

Can *Salvia splendens* ‘Red Vista’ be used in association with the mycorrhizal fungus *Glomus intraradices* for the assisted phytoextraction of lead from the soil in Lima, Peru?

Puede *Salvia splendens* ‘Red Vista’ ser usada en asociación con el hongo micorrízico *Glomus intraradices* para la Fitoextracción asistida de plomo del suelo en Lima, Perú?

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Abstract

In Peru, gasoline containing lead as a main component was used in vehicles up until 2006, when the Government banned its sale. However, since this was preceded by a century of constant use, there is a high chance that most urban soils are polluted with this heavy metal. Hyperaccumulator plants that take up heavy metals from the soil and store them in their tissues without showing any symptoms of toxicity may be the solution to this problem, as the use of these plants for phytoremediation does not require large amounts of money, can be carried out *in situ*, and is environmentally friendly, making it one of the best options for urban areas. However, unfortunately, there are only a few known species of hyperaccumulator plants that can be grown in urban environments. Therefore, we conducted a bioassay at the Soil Fertility Laboratory of the Agronomy Faculty at the National Agrarian University La Molina, Peru, over a period of 4 months to determine the hyperaccumulation capacity of *Salvia splendens* ‘Red Vista’ and to examine whether the mycorrhizal fungus *Glomus intraradices* can enhance the extraction of lead from the soil. After harvest, the plants were divided into three parts (roots, leaves, and inflorescences) to determine the concentrations of lead in the various tissues. We found that *S. splendens* ‘Red Vista’ did not accumulate high amounts of lead in its tissues even when it was associated with *G. intraradices* and consequently should not be considered for use in phytoextraction.

Key words: *Salvia splendens*; *Glomus intraradices*; phytoremediation

Resumen

En Perú, la gasolina usada en vehículos motorizados contuvo plomo como uno de sus principales componentes por mucho tiempo, esto solo cambió luego del año 2006 cuando el gobierno prohibió la comercialización de gasolina con plomo en su composición. Esto sucedió hace solo unos años y luego de poco más de un siglo de constante actividad del parque automotor, de esta forma consideramos que existe una gran posibilidad de que la mayoría de suelos urbanos estén contaminados con este metal pesado. Las plantas hiperacumuladoras podrían ser la solución a este problema; estas plantas extraen y acumulan metales pesados del suelo en su tejido sin mostrar signos de toxicidad. El uso de plantas hiperacumuladoras en estrategias de fitorremediación no necesita de mucho dinero; es realizado *in situ* y es amigable con el ambiente, debido a esto es probablemente una de las mejores opciones para las áreas urbanas. Desafortunadamente no hay muchas especies conocidas de plantas hiperacumuladoras para áreas urbanas. Realizamos este bioensayo para determinar la capacidad de fitoextracción de plomo del suelo de *Salvia splendens* Var. ‘Red Vista’. El bioensayo fue establecido en las instalaciones del Laboratorio de Fertilidad de Suelos de la Universidad Nacional Agraria La Molina, duró cuatro meses y también se usó el hongo micorrízico *Glomus intraradices* en algunos tratamientos para probar la eficiencia de las micorrizas en la extracción de plomo en el suelo. Las plantas fueron cosechadas y divididas en tres partes: Raíces, tallos y hojas, e inflorescencias para observar la dinámica de plomo en la planta. Los resultados finales mostraron que *Salvia splendens* Var. ‘Red Vista’ no acumula grandes cantidades de plomo en su tejido aun cuando está asociado con *Glomus intraradices* y debido a esto no debe ser considerado para técnicas de fitoextracción de plomo.

Palabras Clave: *Salvia splendens*, *Glomus intraradices*, fitorremediación

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Introduction

The use of gasoline containing lead was banned on January 1, 2005 in Peru (Organismo Supervisor de la Inversión en Energía y Minería, 1998; Tamayo, Vásquez, & De la Cruz, 2015). However, the parks and other green areas in this country were exposed to the gaseous byproducts of the combustion of this fuel for a long period prior to this and lead tends to remain in the soil for many years, making it highly probable that they are contaminated with lead and are now the main source of lead pollution in Peru.

