0,0625
Tiempo	5,25	2	2,62	4,56	0,0625
Error	3,45	6	0,58		
Total	8,70	8			

Test:Tukey Alfa:=0,05 DMS:=1,90015

Error: 0,5753 gl: 6

Tiempo	Medias	n	
0,00	0,93	3	A
4,00	1,09	3	A
8,00	2,63	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Stems of *Cucurbita moschata* (B_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,99	0,99	7,23

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	267,56	2	133,78	282,40	<0,0001
Tiempo	267,56	2	133,78	282,40	<0,0001
Error	2,84	6	0,47		
Total	270,40	8			

Test:Tukey Alfa:=0,05 DMS:=1,72421

Error: 0,4737 gl: 6

Tiempo	Medias	n			
0,00	2,07	3	A		
8,00	11,54	3		B	
4,00	14,96	3			C

Letras distintas indican diferencias significativas (p<= 0,05)

Stems of *Cajanus cajan* (C) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,29	0,05	24,96

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,03	2	0,01	1,20	0,3650
Tiempo	0,03	2	0,01	1,20	0,3650
Error	0,07	6	0,01		
Total	0,10	8			

Test:Tukey Alfa:=0,05 DMS:=0,27374

Error: 0,0119 gl: 6

TIEMPO	Medias	n	
4,00	0,37	3	A
8,00	0,43	3	A
0,00	0,51	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Stems of *Cajanus cajan* (A_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,18	0,00	61,54

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,16	2	0,08	0,66	0,5529
Tiempo	0,16	2	0,08	0,66	0,5529
Error	0,75	6	0,13		
Total	0,92	8			

Test:Tukey Alfa:=0,01 DMS:=1,29303

Error: 0,1252 gl: 6

Tiempo	Medias	n	
0,00	0,46	3	A
4,00	0,50	3	A
8,00	0,76	3	A

Letras distintas indican diferencias significativas (p<= 0,01)

Stems of *Cajanus cajan* (B_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,59	0,45	28,05

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,31	2	0,16	4,31	0,0693
Tiempo	0,31	2	0,16	4,31	0,0693
Error	0,22	6	0,04		
Total	0,53	8			

Test:Tukey Alfa:=0,01 DMS:=0,69600

Error: 0,0363 gl: 6

Tiempo	Medias	n	_
8,00	0,49	3	A
4,00	0,61	3	A
0,00	0,93	3	A

Letras distintas indican diferencias significativas (p<= 0,01)

Roots of *Capsicum chinense* (C) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,14	0,00	33,47

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	3,1E-03	2	1,6E-03	0,47	0,6460
Tiempo	3,1E-03	2	1,6E-03	0,47	0,6460
Error	0,02	6	3,3E-03		
Total	0,02	8			

Test:Tukey Alfa:=0,05 DMS:=0,14482

Error: 0,0033 gl: 6

Tiempo	Medias	n	_
0,00	0,15	3	A
8,00	0,17	3	A
4,00	0,20	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Capsicum chinense* (A_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,53	0,37	49,51

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	7,19	2	3,60	3,37	0,1045
Tiempo	7,19	2	3,60	3,37	0,1045
Error	6,40	6	1,07		
Total	13,59	8			

Test:Tukey Alfa:=0,01 DMS:=3,77525

Error: 1,0671 gl: 6

Tiempo	Medias	n	_
4,00	1,16	3	A
8,00	1,81	3	A
0,00	3,29	3	A

Letras distintas indican diferencias significativas (p<= 0,01)

Roots of *Capsicum chinense* (B_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,66	0,55	32,29

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	22,35	2	11,17	5,91	0,0382
Tiempo	22,35	2	11,17	5,91	0,0382
Error	11,35	6	1,89		
Total	33,70	8			

Test:Tukey Alfa:=0,01 DMS:=5,02619

Error: 1,8914 gl: 6

Tiempo	Medias	n	_
4,00	2,54	3	A
0,00	3,89	3	A
8,00	6,35	3	A

Letras distintas indican diferencias significativas (p<= 0,01)

Roots of *Cucurbita moschata* (C) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,65	0,53	27,47

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,05	2	0,02	5,53	0,0434
Tiempo	0,05	2	0,02	5,53	0,0434
Error	0,03	6	4,4E-03		
Total	0,08	8			

Test:Tukey Alfa:=0,05 DMS:=0,16621

Error: 0,0044 gl: 6

Tiempo	Medias	n		
8,00	0,15	3	A	
4,00	0,25	3	A	B
0,00	0,33	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Cucurbita moschata* (A_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,08	0,00	33,39

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	1,31	2	0,65	0,27	0,7739
Tiempo	1,31	2	0,65	0,27	0,7739
Error	14,64	6	2,44		
Total	15,94	8			

Test:Tukey Alfa:=0,01 DMS:=5,70774

Error: 2,4392 gl: 6

Tiempo	Medias	n	
0,00	4,18	3	A
8,00	4,75	3	A
4,00	5,10	3	A

Letras distintas indican diferencias significativas (p<= 0,01)

Roots of *Cucurbita moschata* (B_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,97	0,96	11,81

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	209,76	2	104,88	102,31	<0,0001
Tiempo	209,76	2	104,88	102,31	<0,0001
Error	6,15	6	1,03		
Total	215,91	8			

Test:Tukey Alfa:=0,01 DMS:=3,70027

Error: 1,0251 gl: 6

Tiempo	Medias	n			
0,00	2,26	3	A		
4,00	9,48	3		B	
8,00	13,98	3			C

Letras distintas indican diferencias significativas (p<= 0,01)

Roots of *Cajanus cajan* (C) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,96	0,94	10,27

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,07	2	0,03	69,08	0,0001
Tiempo	0,07	2	0,03	69,08	0,0001
Error	3,0E-03	6	5,0E-04		
Total	0,07	8			

Test:Tukey Alfa:=0,05 DMS:=0,05577

Error: 0,0005 gl: 6

Tiempo	Medias	n		
8,00	0,09	3	A	
4,00	0,27	3		B
0,00	0,29	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Cajanus cajan* (A_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,78	0,70	6,73

