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1 INFLUENCE OF THERMAL REGIME OF SOIL ON THE SULFUR (S) AND 2 SELENIUM (Se) CONCENTRATION IN POTATO PLANTS

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16 **Key Words:** Solanum tuberosum; Mulch; Polyethylene; Phytoremediation; Root-zone
17 temperature

18 19 **ABSTRACT**

20 Three consecutive years of field experiments were carried out to investigate the effect
21 of different root temperatures, induced by the application of mulches on the
22 concentration of sulfur (S) forms (organic-S, total-S and SO₂⁴) and Se in different organs
23 of potato plants (roots, tubers, stems and leaves). Four different plastic covers were
24 used (T1: transparent polyethylene; T2: white polyethylene; T3: white and black
25 coextruded polyethylene, and T4: black polyethylene), using uncovered soil as control
26 (T0). The different treatments had a significant effect on mean root temperatures
27 (T0=16°C, T1=20°C, T2=23°C, T3=27°C and T4=30°C) and induced a significantly different
28 response in the S forms and Se concentration, showing the T3 treatment (27°C) the
29 greatest concentration of total S and organic S in the stems and leaflets. The Se reached
30 higher levels in the roots and tubers in T3. With regard to possibilities in
31 phytoremediation, it is necessary to control the thermal regime of the soil to optimize
32 the accumulation of elements.

33 34 **INTRODUCTION**

35 Root zone temperature strongly influences the growth and uptake of nutrients.^[1]
36 The potato plants require optimal temperatures in the root zone for maximum growth
37 and yield^[2] and one of the techniques used to increase root zone temperature is the
38 application of polyethylene covers (mulch) of different colours and characteristics,
39 which generate a favourable microenvironment (higher temperatures) in the root
40 zone.^[3]

41 In its reduced form, sulfur (S) has an important function in growth and regulation
42 of plant development,^[4] because of its essential role in the synthesis of amino acids,
43 proteins and some secondary metabolites.^[5]

44 While selenium (Se) is not an essential plant nutrient,^[6] and exerts toxic effects
45 in plants principally by interfering with sulfur (S) metabolism,^[7] this elements is essential
46 for maintaining mammalian health. Benefits attributed to proper Se nutrition range

47 from immune system enhancement to cancer suppression.^[8] However, when consumed
48 in high quantities, Se can accumulate in tissues and become toxic.^[9]

49 The current problem of the pollution of agricultural soils and waters causes
50 problems for human health which can be partially solved with the application of
51 technology of phytoremediation.^[10] The objective of this technology is to eliminate
52 contaminants for the environment by using plants.^[11]

53 The aim of the present work was to evaluate the effect of the different root zone
54 temperatures generated by the application of mulches on S status and Se concentrations
55 using field grown potato plants. The aim of the present work was to evaluate the effect
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57 status and Se concentrations using field grown potato plants.

58

59 **MATERIALS AND METHODS**

60 **Crop Design**

61 The experiment was conducted for three consecutive years (1993–1995) in the
62 field (Granada, Spain), using *Solanum tuberosum* L. var. Spunta, planted at the beginning
63 of March and the crop cycle was about 4 months. The climate was semiarid and the area
64 intensively used for agriculture. The soil used showed the following characteristics: sand
65 45.3%, silt 43.2%, and clay 11.2%, pH (H₂O 1:2.5) 8.6; electrical conductivity 1.10dSm⁻¹,
66 CaCO₃ 11.2%; total N (0.1%); P₂O₅ (58μgg⁻¹); K₂O (115μgg⁻¹); DTPA+TEA+CaCl₂ (pH 7.3)
67 extractable Se 24μgkg⁻¹. The characteristics of the irrigation water were: pH 7.6; E.C.
68 1.05dSm⁻¹; Cl 58mgL⁻¹; Na⁺ 25mgL⁻¹; K⁺ 4mgL⁻¹; H₂CO₃ 369mgL⁻¹, Se 1μgL⁻¹, SO₄⁻² 90mgL⁻¹.

