ΦYTON
REVISTA INTERNACIONAL DE BOTÁNICA EXPERIMENTAL
INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY
FUNDACION ROMULO RAGGIO
Gaspar Campos 861, 1638 Vicente López (BA), Argentina
www.revistaphyton.fund-romuloraggio.org.ar

The effects of tin (Sn) additions on the growth of spinach plants

Los efectos del agregado de estaño en el crecimiento de plantas de espinaca

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Abstract. An increase in bioavailable tin in the environment could result in bioaccumulation thereof in agricultural crops, and therefore, have adverse health consequences on humans that eat these crops. The aims of the current study were thus to assess the uptake of Sn by spinach plants, and the subsequent effects this will have on the uptake of Na, Zn, K, Ca, and Mg as well as the growth of spinach plants. Spinach plants were grown in sand culture and received tin at concentrations of 0.02, 0.2, 2 and 20 mg/L along with a nutrient solution. The uptake of tin at detectible concentrations only occurred at the highest concentrations (2 and 20 mg/L), and it was mostly retained in the roots of the plants. Tin additions also resulted in no visual toxicity symptoms, and might be beneficial to biomass production. Further field trials are needed to ensure that these experimental results remain true under field conditions.

Keywords: Beneficial; Heavy metals; Root allocation; Stannous chloride.

Resumen. Un incremento en la biodisponibilidad de estaño en el ambiente podría resultar en la bio-acumulación de estaño en las plantas de cosecha, y así tener consecuencias adversas a la salud en los humanos que consumen la cosecha de dichas plantas. Los propósitos de la presente investigación fueron (1) determinar la absorción de estaño por plantas de espinaca, y (2) los efectos subsiguientes que dicha absorción podría tener en la absorción de Na, Zn, K, Ca, y Mg, y el crecimiento de las plantas de espinaca. Dichas plantas crecieron en arena y recibieron estaño a concentraciones de 0,02; 0,2; 2 y 20 mg/L junto con una solución nutritiva. La absorción de estaño a concentraciones detectables solo ocurrió a las mayores concentraciones (2 y 20 mg/L) y principalmente fue retenido en las raíces de las plantas. El agregado de estaño tampoco determinó síntomas de toxicidad visibles, y podría ser benéfico a la producción de biomasa. Se necesitan estudios a campo adicionales para verificar que estos resultados experimentales persisten bajo dichas condiciones.

Palabras clave: Benéfico; Metales pesados; Distribución a raíces; Cloruro de estaño.

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Recibido / Received 8.I.2014. Aceptado / Accepted 30.X.2014.

INTRODUCTION

Tin (Sn) is a naturally occurring heavy metal in the earths' crust, at an average concentration of 2 mg/kg. It is therefore also a component of many soils due to the natural weathering of bedrock. Normal concentrations of Sn in unpolluted soils range from less than 1 mg/kg to 200 mg/kg and is found in two oxidative states, stannous (+2) and stannic (+4) (WHO, 2005; Kabata- Pendias & Mukerjee, 2007). Shacklette & Boerngen (1984) found that the average Sn concentrations in surface soils in the US ranged between 0.6 and 1.7 ppm, while other studies reported ranges from 1-11 mg/kg (Ure & Bacon, 1978; Kabata-Pendias, 2001). The concentration of Sn in the environment is however highly variable, and is dependent on the use and release of Sn containing products by both natural sources such as the weathering of rocks and volcanic eruptions, and anthropogenic activities such as industrial processes, agriculture and mining (Ashraf et al., 2011).

In the soil, Sn usually has limited to no mobility, and is usually tightly bound in the top soil (Hou et al., 2005; 2006; Nakamaru & Uchida, 2008; Lasley et al., 2010). However, due to the increase in anthropogenic activities such as agriculture, that uses and releases Sn products into the environment, Sn concentrations in certain areas may be highly elevated, with concentrations reaching up to 1000 mg/kg (Schafer & Femfert, 1984; Laughlin & Linden, 1985; Weber, 1985; Snoeij et al., 1986; Bryan & Langston, 1992; Ostrakhovitch & Cherian, 2007). In certain areas in Europe and North America, Sn concentrations in sewage sludge, used for agricultural purposes, ranged from 40-700 mg/kg dry weight, while manure and poultry wastes contained 3.7-7.4 mg/kg and 2.0-4.1 mg/kg respectively, before it is added to agricultural soils (Senesi et al., 1999). Ashraf et al. (2011) conducted a study on the uptake of Sn by three *Cyprus* species. These authors found that as the concentrations of Sn supplied to the plants increased, higher concentrations were taken up and incorporated into the plant tissues. It is therefore important to understand the uptake and allocation of tin by agricultural crops as tin accumulation in these crops is possible (Weber, 1985). The ingestion of relatively high concentrations of Sn is known to cause toxicity in various mammalian species. Toxicity symptoms range from fatigue, headaches, diarrhoea, vomiting, muscular weakness and paralyses, anaemia, excessive damage to the liver and kidneys, and a reduction in various levels of neurotransmitters in the brain (Gerren et al., 1976; Graham et al., 1976; WHO, 1980; 2004; 2005; Snoeij et al., 1987).