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	1,01	2	0,51	10,40	0,0112
Tiempo	1,01	2	0,51	10,40	0,0112
Error	0,29	6	0,05		
Total	1,30	8			

Test:Tukey Alfa:=0,01 DMS:=0,80601

Error: 0,0486 gl: 6

Tiempo	Medias	n	
8,00	2,82	3	A
4,00	3,39	3	A
0,00	3,62	3	A

Letras distintas indican diferencias significativas (p<= 0,01)

Roots of *Cajanus cajan* (B_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,33	0,10	38,99

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	6,55	2	3,28	1,46	0,3047
Tiempo	6,55	2	3,28	1,46	0,3047
Error	13,48	6	2,25		
Total	20,03	8			

Test:Tukey Alfa:=0,01 DMS:=5,47816

Error: 2,2469 gl: 6

Tiempo	Medias	n	
4,00	3,03	3	A
0,00	3,48	3	A
8,00	5,02	3	A

Letras distintas indican diferencias significativas (p<= 0,01)

Leaves of *Capsicum chinense* (A_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,13	0,00	80,48

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,98	2	0,49	0,46	0,6518
Tiempo	0,98	2	0,49	0,46	0,6518
Error	6,39	6	1,06		
Total	7,36	8			

Test:Tukey Alfa:=0,01 DMS:=3,77007

Error: 1,0642 gl: 6

Tiempo	Medias	n	_
4,00	1,03	3	A
0,00	1,06	3	A
8,00	1,75	3	A

Letras distintas indican diferencias significativas (p<= 0,01)

Leaves of *Capsicum chinense* (B_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,56	0,41	37,11

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	4,84	2	2,42	3,81	0,0856
Tiempo	4,84	2	2,42	3,81	0,0856
Error	3,82	6	0,64		
Total	8,66	8			

Test:Tukey Alfa:=0,01 DMS:=2,91464

Error: 0,6360 gl: 6

Tiempo	Medias	n	_
4,00	1,27	3	A
8,00	2,10	3	A
0,00	3,07	3	A

Letras distintas indican diferencias significativas (p<= 0,01)

Leaves of *Cucurbita moschata* (C) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,12	0,00	23,43

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	1,3E-03	2	6,7E-04	0,43	0,6711
Tiempo	1,3E-03	2	6,7E-04	0,43	0,6711
Error	0,01	6	1,6E-03		
Total	0,01	8			

Test:Tukey Alfa:=0,05 DMS:=0,09948

Error: 0,0016 gl: 6

Tiempo	Medias	n	
0,00	0,15	3	A
4,00	0,18	3	A
8,00	0,18	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Cucurbita moschata* (A_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,40	0,20	62,48

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	11,79	2	5,89	2,00	0,2157
Tiempo	11,79	2	5,89	2,00	0,2157
Error	17,65	6	2,94		
Total	29,44	8			

Test:Tukey Alfa:=0,01 DMS:=6,26901

Error: 2,9425 gl: 6

Tiempo	Medias	n	
0,00	1,63	3	A
4,00	2,29	3	A
8,00	4,32	3	A

Letras distintas indican diferencias significativas (p<= 0,01)

Leaves of *Cucurbita moschata* (B_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,98	0,97	10,99

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	57,58	2	28,79	121,01	<0,0001
Tiempo	57,58	2	28,79	121,01	<0,0001
Error	1,43	6	0,24		
Total	59,01	8			

Test:Tukey Alfa:=0,01 DMS:=1,78266

Error: 0,2379 gl: 6

Tiempo	Medias	n		
0,00	2,53	3	A	
4,00	2,77	3	A	
8,00	8,01	3		B

Letras distintas indican diferencias significativas (p<= 0,01)

Leaves of *Cajanus cajan* (C) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,80	0,73	33,80

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,03	2	0,01	11,94	0,0081
Tiempo	0,03	2	0,01	11,94	0,0081
Error	0,01	6	1,1E-03		
Total	0,03	8			

Test:Tukey Alfa:=0,05 DMS:=0,08233

Error: 0,0011 gl: 6

Tiempo	Medias	n		
8,00	0,06	3	A	
4,00	0,06	3	A	
0,00	0,17	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Cajanus cajan* (A_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,54	0,38	76,15

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	38,65	2	19,32	3,50	0,0983
Tiempo	38,65	2	19,32	3,50	0,0983
Error	33,13	6	5,52		
Total	71,77	8			

Test:Tukey Alfa:=0,01 DMS:=8,58742

Error: 5,5213 gl: 6

Tiempo	Medias	n	
4,00	1,45	3	A
0,00	1,80	3	A
8,00	6,01	3	A

Letras distintas indican diferencias significativas (p<= 0,01)

Leaves of *Cajanus cajan* (B_1) at 0, 4, 8 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,42	0,23	24,45

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	1,88	2	0,94	2,17	0,1949
Tiempo	1,88	2	0,94	2,17	0,1949
Error	2,59	6	0,43		
Total	4,47	8			

Test:Tukey Alfa:=0,01 DMS:=2,40055

Error: 0,4315 gl: 6

Tiempo	Medias	n	
4,00	2,04	3	A
0,00	3,00	3	A
8,00	3,02	3	A

Letras distintas indican diferencias significativas (p<= 0,01)

Steams of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (A_1) at 0 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,56	0,41	42,14

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,51	2	0,26	3,83	0,0849
Planta	0,51	2	0,26	3,83	0,0849
Error	0,40	6	0,07		
Total	0,91	8			

Test:Tukey Alfa:=0,05 DMS:=0,64738

Error: 0,0668 gl: 6

Plantas	Medias	n	
C. chinense	0,36	3	A
C. cajan	0,54	3	A
C. moschata	0,93	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Steams of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (A_1) at 4 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,40	0,20	45,76

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,54	2	0,27	1,99	0,2173
Planta	0,54	2	0,27	1,99	0,2173
Error	0,81	6	0,13		
Total	1,35	8			