Lead is an extremely toxic element that can have terrible consequences. The World Health Organization stated that there is no amount of lead in the blood that can be considered safe, with levels as low as 5 µg/dL being capable of causing permanent damage (World Health Organization, 2014). Most of the time, the symptoms of lead poisoning go unnoticed – for example, it can result in the cognitive development of children being severely delayed, as well as behavioral disorders and even hypertension. However, it can also cause more obvious symptoms, such as anemia, fatigue, insomnia, and gastrointestinal disturbances (Word Health Organization, 2014; Londoño, Londoño, & Muñoz, 2017).

The use of hyperaccumulator plants for phytoremediation is probably the best strategy to decontaminate urban soils. Compared with other remediation strategies, it can be carried out *in situ*, is not disruptive, and does not require huge amounts of money. However, because of an urban environment, it is necessary to use only ornamental plants. Few ornamental plant species are currently registered as being capable of extracting heavy metals from the soil. However, Nowak (2007) determined that the ornamental plant *Salvia splendens* ‘Sello Torreador’ has the ability to accumulate up to 300 ppm of lead in its tissue without showing any symptoms of lead toxicity. Consequently, we considered that *S. splendens* ‘Red Vista’ may have the same ability. Therefore, in the present study, we investigated the ability of ‘Red Vista’ to extract lead from the soil, as well as the effects that mycorrhizae have on the phytoextraction of this heavy metal.

Materials and Methods

As indicated in the Guide for Soil Sampling of the Minister of the Environment (Ministerio del Ambiente, 2014), we first performed identification sampling; this type of sampling is performed to identify if the soil is contaminated. Soil samples were collected from two of the main parks in Lima, Peru [Campo de Marte (total area, 38 ha; $n = 42$ soil samples) and Kennedy Park (total area, 2.5 ha; $n = 19$ soil samples)] following the Guide for Soil Sampling of the Minister of the Environment to determine the average concentration of lead in the soil and the sites with the highest concentrations of lead. Identification sampling showed that 38 of the 42 samples obtained from Campo de Marte and four of the 19 samples obtained from Kennedy Park had concentrations of lead that exceeded the limits set by the Environmental Quality Standards for the soil of parks and residential areas (140 ppm). Detailed sampling was the second type of sampling performed, which was performed to identify the area of soil impacted by contamination. It was performed near the high-concentration sites in each park (Campo de Marte, $n = 64$ soil samples; Kennedy Park, $n = 13$ soil samples) identified after the identification sampling. During both sets of sampling, the soil samples were taken from the top 10 cm of the soil and were sent to the Soil, Plant, Water and Fertilizer Analysis Laboratory of the National Agrarian University La Molina, Lima, Peru (12°4'24"S, 76°56'10"W; UTM 18L 0288166 8663907; 244 meters above sea level) for analysis. In addition, 100 kg of soil was taken from the top 10 cm of the soil profile in each park (in the case of Campo de Marte, only from sites with lead concentrations >140 ppm), which was the soil used in the bioassay. Samples from high-concentration sites at Campo de Marte were used to represent urban soils with high lead concentrations, whereas samples from Kennedy Park were used to represent urban soils with low lead concentrations. The soil characteristics and the levels of other heavy metals of the soil obtained in each park are shown in Tables 1 and 2.

Two types of soil (contaminated soil from Campo de Marte and uncontaminated soil from Kennedy Park), three types of water (deionized water, deionized water with 0.1 ppm