The aim of the current study was thus to assess the uptake of Sn by spinach plants, and the subsequent effects this will have on the uptake of Na, Zn, K, Ca, and Mg as well as the growth of spinach plants.

MATERIALS AND METHODS

Growing conditions and sample preparation. Spinach (*Spinacea oleracea* L.) was grown in 15 cm pots in a complete random block design, in sand culture. The pH of the soil was adjusted to 6.5 by washing it with deionised water (pH 6.5). The experiments were conducted in a growth cabinet with a photoperiod of 12 h/12 h day/night cycle, 20 °C/10 °C day/night temperature cycle. The plants were watered daily with deionised water until the seedlings were established. After establishment, the plants received the different treatments which consisted of a nutrient solution (Chemicult-Kompel), with the addition of Sn as stannous chloride ($SnCl_2.2H_2O$) at concentrations of 0.02 mg/L (treatment 1), 0.2 mg/L (treatment 2), 2 mg/L (treatment 3) and 20 mg/L (treatment 4). The stock nutrient solution was used as the control treatment (Table 1). Each treatment was replicated four times within the random block design.

Tabla 1. Elemental make-up, before dilution, of the nutrient solution (Chemicult-Kompel) applied to the vegetable crops.
Tabla 1. Constitución de la solución nutritiva (Chemicult-Kompel) aplicada a las plantas de cosecha antes de su dilución.

Concentration (g/kg)									
N	P	K	Mg	Fe	B	Zn	Cu	Mn	Mo
146	43	274	29	1.8	0.24	0.05	0.02	0.24	0.01

The plants were harvested after nine weeks and separated into roots and shoots. The roots were washed with deionised water after which both roots and shoots were oven dried at 50 °C to a constant mass. After oven drying, the dry mass of both roots and shoots were determined, after which the dried material was milled using a stainless steel laboratory blender, and stored for nutrient determination. The milled samples were digested using a sulphuric-peroxide digestion method as described by Moore and Chapman (1986) after which the digested samples were filtered through Whatman no. 1 filter paper, and diluted to 100 mL with deionised water. An Atomic Absorption Spectrophotometer (AAS) was used to determine the concentrations of Sn in furnace mode, and Na, K, Ca, Mg and Zn in flame mode.

Statistical analyses. The Statistical Package for the Social Sciences version 21 (SPSS Inc., Chicago IL) was used to test the data for normality using a Shapiro-Willks test, after which a Kruskal-Wallis (H) analyses was performed to determine whether there were statistically significant differences ($p \leq 0.05$) in Sn, Na, K, Ca, Mg and Zn concentrations between the different treatments and also between the roots and shoot concentrations. Log 10 of the Sn concentrations supplied to the plants in the different treatments were calculated in order to perform a Spearmans Rho (ρ) test to determine the relationship between the Sn additions and dry mass of the roots and shoots of the spinach plants.

RESULTS AND DISCUSSION

Sn uptake and accumulation. In this study, the addition of Sn at concentrations containing less than 2 mg/L resulted in no detectible concentrations of Sn in both roots and shoots of the spinach plants (Table 2). However, at the concentrations where Sn was taken up at detectable concentrations by the plants, concentrations in the roots and shoots were significantly higher than that found in the control treatment (Table 2).

Within these plants, it was found that Sn was accumulated in significantly higher concentrations in the roots of the plants, compared to the shoots (Table 3). This suggests that Sn is retained in the apoplastic regions in the roots of the plants, which result in poor transport to the aerial parts of the plants (Prasad, 2004). Our findings correspond to that of Cohen, (1940) and Romney et al. (1975) who worked on peas and corn plants and bush beans, respectively. These authors suggested that Sn, even when supplied at relatively high concentrations to the plants, is not readily available to the plants. These authors did however find that when Sn was applied at high enough concentrations, Sn was taken up by the plants, but was primarily accumulated in the roots, rather than the shoots. Ashraf et al. (2011), however, found that when Sn was supplied to three different *Cyprus* species, Sn concentrations were significantly higher in the above ground parts of the plants, when compared to the roots. Prasad (2004) stated that certain heavy metals, although primarily stored in the apoplastic regions of the roots of the plants, could be taken up at high concentrations, saturating the apoplastic regions in the roots, resulting in the transfer of higher concentrations to the above ground parts of the plants. This corresponds to our findings where at the highest Sn concentrations supplied to the plants (20 mg/L), higher Sn concentrations was detected in the shoots of the plants when compared to the other Sn treatments (Table 2 and 3).