Test:Tukey Alfa:=0,05 DMS:=0,92029

Error: 0,1350 gl: 6

Planta	Medias	n	
C. cajan	0,50	3	A
C. chinense	0,82	3	A
C. moschata	1,09	3	A

Steams of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (A_1) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,68	0,57	49,06

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	6,39	2	3,19	6,40	0,0325
Planta	6,39	2	3,19	6,40	0,0325
Error	2,99	6	0,50		
Total	9,38	8			

Test:Tukey Alfa:=0,05 DMS:=1,76962

Error: 0,4990 gl: 6

Planta	Medias	n		
C. cajan	0,76	3	A	
C. chinese	0,93	3	A	B
C. moschata	2,63	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Steams of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (B_1) at 0 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,74	0,65	12,82

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,46	2	0,23	8,51	0,0177
Planta	0,46	2	0,23	8,51	0,0177
Error	0,16	6	0,03		
Total	0,63	8			

Test:Tukey Alfa:=0,05 DMS:=0,41322

Error: 0,0272 gl: 6

Planta	Medias	n		
C. cajan	0,97	3	A	
C. moschata	1,43	3		B
C. chinense	1,46	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Steams of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (B_1) at 4 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,99	0,99	13,00

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	405,00	2	202,50	400,23	<0,0001
Planta	405,00	2	202,50	400,23	<0,0001
Error	3,04	6	0,51		
Total	408,03	8			

Test:Tukey Alfa:=0,05 DMS:=1,78191

Error: 0,5060 gl: 6

Planta	Medias	n		
C. chinense	0,72	3	A	
C. cajan	0,74	3	A	
C. moschata	14,96	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Steams of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (B_1) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,99	0,99	11,74

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	218,05	2	109,02	370,71	<0,0001
Planta	218,05	2	109,02	370,71	<0,0001
Error	1,76	6	0,29		
Total	219,81	8			

Test:Tukey Alfa:=0,05 DMS:=1,35854

Error: 0,2941 gl: 6

Planta	Medias	n	
C. chinense	0,49	3	A
C. cajan	1,83	3	A
C. moschata	11,54	3	B

Letras distintas indican diferencias significativas(p<= 0,05)

Roots of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (A_1) at 0 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,18	0,00	25,82

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	1,20	2	0,60	0,66	0,5512
Planta	1,20	2	0,60	0,66	0,5512
Error	5,47	6	0,91		
Total	6,67	8			

Test:Tukey Alfa:=0,05 DMS:=2,39176

Error: 0,9115 gl: 6

Planta	Medias	n	
C. chinense	3,29	3	A
C. cajan	3,62	3	A
C. moschata	4,18	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (A_1) at 4 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,89	0,86	12,27

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	2,19	2	1,09	24,65	0,0013
Planta	2,19	2	1,09	24,65	0,0013
Error	0,27	6	0,04		
Total	2,45	8			

Test:Tukey Alfa:=0,05 DMS:=0,52738

Error: 0,0443 gl: 6

Planta	Medias	n		
C. chinense	1,06	3	A	
C. cajan	1,84	3		B
C. moschata	2,25	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (A_1) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,52	0,35	24,20

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,68	2	0,34	3,20	0,1132
Planta	0,68	2	0,34	3,20	0,1132
Error	0,63	6	0,11		
Total	1,31	8			

Test:Tukey Alfa:=0,05 DMS:=0,81379

Error: 0,1055 gl: 6

Planta	Medias	n	
C. chinense	1,01	3	A
C. cajan	1,34	3	A
C. moschata	1,68	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (B_1) at 0 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,32	0,09	38,85

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	4,31	2	2,15	1,39	0,3201
Planta	4,31	2	2,15	1,39	0,3201
Error	9,32	6	1,55		
Total	13,63	8			

Test:Tukey Alfa:=0,05 DMS:=3,12252

Error: 1,5537 gl: 6

Planta	Medias	n	
C. moschata	2,26	3	A
C. cajan	3,48	3	A
C. chinense	3,89	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (B_1) at 4 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,86	0,82	30,91

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	89,96	2	44,98	18,70	0,0026
Planta	89,96	2	44,98	18,70	0,0026
Error	14,43	6	2,40		
Total	104,39	8			

Test:Tukey Alfa:=0,05 DMS:=3,88492

Error: 2,4049 gl: 6

Planta	Medias	n		
C. chinense	2,54	3	A	
C. cajan	3,03	3	A	
C. moschata	9,48	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (B_1) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,95	0,93	12,99

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	140,21	2	70,11	58,19	0,0001
Planta	140,21	2	70,11	58,19	0,0001
Error	7,23	6	1,20		
Total	147,44	8			

Test:Tukey Alfa:=0,05 DMS:=2,74980

Error: 1,2049 gl: 6

Planta	Medias	n		
C. cajan	5,02	3	A	
C. chinense	6,35	3	A	
C. moschata	13,98	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (A_1) at 0 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,12	0,00	68,65

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,88	2	0,44	0,42	0,6756
Planta	0,88	2	0,44	0,42	0,6756
Error	6,33	6	1,06		
Total	7,22	8			

Test:Tukey Alfa:=0,05 DMS:=2,57348

Error: 1,0553 gl: 6

Planta	Medias	n	_
C. chinense	1,06	3	A
C. moschata	1,63	3	A
C. cajan	1,80	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (A_1) at 4 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,32	0,09	58,29

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	2,44	2	1,22	1,42	0,3132
Planta	2,44	2	1,22	1,42	0,3132
Error	5,16	6	0,86		
Total	7,60	8			

Test:Tukey Alfa:=0,05 DMS:=2,32328

Error: 0,8601 gl: 6

Planta	Medias	n	_
C. chinense	1,03	3	A
C. cajan	1,45	3	A
C. moschata	2,29	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (A_1) at 8 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,38	0,17	68,54