Table 1. Characteristics of the soils obtained from Campo de Marte and Kennedy Park

| Source | pH (1:1) | E.C. (1:1) (dS/m) | CaCO ₃ (%) | M.O. % | P (ppm) | K (ppm) | Mechanical analysis | | | Textural classification | CEC | Exchangeable cations | | | | | Sum of cations |
|----------------|----------|-------------------|-----------------------|--------|---------|---------|---------------------|------|------|-------------------------|------|----------------------|------------------|----------------|-----------------|----------------------------------|----------------|
| | | | | | | | Sand | Loam | Clay | | | Ca ²⁺ | Mg ²⁺ | K ⁺ | Na ⁺ | Al ³⁺ +H ⁺ | |
| | | | | | | | % | % | % | | | mEq/100 g | | | | | |
| Kennedy Park | 7.49 | 0.51 | 1 | 2.67 | 48.5 | 137 | 75 | 14 | 11 | Sandy loam | 15.5 | 13.33 | 1.67 | 0.34 | 0.18 | 0 | 15.52 |
| Campo de Marte | 7.3 | 0.7 | 0.3 | 9.47 | 21 | 236 | 59 | 20 | 21 | Clay sandy loam | 27 | 23.9 | 1.82 | 0.56 | 0.27 | 0 | 26.56 |

Table 2. Analysis of the metal concentrations (ppm) in soil samples obtained from Campo de Marte and Kennedy Park.

| Source | Cu | Fe | Zn | Mn | Cd | Cr | Pb |
|----------------|--------|-------|--------|------|------|-------|--------|
| Kennedy Park | 16.60 | 45.10 | 23.70 | 4.50 | 1.46 | 24.7 | 71.61 |
| Campo de Marte | 296.00 | 82.00 | 255.00 | 2.50 | 9.36 | 91.49 | 200.86 |

of lead, and regular irrigation water with 0.1 ppm of lead), and two levels of mycorrhizae (0 and 2.5 g/kg of soil) were tested in the bioassay. To produce lead-contaminated water, lead acetate was added to the deionized water, which contained 0 ppm of lead, and the irrigation water, which contained 0.035 ppm of lead (see Table 3), to give a final concentration of 0.1 ppm of lead.

Table 3: Analysis of the regular irrigation water that was used in the bioassay

| Analysis of the regular irrigation water that was used in the bioassay | | |
|--|-------|-------|
| pH | | 7.41 |
| E.C. | dS/m | 0.67 |
| Ca | mEq/L | 4.87 |
| Mg | mEq/L | 0.56 |
| K | mEq/L | 0.14 |
| Na | mEq/L | 1.25 |
| Sum of cations | | 6.82 |
| Nitrates | mEq/L | 0.01 |
| Carbonates | mEq/L | 0 |
| Bicarbonates | mEq/L | 2.95 |
| Sulfates | mEq/L | 1.29 |
| Chlorides | mEq/L | 2.5 |
| Sum of anions | | 6.75 |
| Na | % | 18.34 |
| SAR | | 0.76 |
| B | ppm | 0.24 |
| Classification | | C2-S1 |
| Cu | ppm | 0.001 |
| Zn | ppm | 0.078 |
| Mn | ppm | 0.002 |
| Fe | ppm | 0.001 |
| Pb | ppm | 0.035 |
| Cd | ppm | 0.029 |
| Cr | ppm | 0 |

Salvia splendens 'Red Vista' seeds were placed individually in the cells of seedbed trays that contained a mixture of sand and moss (1:4, v:v) as a substrate. In the case of the mycorrhizal treatment, this substrate was mixed with the product Mycosym Triton (active component: mycorrhizal fungus *G. intraradices*; inert substrate: lightweight expanded clay aggregate) at a concentration of 2.5 g per kilogram of substrate. The *S. splendens* seeds were planted in wet substrate and were watered with deionized water while they were in the seedbed trays. After 26 days, each seedling had at least two true leaves, at which time 36 of the plants were transplanted into separate pots (4 kg capacity; $n = 3$ pots per treatment).

The bioassay was carried out in the facilities of the Soil Fertility Laboratory of the National Agrarian University La Molina from October 22, 2016 to January 20, 2017. The plants were watered every Monday, Wednesday, and Friday; and were measured at the same time to determine the lengths of the stems and inflorescences. Each plant was then harvested 90 days after sowing in the seedbed trays and separated into three parts: inflorescence, stem and leaves, and roots. Each part of the plant was weighed and washed with deionized water and then placed in a laboratory oven at 70°C for 48 hours, after which the weight was remeasured. Each part was then ground and put in an Erlenmeyer flask for acid digestion. The atomic absorption spectrophotometry technique was used to measure the lead concentration in each plant tissue.