The effects of Sn fertilisation on nutrient uptake and biomass production. The addition of Sn to spinach plants resulted in no visual toxicity symptoms such as chlorosis and necrosis of the leaves, and also did not affect the uptake of Na, K, Ca, Mg and Zn by the plants (Table 2). Romney et al. (1975) however, noted that the addition of Sn to bush bean plants resulted in an increase in manganese and zinc concentrations and a decrease in iron concentrations while Cohen (1940), indicated that at high Sn concentrations (>1-100 mg/L), various toxicity symptoms occurred in pea and corn plants. In this study, a strong positive correlation was however found between the Sn concentrations

Table 2. Element concentrations (g/kg) in the roots and shoots of spinach plants. Mean concentrations with the same letters are not statistically significantly different from one another (p≥0.05). Comparisons were made between the different Sn treatments for the same element and plant organ and not between elements and organs (i.e. roots and shoots).
Tabla 2. Concentraciones de elementos (g/kg) en las raíces y tallos de plantas de espinaca. Las concentraciones promedio seguidos por la misma letra no son significativamente diferentes (p≥0,05). Las comparaciones se hicieron entre los diferentes tratamientos de Sn para el mismo elemento y órgano vegetal, y no entre elementos y órganos (es decir, raíces y tallos).

Sn treatment (mg/L)	**Mean Concentration (g/kg) ± Standard Error**											
	Sn		Na		K		Ca		Mg		Zn	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
0	0.000 ± 0.000 a	0.000 ± 0.000 a	3.6 ± 0.38 a	2.2 ± 0.28 a	60.6 ± 3.33 a	25.7 ± 1.54 a	11.1 ± 0.38 a	8.8 ± 0.46 a	5.6 ± 0.38 a	3.4 ± 0.12 a	0.05 ± 0.005 a	0.03 ± 0.003 a
0.02	0.000 ± 0.000 a	0.000 ± 0.000 a	3.6 ± 0.29 a	1.3 ± 0.07 a	63.1 ± 3.81 a	22.0 ± 2.32 a	10.5 ± 0.55 a	9.3 ± 1.47 a	5.4 ± 0.52 a	2.9 ± 0.34 a	0.04 ± 0.003 a	0.03 ± 0.007 a
0.2	0.000 ± 0.000 a	0.000 ± 0.000 a	3.1 ± 0.21 a	1.2 ± 0.02 a	60.0 ± 4.22 a	25.1 ± 1.01 a	10.3 ± 0.57 a	7.7 ± 0.86 a	5.3 ± 0.43 a	2.6 ± 0.23 a	0.04 ± 0.004 a	0.02 ± 0.001 a
2	0.000 ± 0.000 a	0.003 ± 0.001 ab	3.4 ± 0.26 a	1.2 ± 0.05 a	58.8 ± 3.86 a	17.0 ± 2.23 a	10.3 ± 0.74 a	9.4 ± 0.58 a	5.2 ± 0.45 a	3.0 ± 0.16 a	0.04 ± 0.003 a	0.03 ± 0.006 a
20	0.002 ± 0.001 b	0.014 ± 0.005 b	3.2 ± 0.04 a	1.2 ± 1.04 a	60.1 ± 3.35 a	21.6 ± 2.95 a	10.8 ± 1.74 a	8.9 ± 0.86 a	5.8 ± 0.45 a	3.4 ± 1.23 a	0.04 ± 0.019 a	0.03 ± 0.003 a
H_4	**16.941**	**16.26**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
p	**0.003**	**0.003**										

ns = not significant (p≥0.05), H = Kruskal Wallis statistic, 4 = degrees of freedom.
ns = no significativo (p≥0,05), H = prueba de Kruskal Wallis, 4 = grados de libertad.

Table 3. Allocation (g/kg) of tin in the roots and shoots of spinach plants. Allocations with the same letters are not statistically significantly different from one another ($p \geq 0.05$). Comparisons were made within each treatment between the roots and shoots.

Tabla 3. Distribución (g/kg) de estaño entre raíces y tallos de plantas de espinaca. Valores seguidos por la misma letra no son significativamente diferentes ($p \geq 0,05$). Las comparaciones se hicieron dentro de cada tratamiento entre las raíces y los tallos.