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	27,63	2	13,82	1,81	0,2419
Planta	27,63	2	13,82	1,81	0,2419
Error	45,68	6	7,61		
Total	73,31	8			

Test:Tukey Alfa:=0,05 DMS:=6,91183

Error: 7,6125 gl: 6

Planta	Medias	n	
C. chinense	1,75	3	A
C. moschata	4,32	3	A
C. cajan	6,01	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (B_1) at 0 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,27	0,03	16,94

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,52	2	0,26	1,11	0,3886
Planta	0,52	2	0,26	1,11	0,3886
Error	1,41	6	0,24		
Total	1,94	8			

Test:Tukey Alfa:=0,05 DMS:=1,21623

Error: 0,2357 gl: 6

Planta	Medias	n	
C. moschata	2,53	3	A
C. cajan	3,00	3	A
C. chinense	3,07	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (B_1) at 4 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,56	0,42	32,48

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	3,37	2	1,68	3,88	0,0830
Planta	3,37	2	1,68	3,88	0,0830
Error	2,61	6	0,43		
Total	5,97	8			

Test:Tukey Alfa:=0,05 DMS:=1,65121

Error: 0,4345 gl: 6

Planta	Medias	n	
C. chinense	1,27	3	A
C. cajan	2,04	3	A
C. moschata	2,77	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Capsicum chinense, Cucurbita moschata and Cajanus cajan* (B_1) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,94	0,92	18,21

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	60,67	2	30,34	47,75	0,0002
Planta	60,67	2	30,34	47,75	0,0002
Error	3,81	6	0,64		
Total	64,48	8			

Test:Tukey Alfa:=0,05 DMS:=1,99666

Error: 0,6353 gl: 6

Planta	Medias	n		
C. chinense	2,10	3	A	
C. cajan	3,02	3	A	
C. moschata	8,01	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Stem of *Capsicum chinense* (C, A_1, B_1) at 0 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,89	0,85	26,34

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	2,09	2	1,04	24,19	0,0013
Tratamiento	2,09	2	1,04	24,19	0,0013
Error	0,26	6	0,04		
Total	2,35	8			

Test:Tukey Alfa:=0,05 DMS:=0,52023

Error: 0,0431 gl: 6

Tratamiento	Medias	n	
A_1	0,36	3	A
C	0,54	3	A
B_1	1,46	3	B

Letras distintas indican diferencias significativas (p<= 0,05)

Stem of *Capsicum chinense* (C, A_1, B_1) at 4 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,64	0,52	47,55

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,77	2	0,39	5,41	0,0454
Tratamiento	0,77	2	0,39	5,41	0,0454
Error	0,43	6	0,07		
Total	1,20	8			

Test:Tukey Alfa:=0,05 DMS:=0,66925

Error: 0,0714 gl: 6

Tratamiento	Medias	n	
C	0,15	3	A
B_1	0,72	3	A
A_1	0,82	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Stem of *Capsicum chinense* (C, A_1, B_1) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,49	0,33	53,78

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	2,21	2	1,11	2,93	0,1296
Tratamiento	2,21	2	1,11	2,93	0,1296
Error	2,27	6	0,38		
Total	4,48	8			

Test:Tukey Alfa:=0,05 DMS:=1,54026

Error: 0,3780 gl: 6

Tratamiento	Medias	n	
C	0,67	3	A
A_1	0,93	3	A
B_1	1,83	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Stem of *Cucurbita moschata* (C, A_1, B_1) at 0 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,84	0,79	36,07

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	4,93	2	2,46	15,88	0,0040
Tratamiento	4,93	2	2,46	15,88	0,0040
Error	0,93	6	0,16		
Total	5,86	8			

Test:Tukey Alfa:=0,05 DMS:=0,98685

Error: 0,1552 gl: 6

Tratamiento	Medias	n		
C	0,28	3	A	
A_1	0,93	3	A	
B_1	2,07	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Stem of *Cucurbita moschata* (C, A_1, B_1) at 4 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,99	0,99	13,27

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	403,55	2	201,78	379,75	<0,0001
Tratamiento	403,55	2	201,78	379,75	<0,0001
Error	3,19	6	0,53		
Total	406,74	8			

Test:Tukey Alfa:=0,05 DMS:=1,82606

Error: 0,5313 gl: 6

Tratamiento	Medias	n		
C	0,43	3	A	
A_1	1,09	3	A	
B_1	14,96	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Stem of *Cajanus cajan* (C, A_1, B_1) at 4 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,60	0,47	46,62

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,25	2	0,12	4,55	0,0626
Tratamiento	0,25	2	0,12	4,55	0,0626
Error	0,16	6	0,03		
Total	0,41	8			

Test:Tukey Alfa:=0,05 DMS:=0,41223

Error: 0,0271 gl: 6

Tratamiento	Medias	n	
C	0,13	3	A
A_1	0,40	3	A
B_1	0,53	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Stem of *Cajanus cajan* (C, A_1, B_1) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,47	0,30	62,44

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,49	2	0,25	2,71	0,1448
Tratamiento	0,49	2	0,25	2,71	0,1448
Error	0,55	6	0,09		
Total	1,04	8			

Test:Tukey Alfa:=0,05 DMS:=0,75619

Error: 0,0911 gl: 6

Tratamiento	Medias	n	
C	0,19	3	A
B_1	0,49	3	A
A_1	0,76	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Capsicum chinense* (C, A_1, B_1) at 0 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,73	0,64	50,10

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	24,17	2	12,09	8,07	0,0199
Tratamiento	24,17	2	12,09	8,07	0,0199
Error	8,99	6	1,50		
Total	33,17	8			

Test:Tukey Alfa:=0,05 DMS:=3,06652

Error: 1,4984 gl: 6

Tratamiento	Medias	n		
C	0,15	3	A	
A_1	3,29	3		B
B_1	3,89	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Capsicum chinense* (C, A_1, B_1) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,96	0,94	24,99

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	61,46	2	30,73	63,83	0,0001
Tratamiento	61,46	2	30,73	63,83	0,0001
Error	2,89	6	0,48		
Total	64,35	8			