A completely randomized experimental design was used with a 3×2 factorial arrangement and three replications for each type of soil, giving a total of 18 experimental units in soil obtained from Campo de Marte and Kennedy Park, respectively (see Table 4).

Table 4: Description of the treatments

| Soil | Treatment | Quality of water | Mycorrhizae |
|----------------|-----------|------------------|------------------------|
| Campo de Marte | T1 | Deionized | No mycorrhizae |
| | T2 | Deionized | Mycorrhizae (2.5 g/kg) |
| | T3 | Deionized + Pb | No mycorrhizae |
| | T4 | Deionized + Pb | Mycorrhizae (2.5 g/kg) |
| | T5 | Irrigation + Pb | No mycorrhizae |
| | T6 | Irrigation + Pb | Mycorrhizae (2.5 g/kg) |
| Kennedy Park | T7 | Deionized | No mycorrhizae |
| | T8 | Deionized | Mycorrhizae (2.5 g/kg) |
| | T9 | Deionized + Pb | No mycorrhizae |
| | T10 | Deionized + Pb | Mycorrhizae (2.5 g/kg) |
| | T11 | Irrigation + Pb | No mycorrhizae |
| | T12 | Irrigation + Pb | Mycorrhizae (2.5 g/kg) |

A homogeneity of variances test and normality test were initially performed on the data. We performed analysis of variance (ANOVA) to determine the influence of the variables on lead concentrations in the tissues and to determine the influence of the interaction between these variables on lead concentrations in the tissues. It is considered that in the case that the p value is greater than 0.05, the null hypothesis cannot be rejected; in other words, there are no significant differences in the treatments

analyzed. The initial and final concentrations of lead in the soil were then compared using ANOVA and the Student's one-sample t-test. All statistical analyses were performed in SPSS (IBM SPSS Statistics 23) using a significance level of $p < 0.05$.

Results

Campo de Marte

Concentration of lead in the root tissue

An ANOVA test was performed to determine the influence of each variable. The variable water (p value: 0.084) and the interaction between variable water and variable mycorrhizae (p value: 0.054) did not have a significant influence on the concentration of lead in the root tissue. However, plants that were grown in association with the mycorrhizal fungus *G. intraradices* (p value: 0.027) had a significantly higher concentration of lead in their root tissues (53.97 ppm) than those that were grown without mycorrhizae (39.41 ppm) (Table 5).

Table 5. Average concentrations of lead (ppm) in the root tissues of plants grown in soil obtained from Campo de Marte under different water and mycorrhizal regimes

| | Mycorrhizae | Water | | | Avg* |
|----------------|----------------|--------------|----------------|-----------------|--------------|
| | | Deionized | Deionized + Pb | Irrigation + Pb | |
| Campo de Marte | No Mycorrhizae | 29.59 | 55.34 | 33.30 | 39.41 |
| | Mycorrhizae | 66.33 | 53.41 | 42.17 | 53.97 |
| | Avg* | 47.96 | 54.37 | 37.73 | 46.69 |

*Avg: Average

Concentration of lead in the stem and leaf tissues

An ANOVA test was performed to determine the influence of each variable. The variable water (p value: $0.889 > 0.05$), the variable mycorrhizae (p value: $0.123 > 0.05$), and the interaction between variable water and variable mycorrhizae (p value: $0.661 > 0.05$) did not have a significant influence on the concentration of lead in the stem and leaf tissues.

Table 6. Average concentrations of lead (ppm) in the stem and leaf tissues of plants grown in soil obtained from Campo de Marte under different water and mycorrhizal regimes

| | Mycorrhizae | Water | | | Avg* |
|----------------|----------------|--------------|----------------|-----------------|--------------|
| | | Deionized | Deionized + Pb | Irrigation + Pb | |
| Campo de Marte | No Mycorrhizae | 11.86 | 10.71 | 12.49 | 11.69 |
| | Mycorrhizae | 10.36 | 10.17 | 9.5 | 10.01 |
| | Avg* | 11.11 | 10.44 | 10.997 | 10.85 |

*Avg: Average

Plants that were grown in soil obtained from Campo de Marte did not translocate a significant amount of lead to the stem and leaves under any of the water or mycorrhizal conditions, with no significant difference between any of the treatments (Table 6).