Sn treatment (mg/L)	Allocation (g/kg) ± Standard Error		Significance
	Roots	Shoots	
0	0.000 ± 0.000 a	0.000 ± 0.000 a	**ns**
0.02	0.000 ± 0.000 a	0.000 ± 0.000 a	**ns**
0.2	0.000 ± 0.000 a	0.000 ± 0.000 a	**ns**
2	0.002 ± 0.001 b	0.000 ± 0.000 a	**p=0.014**
20	0.008 ± 0.004 b	0.0004 ± 0.0001 a	**p=0.020**

ns = not significant ($p \geq 0.05$).
ns = no significativo ($p \geq 0,05$).

supplied to the plants and the dry matter production of both roots and shoots of the spinach plants (Fig. 1). This increase in the dry matter production suggests that Sn might have some beneficial effects on biomass production. Cohen (1940) showed similar results, and found that the addition of Sn to pea and corn plants at low concentrations (0.2 and 1 mg/L) increased root growth as well as the height of the corn plants.

CONCLUSION AND RECOMMENDATIONS

We investigated the uptake and accumulation of Sn by spinach plants. Generally, at the lowest Sn concentrations supplied to the plants, Sn could not be detected in the plant tissues. However, at the higher Sn concentrations supplied to the plants, Sn was taken up by the plants, but were primarily allocated in their roots. It is suggested that further studies be conducted on a wider variety of agricultural crops in order to establish the uptake of Sn by these plants, and in which

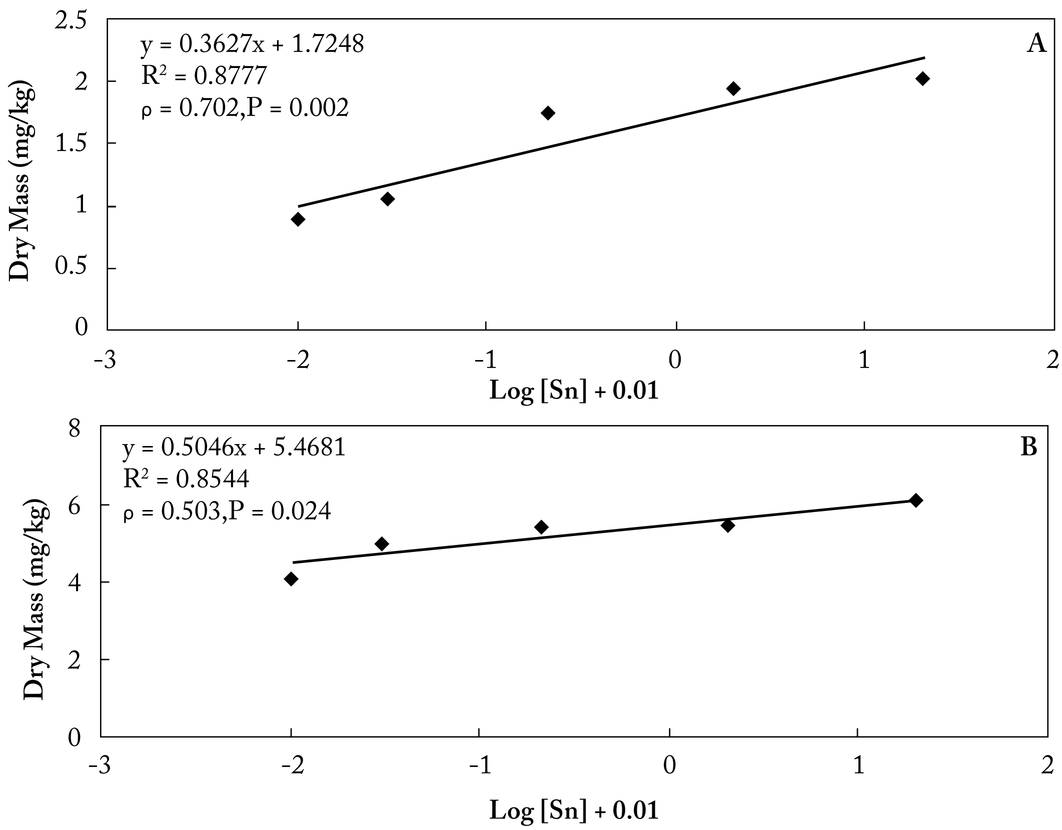

Fig. 1. Statistically significant ($p<0.05$) correlations (Spearmans Rho = ρ) in root (A) and shoot (B) dry matter production and tin (Sn) treatments supplied to spinach plants.

Fig. 1. Correlaciones estadísticamente significativas ($p<0,05$) (Spearmans Rho = ρ) entre los tratamientos de estaño (Sn) a los que se expusieron las plantas de espinaca y su producción de materia seca radical (A) y aérea (B).

organs, edible or non-edible portions, it will be primarily allocated. Also, it is suggested that similar studies be conducted under field conditions to determine whether the results obtained in this study will remain true under field conditions.

ACKNOWLEDGEMENTS

Mosilo Machere is thanked for her contribution in the growth experiments. The authors also want to thank the reviewers of this manuscript for their inputs and guidance. The National Research Foundation provided the funding to do this study. The opinions expressed and conclusions arrived at, are however those of the authors.

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