Test:Tukey Alfa:=0,05 DMS:=1,73816

Error: 0,4814 gl: 6

Tratamiento	Medias	n		
C	0,17	3	A	
A_1	1,81	3	A	
B_1	6,35	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Cucurbita moschata* (C, A_1, B_1) at 0 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,97	0,96	14,86

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	22,25	2	11,13	99,16	<0,0001
Tratamiento	22,25	2	11,13	99,16	<0,0001
Error	0,67	6	0,11		
Total	22,93	8			

Test:Tukey Alfa:=0,05 DMS:=0,83914

Error: 0,1122 gl: 6

Tratamiento	Medias	n			
C	0,33	3	A		
B_1	2,26	3		B	
A_1	4,18	3			C

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Cucurbita moschata* (C, A_1, B_1) at 4 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,95	0,93	21,18

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	127,87	2	63,93	58,27	0,0001
Tratamiento	127,87	2	63,93	58,27	0,0001
Error	6,58	6	1,10		
Total	134,45	8			

Test:Tukey Alfa:=0,05 DMS:=2,62401

Error: 1,0972 gl: 6

Tratamiento	Medias	n			
C	0,25	3	A		
A_1	5,10	3		B	
B_1	9,48	3			C

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Cajanus cajan* (C, A_1, B_1) at 0 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,92	0,90	19,03

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	3,10	2	1,55	36,68	0,0004
Tratamiento	3,10	2	1,55	36,68	0,0004
Error	0,25	6	0,04		
Total	3,35	8			

Test:Tukey Alfa:=0,05 DMS:=0,51482

Error: 0,0422 gl: 6

Tratamiento	Medias	n		
C	0,25	3	A	
B_1	1,46	3		B
A_1	1,53	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Cajanus cajan* (C, A_1, B_1) at 4 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,76	0,68	43,04

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	17,46	2	8,73	9,47	0,0139
Tratamiento	17,46	2	8,73	9,47	0,0139
Error	5,53	6	0,92		
Total	22,99	8			

Test:Tukey Alfa:=0,05 DMS:=2,40494

Error: 0,9216 gl: 6

Tratamiento	Medias	n		
C	0,27	3	A	
B_1	3,03	3		B
A_1	3,39	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Capsicum chinense* (C, A_1, B_1) at 0 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,60	0,47	59,56

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	8,87	2	4,44	4,49	0,0643
Tratamiento	8,87	2	4,44	4,49	0,0643
Error	5,93	6	0,99		
Total	14,81	8			

Test:Tukey Alfa:=0,05 DMS:=2,49136

Error: 0,9890 gl: 6

Tratamiento	Medias	n	
C	0,88	3	A
A_1	1,06	3	A
B_1	3,07	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Capsicum chinense* (C, A_1, B_1) at 4 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,74	0,65	40,30

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	1,94	2	0,97	8,59	0,0174
Tratamiento	1,94	2	0,97	8,59	0,0174
Error	0,68	6	0,11		
Total	2,62	8			

Test:Tukey Alfa:=0,05 DMS:=0,84156

Error: 0,1129 gl: 6

Tratamiento	Medias	n		
C	0,19	3	A	
A_1	1,03	3		B
B_1	1,27	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Capsicum chinense* (C, A_1, B_1) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,38	0,17	76,75

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	4,41	2	2,21	1,80	0,2438
Tratamiento	4,41	2	2,21	1,80	0,2438
Error	7,34	6	1,22		
Total	11,75	8			

Test:Tukey Alfa:=0,05 DMS:=2,77134

Error: 1,2238 gl: 6

Tratamiento	Medias	n	
C	0,47	3	A
A_1	1,75	3	A
B_1	2,10	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Cucurbita moschata* (C, A_1, B_1) at 0 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,63	0,50	64,35

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	8,63	2	4,31	5,05	0,0518
Tratamiento	8,63	2	4,31	5,05	0,0518
Error	5,13	6	0,85		
Total	13,76	8			

Test:Tukey Alfa:=0,05 DMS:=2,31612

Error: 0,8548 gl: 6

Tratamiento	Medias	n		
C	0,15	3	A	
A_1	1,63	3	A	B
B_1	2,53	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Cucurbita moschata* (C, A_1, B_1) at 4 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,69	0,59	52,87

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	11,43	2	5,71	6,71	0,0295
Tratamiento	11,43	2	5,71	6,71	0,0295
Error	5,11	6	0,85		
Total	16,53	8			

Test:Tukey Alfa:=0,05 DMS:=2,31176

Error: 0,8516 gl: 6

Tratamiento	Medias	n		
C	0,18	3	A	
A_1	2,29	3	A	B
B_1	2,77	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Cajanus cajan* (C, A_1, B_1) at 0 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	1,00	0,99	5,61

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	12,08	2	6,04	699,08	<0,0001
Tratamiento	12,08	2	6,04	699,08	<0,0001
Error	0,05	6	0,01		
Total	12,13	8			

Test:Tukey Alfa:=0,05 DMS:=0,23283

Error: 0,0086 gl: 6

Tratamiento	Medias	n			
C	0,17	3	A		
A_1	1,80	3		B	
B_1	3,00	3			C

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Cajanus cajan* (C, A_1, B_1) at 4 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,76	0,68	48,58

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	6,19	2	3,10	9,33	0,0144
Tratamiento	6,19	2	3,10	9,33	0,0144
Error	1,99	6	0,33		
Total	8,18	8			

Test:Tukey Alfa:=0,05 DMS:=1,44256

Error: 0,3316 gl: 6

Tratamiento	Medias	n		
C	0,06	3	A	
A_1	1,45	3	A	B
B_1	2,04	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Cajanus cajan* (C, A_1, B_1) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,61	0,48	78,25

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	53,17	2	26,58	4,74	0,0583
Tratamiento	53,17	2	26,58	4,74	0,0583
Error	33,68	6	5,61		
Total	86,85	8			