Concentration of lead in the inflorescence tissue

An ANOVA test was performed to determine the influence of each variable. The variable water (p value: 0.256), the variable mycorrhizae (p value: 0.123), and the interaction between variable water and variable mycorrhizae (p value: 0.874) did not have a significant influence on the concentration of lead in the inflorescence tissue. Plants that were grown in soil obtained from Campo de Marte did not translocate a significant amount of lead to the inflorescence under any of the water or mycorrhizal conditions, with no significant difference between any of the treatments (Table 7).

Table 7. Average concentrations of lead (ppm) in the inflorescence tissues of plants grown in soil obtained from Campo de Marte under different water and mycorrhizal regimes

| | Mycorrhizae | Water | | | Avg* |
|----------------|----------------|--------------|----------------|-----------------|--------------|
| | | Deionized | Deionized + Pb | Irrigation + Pb | |
| Campo de Marte | No Mycorrhizae | 16.12 | 14.80 | 13.07 | 14.66 |
| | Mycorrhizae | 13.14 | 13.03 | 9.06 | 11.75 |
| | Avg* | 14.63 | 13.91 | 11.07 | 13.20 |

*Avg: Average

Length of the stem

An ANOVA test was performed to determine the influence of each variable. The variable water (p value: 0.187), the variable mycorrhizae (p value: 0.255), and the interaction between variable water and variable mycorrhizae (p value: 0.981) did not have a significant influence on the length of the stem. The presence of lead in the soil obtained from Campo de Marte stunted the growth of the aerial parts of the plants, with no significant difference in the length of the stem between any of the treatments (Table 8).

Table 8. Average lengths (cm) of the stems of plants grown in soil obtained from Campo de Marte under different water and mycorrhizal regimes

| | Mycorrhizae | Water | | | Avg* |
|----------------|----------------|--------------|----------------|-----------------|--------------|
| | | Deionized | Deionized + Pb | Irrigation + Pb | |
| Campo de Marte | No Mycorrhizae | 10.77 | 9.5 | 11.23 | 10.5 |
| | Mycorrhizae | 9.77 | 8.83 | 10.4 | 9.67 |
| | Avg* | 10.27 | 9.165 | 10.815 | 10.08 |

*Avg: Average

Length of the inflorescence

An ANOVA test was performed to determine the influence of each variable. The variable water (p value: 0.899), the variable mycorrhizae (p value: 0.369), and the interaction between variable water and variable mycorrhizae (p value: 0.222) did not have a significant influence on the length of the stem. The presence of lead in the soil obtained from Campo de Marte also had a stunting effect on the length of the inflorescence for all treatments (Table 9), decreasing the ornamental value of the plants.

Table 9. Average lengths (cm) of the inflorescences of plants grown in soil obtained from Campo de Marte under different water and mycorrhizal regimes

| | Mycorrhizae | Water | | | Avg* |
|----------------|----------------|--------------|----------------|-----------------|--------------|
| | | Deionized | Deionized + Pb | Irrigation + Pb | |
| Campo de Marte | No Mycorrhizae | 14.20 | 17.67 | 13.87 | 15.24 |
| | Mycorrhizae | 13.70 | 12.00 | 15.43 | 13.71 |
| | Avg* | 13.95 | 14.83 | 14.65 | 14.48 |

*Avg: Average

Kennedy Park

Concentration of lead in the root tissue

An ANOVA test was performed to determine the influence of each variable. The variable water (p value: 0.002), the variable mycorrhizae (p value: 0.045), and the interaction between variable water and variable mycorrhizae (p value: 0.010) did have a significant influence on the concentration of lead in the root tissue. Plants that were grown in association with *G. intraradices* accumulated more lead in their roots, as did plants that were watered with contaminated water, with plants that received both treatments accumulating the most lead (Table 10).