Test:Tukey Alfa:=0,05 DMS:=5,93538

Error: 5,6136 gl: 6

Tratamiento	Medias	n		
C	0,06	3	A	
B_1	3,02	3	A	B
A_1	6,01	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Stems of *Capsicum chinense, Cucurbita moschata* and *Cajanus cajan* (A_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,86	0,82	19,57

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	2,12	2	1,06	19,00	0,0025
Plantas	2,12	2	1,06	19,00	0,0025
Error	0,33	6	0,06		
Total	2,45	8			

Test:Tukey Alfa:=0,05 DMS:=0,59117

Error: 0,0557 gl: 6

Plantas	Medias	n		
C. cajan	0,84	3	A	
C. chinense	0,89	3	A	
C. moschata	1,89	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Capsicum chinense, Cucurbita moschata* and *Cajanus cajan* (A_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,88	0,84	23,38

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	36,36	2	18,18	21,76	0,0018
Plantas	36,36	2	18,18	21,76	0,0018
Error	5,01	6	0,84		
Total	41,37	8			

Test:Tukey Alfa:=0,05 DMS:=2,28963

Error: 0,8354 gl: 6

Plantas	Medias	n		
C. cajan	2,21	3	A	
C. chinense	2,79	3	A	
C. moschata	6,73	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Capsicum chinense, Cucurbita moschata* and *Cajanus cajan* (A_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,95	0,94	11,60

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	10,92	2	5,46	59,27	0,0001
Plantas	10,92	2	5,46	59,27	0,0001
Error	0,55	6	0,09		
Total	11,47	8			

Test:Tukey Alfa:=0,05 DMS:=0,76035

Error: 0,0921 gl: 6

Plantas	Medias	n		
C. chinense	1,10	3	A	
C. moschata	3,08	3		B
C. cajan	3,68	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Stems of *Capsicum chinense, Cucurbita moschata* and *Cajanus cajan* (B_2) at 8 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,99	0,99	8,91

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	13,89	2	6,95	293,53	<0,0001
Plantas	13,89	2	6,95	293,53	<0,0001
Error	0,14	6	0,02		
Total	14,03	8			

Test:Tukey Alfa:=0,05 DMS:=0,38534

Error: 0,0237 gl: 6

Plantas	Medias	n		
C. cajan	0,78	3	A	
C. chinense	0,92	3	A	
C. moschata	3,48	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Capsicum chinense, Cucurbita moschata* and *Cajanus cajan* (B_2) at 8 weeks

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Valor	9	0,98	0,97	14,75

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	831,45	2	415,73	149,13	<0,0001
Plantas	831,45	2	415,73	149,13	<0,0001
Error	16,73	6	2,79		
Total	848,18	8			

Test:Tukey Alfa:=0,05 DMS:=4,18264

Error: 2,7877 gl: 6

Plantas	Medias	n		
C. chinense	3,19	3	A	
C. cajan	5,96	3	A	
C. moschata	24,82	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Capsicum chinense, Cucurbita moschata* and *Cajanus cajan* (B_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	9	0,87	0,83	39,39

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	1147,33	2	573,67	20,60	0,0021
Plantas	1147,33	2	573,67	20,60	0,0021
Error	167,09	6	27,85		
Total	1314,42	8			

Test:Tukey Alfa:=0,05 DMS:=13,21973

Error: 27,8476 gl: 6

Plantas	Medias	n		
C. chinense	2,45	3	A	
C. moschata	8,80	3	A	
C. cajan	28,94	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Stems of *Capsicum chinense* (A_1, A_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,09	0,00	29,02

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,02	1	0,02	0,38	0,5707
Tratamiento	0,02	1	0,02	0,38	0,5707
Error	0,25	4	0,06		
Total	0,28	5			

Test:Tukey Alfa:=0,05 DMS:=0,56871

Error: 0,0629 gl: 4

Tratamiento	Medias	n	
A_2	0,80	3	A
A_1	0,93	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Stems of *Cucurbita moschata* (A_1, A_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,19	0,00	41,73

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	1,64	1	1,64	0,95	0,3854
Tratamiento	1,64	1	1,64	0,95	0,3854
Error	6,91	4	1,73		
Total	8,55	5			

Test:Tukey Alfa:=0,05 DMS:=2,98009

Error: 1,7277 gl: 4

Tratamiento	Medias	n	
A_1	2,63	3	A
A_2	3,67	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Stems of *Cajanus cajan* (A_1, A_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,01	0,00	49,13

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,01	1	0,01	0,04	0,8450
Tratamiento	0,01	1	0,01	0,04	0,8450
Error	0,52	4	0,13		
Total	0,53	5			

Test:Tukey Alfa:=0,05 DMS:=0,81786

Error: 0,1301 gl: 4

Tratamiento	Medias	n	
A_2	0,70	3	A
A_1	0,76	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Stems of *Capsicum chinense* (B_1, B_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,42	0,27	51,17

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	1,38	1	1,38	2,89	0,1646
Tratamiento	1,38	1	1,38	2,89	0,1646
Error	1,91	4	0,48		
Total	3,29	5			

Test:Tukey Alfa:=0,05 DMS:=1,56626

Error: 0,4772 gl: 4

Tratamiento	Medias	n	
B_2	0,87	3	A
B_1	1,83	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Stems of *Cajanus cajan* (B_1, B_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,30	0,12	20,23

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,02	1	0,02	1,68	0,2652
Tratamiento	0,02	1	0,02	1,68	0,2652
Error	0,05	4	0,01		
Total	0,07	5			

Test:Tukey Alfa:=0,05 DMS:=0,25383

Error: 0,0125 gl: 4

Tratamiento	Medias	n	
B_1	0,49	3	A
B_2	0,61	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Capsicum chinense* (A_1, A_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,26	0,08	43,50

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	1,43	1	1,43	1,43	0,2976
Tratamiento	1,43	1	1,43	1,43	0,2976
Error	4,00	4	1,00		
Total	5,43	5			

Test:Tukey Alfa:=0,05 DMS:=2,26688

Error: 0,9997 gl: 4

Tratamiento	Medias	n	
A_1	1,81	3	A
A_2	2,79	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Cajanus cajan* (A_1, A_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,20	0,00	29,87

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,56	1	0,56	1,00	0,3740
Tratamiento	0,56	1	0,56	1,00	0,3740
Error	2,26	4	0,57		
Total	2,83	5			

Test:Tukey Alfa:=0,05 DMS:=1,70432

Error: 0,5651 gl: 4

Tratamiento	Medias	n	
A_2	2,21	3	A
A_1	2,82	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Capsicum chinense* (B_1, B_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,56	0,45	35,75

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	14,97	1	14,97	5,15	0,0858
Tratamiento	14,97	1	14,97	5,15	0,0858
Error	11,63	4	2,91		
Total	26,60	5			

Test:Tukey Alfa:=0,05 DMS:=3,86614

Error: 2,9077 gl: 4

Tratamiento	Medias	n	
B_2	3,19	3	A
B_1	6,35	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Cucurbita moschata* (B_1, B_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,97	0,96	6,14

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	176,30	1	176,30	124,40	0,0004
Tratamiento	176,30	1	176,30	124,40	0,0004
Error	5,67	4	1,42		
Total	181,97	5			

Test:Tukey Alfa:=0,05 DMS:=2,69910

Error: 1,4172 gl: 4

Tratamiento	Medias	n		
B_1	13,98	3	A	
B_2	24,82	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Roots of *Cajanus cajan* (B_1, B_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,16	0,00	23,50

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	1,31	1	1,31	0,78	0,4257
Tratamiento	1,31	1	1,31	0,78	0,4257
Error	6,66	4	1,66		
Total	7,96	5			

Test:Tukey Alfa:=0,05 DMS:=2,92458

Error: 1,6639 gl: 4

Tratamiento	Medias	n	
B_1	5,02	3	A
B_2	5,96	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Capsicum chinense* (A_1, A_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,07	0,00	74,75

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,39	1	0,39	0,31	0,6061
Tratamiento	0,39	1	0,39	0,31	0,6061
Error	4,98	4	1,25		
Total	5,37	5			

Test:Tukey Alfa:=0,05 DMS:=2,53074

Error: 1,2459 gl: 4

Tratamiento	Medias	n	
A_2	1,24	3	A
A_1	1,75	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Cucurbita moschatae* (A_1, A_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,80	0,76	22,53

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	39,72	1	39,72	16,47	0,0154
Tratamiento	39,72	1	39,72	16,47	0,0154
Error	9,65	4	2,41		
Total	49,36	5			

Test:Tukey Alfa:=0,05 DMS:=3,52069

Error: 2,4113 gl: 4

Tratamiento	Medias	n		
A_1	4,32	3	A	
A_2	9,46	3		B

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Cajanus cajan* (A_1, A_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,62	0,52	37,32

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	87,59	1	87,59	6,51	0,0632
Tratamiento	87,59	1	87,59	6,51	0,0632
Error	53,84	4	13,46		
Total	141,43	5			

Test:Tukey Alfa:=0,05 DMS:=8,31778

Error: 13,4590 gl: 4

Tratamiento	Medias	n	
A1	6,01	3	A
A2	13,65	3	A

Letras distintas indican diferencias significativas (p<= 0,05)

Leaves of *Capsicum chinense* (B_1, B_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,02	0,00	61,98

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0,18	1	0,18	0,09	0,7768
Tratamiento	0,18	1	0,18	0,09	0,7768
Error	7,98	4	1,99		
Total	8,16	5			

Test:Tukey Alfa:=0,01 DMS:=5,30735

Error: 1,9940 gl: 4

Tratamiento	Medias	n	
B_1	2,10	3	A
B_2	2,45	3	A

Letras distintas indican diferencias significativas (p<= 0,01)

Leaves of *Cajanus cajan* (B_1, B_2) at 8 weeks

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Valor	6	0,89	0,86	35,57

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	1007,73	1	1007,73	31,19	0,0050
Tratamiento	1007,73	1	1007,73	31,19	0,0050
Error	129,22	4	32,31		
Total	1136,95	5			

Test:Tukey Alfa:=0,01 DMS:=21,36303

Error: 32,3061 gl: 4

Tratamiento	Medias	n		
B_1	3,02	3	A	
B_2	28,94	3		B

Letras distintas indican diferencias significativas (p<= 0,01)

APPENDIX J STUDENT-T TEST

Stems of *Capsicum chinense* (C) at 0, 4 and 8 weeks

Clasific	Variable	Grupo(1)	Grupo(2)	n(1)	n(2)	media(1)	media(2)	p(Var.Hom.)	T	gl	p	prueba
TIEMPO	VALOR	{0,00}	{4,00}	3	3	0,34	0,15	0,0027	1,01	2	0,4189	Bilateral
TIEMPO	VALOR	{0,00}	{8,00}	3	3	0,34	0,67	0,5648	-0,98	4	0,3826	Bilateral
TIEMPO	VALOR	{4,00}	{8,00}	3	3	0,15	0,67	0,0011	-1,79	2	0,2153	Bilateral

Stems of *Cucurbita moschata* (C) at 0, 4 and 8 weeks

Clasific	Variable	Grupo(1)	Grupo(2)	n(1)	n(2)	media(1)	media(2)	p(Var.Hom.)	T	gl	p	prueba
TIEMPO	VALOR	{0,00}	{4,00}	3	3	0,28	0,43	0,0956	-0,72	4	0,5104	Bilateral
TIEMPO	VALOR	{0,00}	{8,00}	3	3	0,28	0,25	0,1573	0,57	4	0,5962	Bilateral
TIEMPO	VALOR	{4,00}	{8,00}	3	3	0,43	0,25	0,0085	0,87	2	0,4752	Bilateral