Table 10. Average concentrations of lead (ppm) in the root tissues of plants grown in soil obtained from Kennedy Park under different water and mycorrhizal regimes

| | Mycorrhizae | Water | | | Avg* |
|--------------|----------------|--------------|----------------|-----------------|--------------|
| | | Deionized | Deionized + Pb | Irrigation + Pb | |
| Kennedy Park | No Mycorrhizae | 18.53 | 26.02 | 21.76 | 22.10 |
| | Mycorrhizae | 17.823 | 25.185 | 34.925 | 25.98 |
| | Avg* | 18.18 | 25.60 | 28.34 | 24.04 |

*Avg: Average

Concentration of lead in the stem and leaf tissues

An ANOVA test was performed to determine the influence of each variable. The variable water (p value: 0.121) and the interaction between variable water and variable

mycorrhizae (p value: 0.946) did not have a significant influence on the concentration of lead in the stem and leaf tissues; however, the variable mycorrhizae (p value: 0.012) did have a significant influence on the concentration of lead in the stem and leaf tissues. Plants that were grown in association with *G. intraradices* accumulated significantly more lead than those that were grown without mycorrhizae. (Table 11)

Table 11. Average concentrations of lead (ppm) in the stem and leaf tissues of plants grown in soil obtained from Kennedy Park under different water and mycorrhizal regimes

| | Mycorrhizae | Water | | | Avg* |
|--------------|----------------|-------------|----------------|-----------------|--------------|
| | | Deionized | Deionized + Pb | Irrigation + Pb | |
| Kennedy Park | No Mycorrhizae | 7.44 | 10.75 | 9.82 | 9.34 |
| | Mycorrhizae | 11.02 | 15.4 | 14.35 | 13.59 |
| | Avg* | 9.23 | 13.08 | 12.08 | 11.46 |

*Avg: Average

Concentration of lead in the inflorescence tissue

An ANOVA test was performed to determine the influence of each variable. The variable water (p value: 0.135), the variable mycorrhizae (p value: 0.107), and the interaction between variable water and variable mycorrhizae (p value: 0.068) did not have a significant influence on the concentration of lead in the inflorescence tissue. Plants that were grown in soil obtained from Kennedy Park did not translocate a significant amount of lead to their inflorescences, with no significant differences between any of the treatments (Table 12).

Table 12. Average concentrations of lead (ppm) in the inflorescence tissues of plants grown in soil obtained from Kennedy Park under different water and mycorrhizal regimes

| | Mycorrhizae | Water | | | Avg* |
|--------------|----------------|-------------|----------------|-----------------|--------------|
| | | Deionized | Deionized + Pb | Irrigation + Pb | |
| Kennedy Park | No Mycorrhizae | 10.52 | 10.02 | 10.34 | 10.29 |
| | Mycorrhizae | 9.36 | 11.91 | 12.54 | 11.27 |
| | Avg* | 9.94 | 10.97 | 11.44 | 10.78 |

*Avg: Average

Length of the stem

An ANOVA test was performed to determine the influence of each variable. The variable water (p value: 0.683), the variable mycorrhizae (p value: 0.187), and the interaction between variable water and variable mycorrhizae (p value: 0.287) did not have a significant influence on the length of the stem. The length of the stem was similar for all of the plants that were grown in soil obtained from Kennedy Park, with no significant differences between any of the treatments (Table 13).

Table 13. Average lengths (cm) of the stems of plants grown in soil obtained from Kennedy Park under different water and mycorrhizal regimes

| | Mycorrhizae | Water | | | Avg* |
|--------------|----------------|--------------|----------------|-----------------|-------------|
| | | Deionized | Deionized + Pb | Irrigation + Pb | |
| Kennedy Park | No Mycorrhizae | 10.33 | 10.53 | 11.47 | 10.78 |
| | Mycorrhizae | 11.03 | 9 | 7.6 | 9.21 |
| | Avg* | 10.68 | 9.77 | 9.54 | 9.99 |

*Avg: Average

Length of the inflorescence

An ANOVA test was performed to determine the influence of each variable. The variable water (p value: 0.126) and the interaction between variable water and variable mycorrhizae (p value: 0.583) did not have a significant influence on the length of the inflorescence; however, the variable mycorrhizae (p value: 0.47) did have a significant influence on the length of the inflorescence. Plants that were grown in association with *G. intraradices* had a lower average inflorescence length than plants that were grown without mycorrhizae and showed symptoms of lead toxicity that were similar to the plants that were grown in contaminated soil obtained from Campo de Marte. (Table 14).

Table 14. Average lengths (cm) of the inflorescences of plants grown in soil obtained from Kennedy Park under different water and mycorrhizal regimes

| | Mycorrhizae | Water | | | Avg* |
|--------------|----------------|--------------|----------------|-----------------|--------------|
| | | Deionized | Deionized + Pb | Irrigation + Pb | |
| Kennedy Park | No Mycorrhizae | 22.47 | 19.07 | 18.90 | 20.14 |
| | Mycorrhizae | 19.07 | 16.37 | 11.00 | 15.48 |
| | Avg* | 20.77 | 17.72 | 14.95 | 17.81 |

*Avg: Average

Discussion

The concentration of lead in soil obtained from Campo de Marte did not affect the growth of *S. splendens* ‘Red Vista’ plants (Figure 1) but did affect other characteristics associated with their ornamental value (i.e., the volume and number of secondary inflorescences).

Salvia splendens ‘Red Vista’ plants growing in soil obtained from Campo de Marte accumulated an average of 46.69 ppm of lead in the roots, 10.85 ppm of lead in the stems and leaves, and 13.20 ppm of lead in the inflorescences. The concentration of lead in the soil increased from 200.86 ppm to 230.40 ppm during the bioassay. The results of the Student’s one-sample t-test indicated that there was a

significant difference between the initial concentration of lead in the soil and the final concentration of lead in the soil (p value: 0.000; degrees of freedom: 17. The p value was less than 0.05; therefore, there was a statistically significant difference), indicating that most of the lead added through contaminated water stayed in the soil, which suggests that only a very small amount of lead was available for the plant and most of it was retained in the root tissue. The soil at Campo de Marte contained 9.47% organic matter and had a cation exchange capacity of 26.56 cmol+/kg and a pH of 7.3. Lead is a heavy metal that has a high affinity to form stable compounds with organic matter that cannot be taken up by plants, and at a pH of 4.0 or higher, lead tends to be strongly retained by the humic matter (Steinnes, 2013). Furthermore, this soil also contained a high concentration of copper (296 ppm), which tends to precipitate with lead to form very stable compounds. Therefore, since *S. splendens* ‘Red Vista’ did not have high concentrations of lead in its tissues and exhibited symptoms of lead toxicity that were similar to those described by Nowak (2007), we should assume that this variety cannot be considered a phytoextractor plant and should not be considered for use in phytoremediation strategies at least under similar conditions to the current bioassay.

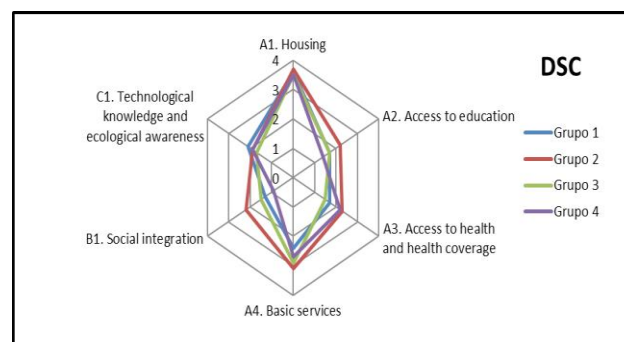


Figure 1. Growth of plants in soil obtained from Campo de Marte

The concentration of lead in the soil obtained from Kennedy Park did not affect the growth (Figure 2) or ornamental value of the *S. splendens* ‘Red Vista’ plants except in treatments T10 and T12 (treatments with mycorrhizae and water contaminated with lead), in which case they exhibited very similar symptoms to the plants that were grown in soil obtained from Campo de Marte.