Leaves of *Capsicum chinense* (C) at 0, 4 and 8 weeks

Clasific	Variable	Grupo(1)	Grupo(2)	n(1)	n(2)	media(1)	media(2)	p(Var.Hom.)	T	gl	p	prueba
TIEMPO	VALOR	{0,00}	{4,00}	3	3	0,88	0,19	0,0017	0,91	2	0,4577	Bilateral
TIEMPO	VALOR	{0,00}	{8,00}	3	3	0,88	0,47	0,2069	0,51	4	0,6371	Bilateral
TIEMPO	VALOR	{4,00}	{8,00}	3	3	0,19	0,47	0,0149	-1,10	2	0,3861	Bilateral

Stems of *Cucurbita moschata* (C, A_1 and B_1) at 8 weeks

Clasific	Variable	Grupo(1)	Grupo(2)	n(1)	n(2)	media(1)	media(2)	p(Var.Hom.)	T	p	prueba
TRATAMIENTO	VALOR	{0,00}	{100,00}	3	3	0,25	2,63	0,0010	-3,81	0,0624	Bilateral
TRATAMIENTO	VALOR	{0,00}	{200,00}	3	3	0,25	11,54	0,0187	-77,78	0,0002	Bilateral
TRATAMIENTO	VALOR	{100,00}	{200,00}	3	3	2,63	11,54	0,1017	-13,90	0,0002	Bilateral

Stems of *Cajanus cajan* (C, A_1 and B_1) at 0 weeks

Clasific	Variable	Grupo(1)	Grupo(2)	n(1)	n(2)	media(1)	media(2)	p(Var.Hom.)	T	gl	p	prueba
TRATAMIENTO	VALOR	{0,00}	{100,00}	3	3	0,28	0,36	0,3341	-0,33	4	0,7570	Bilateral
TRATAMIENTO	VALOR	{0,00}	{200,00}	3	3	0,28	0,93	0,0414	-6,17	2	0,0253	Bilateral
TRATAMIENTO	VALOR	{100,00}	{200,00}	3	3	0,36	0,93	0,0084	-2,42	2	0,1362	Bilateral

Roots of *Capsicum chinense* C, A_1 and B_1) at 4 weeks

Clasific	Variable	Grupo(1)	Grupo(2)	n(1)	n(2)	media(1)	media(2)	p(Var.Hom.)	T	gl	p	prueba
TRATAMIENTO	VALOR	{0,00}	{100,00}	3	3	0,20	1,16	0,0151	-3,35	2	0,0785	Bilateral
TRATAMIENTO	VALOR	{0,00}	{200,00}	3	3	0,20	2,54	0,0014	-2,47	2	0,1320	Bilateral
TRATAMIENTO	VALOR	{100,00}	{200,00}	3	3	1,16	2,54	0,1653	-1,40	4	0,2340	Bilateral

Roots of *Cucurbita moschata* (C, A_1 and B_1) at 8 weeks

Clasific	Variable	Grupo(1)	Grupo(2)	n(1)	n(2)	media(1)	media(2)	p(Var.Hom.)	T	gl	p	prueba
TRATAMIENTO	VALOR	{0,00}	{100,00}	3	3	0,15	4,75	0,0041	-3,39	2	0,0770	Bilateral
TRATAMIENTO	VALOR	{0,00}	{200,00}	3	3	0,15	13,98	0,0179	-21,38	2	0,0022	Bilateral
TRATAMIENTO	VALOR	{100,00}	{200,00}	3	3	4,75	13,98	0,3678	-6,14	4	0,0036	Bilateral

Roots of *Cajanus cajan* (C, A_1 and B_1) at 8 weeks

Clasific	Variable	Grupo(1)	Grupo(2)	n(1)	n(2)	media(1)	media(2)	p(Var.Hom.)	T	gl	p	prueba
TRATAMIENTO	VALOR	{0,00}	{100,00}	3	3	0,09	2,82	0,0074	-16,71	2	0,0036	Bilateral
TRATAMIENTO	VALOR	{0,00}	{200,00}	3	3	0,09	5,02	0,0004	-7,02	2	0,0197	Bilateral
TRATAMIENTO	VALOR	{100,00}	{200,00}	3	3	2,82	5,02	0,1023	-3,05	4	0,0379	Bilateral

Leaves of *Cucurbita moschata* (C, A_1 and B_1) at 8 weeks

Clasific	Variable	Grupo(1)	Grupo(2)	n(1)	n(2)	media(1)	media(2)	p(Var.Hom.)	T	gl	p	prueba
TRATAMIENTO	VALOR	{0,00}	{100,00}	3	3	0,18	4,32	0,0007	-3,41	2	0,0763	Bilateral
TRATAMIENTO	VALOR	{0,00}	{200,00}	3	3	0,18	8,01	0,3575	-141,87	4	<0,0001	Bilateral
TRATAMIENTO	VALOR	{100,00}	{200,00}	3	3	4,32	8,01	0,0034	-3,04	2	0,0933	Bilateral

Stems of *Cucurbita moschata* (B_1 and B_2) at 8 weeks

Clasific	Variable	Grupo(1)	Grupo(2)	n(1)	n(2)	media(1)	media(2)	p(Var.Hom.)	T	gl	p	prueba
TRATAMIENTO	valor	{1,00}	{2,00}	3	3	11,54	12,14	0,1133	-0,98	4	0,3807	Bilateral

Roots of *Cucurbita moschata* (A_1 and A_2) at 8 weeks

Clasific	Variable	Grupo(1)	Grupo(2)	n(1)	n(2)	media(1)	media(2)	p(Var.Hom.)	T	gl	p	prueba
TRATAMIENTO	valor	{1,00}	{2,00}	3	3	4,75	6,73	0,0002	-1,46	2	0,2822	Bilateral

Leaves of *Cucurbita moschata* (B_1 and B_2) at 8 weeks

Clasific	Variable	Grupo(1)	Grupo(2)	n(1)	n(2)	media(1)	media(2)	p(Var.Hom.)	T	gl	p	prueba
TRATAMIENTO	valor	{1,00}	{2,00}	3	3	8,01	8,80	0,0009	-0,33	2	0,7717	Bilatera