Salvia splendens ‘Red Vista’ plants that were grown in soil obtained from Kennedy Park accumulated an average of 24.04 ppm of lead in their roots, 11.46 ppm of lead in their stems and leaves, and 10.78 ppm of lead in their inflorescences. The concentration of lead in the soil decreased from 71.61 ppm to 68.795 ppm during the bioassay, and the results of the Student’s one-sample t-test indicated that there was no significant difference between the initial concentration of lead in the soil and the final concentration of lead in the soil (p value: 0.054; degrees of

freedom: 17. The p value was higher than 0.05; therefore, there is no statistically significant difference), indicating that most of the lead added through contaminated water did not stay in the soil and most of it was not taken by the plant). The Kennedy Park soil contained 2.67% organic matter, had a sandy loam texture, and did not contain high concentrations of any element that would precipitate with lead to form very stable compounds. Consequently, the lead that was added to the water was not strongly absorbed by the soil, which contained only a low concentration of lead, but rather was quickly washed out of the soil by water used for irrigation or taken up by the plants, sometimes causing them to show symptoms of lead toxicity that were similar to plants that were grown in contaminated soil (observed in T10 and T12).

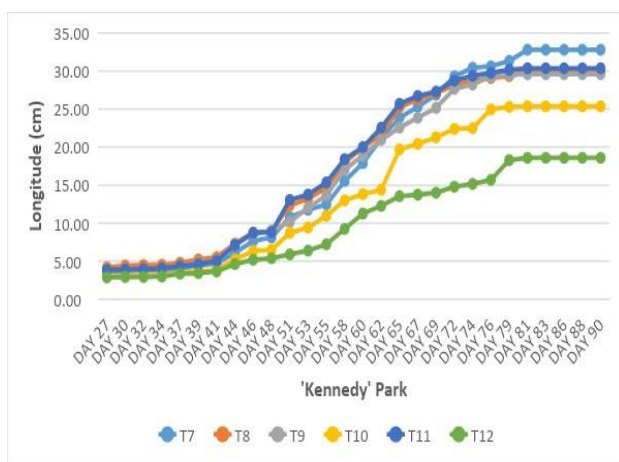


Figure 2. Growth of plants in soil obtained from Kennedy Park

Although the average length of the stem was similar across all treatments, the final average length of the inflorescence was lower in plants that were grown in association with mycorrhizae (15.48 cm) than in those that were grown without mycorrhizae (20.14 cm). Mycorrhizal fungi, in this case *G. intraradices*, allow plants to explore a greater volume of soil and increase the amount of nutrients and other elements the plant can extract. The plants did not translocate a significant amount of lead to their inflorescences but the presence of mycorrhizae helped them to accumulate lead in their roots, which probably explains why the translocation of valuable nutrients decreased, leading to a shorter inflorescence. When lead was added to the water (treatments T10 and T12), a reduction in the ornamental value was observed in addition to the reduction in inflorescence length. Thus, the mycorrhizae had a negative effect on the plants, which was best observed when these plants were watered with lead-contaminated water.

Translocation and bioconcentration factors

The translocation factor was calculated by dividing the concentration of lead in the aerial parts of the plant by that in the roots, while the bioconcentration factor was calculated by dividing the concentration of lead in the

roots by that in the soil (Tu, Ma, & Bondada, 2003; Rizzi, Petruzzelli, Poggio & Vigna Guidi, 2004; Maldonado, Favela, Rivera & Volke 2011). The translocation factor was 0.26 for plants grown in soil from Campo de Marte and 0.46 for plants grown in soil from Kennedy Park, while the bioconcentration factor was 0.21 for plants grown in soil from Campo de Marte and 0.34 for plants grown in soil from Kennedy Park. Therefore, since values of at least one are required to consider a species as having some kind of phytoextraction ability, these values indicate that *S. splendens* 'Red Vista' is unable to extract high amounts of lead from the soil and thus cannot be considered a hyperaccumulator species.

Conclusion

The ornamental plant *S. splendens* 'Red Vista' was unable to extract high amounts of lead from the soil even when it was grown in association with the mycorrhizal fungus *G. intraradices*, demonstrating that under the tested conditions it does not behave as a hyperaccumulator plant.

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