69 The experimental design was a factorial arrangement in a randomized complete
70 block with 5 treatments replicated 4 times (20 plots). Each plot occupied an area of
71 78.4m², with a planting density of 4.2 plants m⁻². Plants were spaced 30cm apart, with
72 80cm between rows. The soil temperature was measured at the 15-cm in depth, using
73 probes (107 type) from Campbell Scientific TM. Root zone temperature was measured
74 (6 measurements at 4-h intervals) every 3 days of the crop cycle.

75 The different treatments consisted of covering the soil surface of each plot with
76 plastic mulches (polyethylene sheets), making a tight seal with the soil: transparent
77 polyethylene (25mm in thickness, T1), white polyethylene (25mm in thickness, T2),
78 coextruded black and white polyethylene (50mm in thickness, T3), and black
79 polyethylene (25mm in thickness, T4). Finally, no plastic was applied in the control
80 treatment (T0).

81 The fertilization used was the same as is habitually applied by farmers in the
82 zone. In the month of February in all three years, N (NH₄NO₃) and P and K (K₂HPO₄) were
83 applied (27gm⁻²). Afterwards, at the end of the month of April, 25gm⁻² of NH₄NO₃ were
84 applied. Fertigation was complemented with the following micronutrients: Fe: 0.5mgL⁻¹
85 ¹; B: 0.1mgL⁻¹; Mn: 0.1mgL⁻¹; Zn: 0.075mgL⁻¹; Cu: 0.075mgL⁻¹ and Mo: 0.05mgL⁻¹. Iron was
86 applied as FeEDDHA, B as H₃BO₃ and the remaining micronutrients as sulphates.

87

88 **Plant Sampling**

89 The plant material (stems, leaves, roots and tubers) were sampled 6 times every
90 two weeks, throughout the plant development for the three years of experiments. For
91 each sampling, 10 plants were collected from each replicate per treatment. Leaf samples
92 were taken only from plants with fully expanded leaves of the same size. Leaves were

93 picked at about one third of the plant height from the plant apex. Roots, leaves, stems
94 and tubers were rinsed three times in distilled water after decontamination with non-
95 ionic detergent at 1%, then blotted on filter paper. Then a sample was dried in a forced
96 air oven at 70°C for 24h, ground in a wiley mill and then placed in plastic bags for the
97 further analyses.

98

99 **Plant Analysis**

100 Se Determination:

101 For the assay of total Se concentration, oven-dried and pulverized plant material
102 was digested with concentrated nitric acid and measurements were made using an
103 atomic absorption spectrophotometer equipped with a graphite furnace.^[12] Reagent
104 blanks for analysis were also prepared performing the entire extraction procedure but
105 in the absence of the samples.

106

107 Sulfate Determination:

108 Sulfate (SO_4^{2-}) was determined in aqueous extraction of 0.2g of dried ground
109 material in 10mL of MILLIPORE-filtered water, shaking 120min at room temperature and
110 then filtered with Whatman-n1 filter paper. SO_4^{2-} was determined from the aqueous
111 extract obtained and measured by turbidimetry of the BaSO_4 maintained in suspension
112 by means of tensioactive agent (gum arabic) according to Novozamsky and Van Eck.^[13]

113

114 Organic-S and Total-S Determination:

115 A 0.1g dry weight sub-sample was digested with nitric acid mineralization and
116 H_2O_2 .^[14] After dilution with deionized water, in the product, organic S was measured as
117 described previously for the sulphate,^[13] against a pattern curve. Total S were
118 recalculated from the sum of organic S and sulphate.

119

120 **Statistical Analyses**

121 Analysis of variance was used to assess the significance of treatment means.
122 Significant differences according to the Duncan's Multiple Range Test (DMRT) are
123 indicated with different letters in the tables. Level of significance are represented by *
124 at $p < 0.05$, ** at $p < 0.01$, *** at $p < 0.001$, and ns: not significant.

125

126 **RESULTS AND DISCUSSION**

127 Table 1 shows the mean root zone temperatures (RZT) generated under the
128 different treatments with the highest value in T4 (30°C), and the lowest in T0 (16°C). The
129 effect of the different mulches on root-zone temperatures were similar to those of Ham
130 et al.,^[15] who reported that black polyethylene (our T4), absorbs roughly 96% of the
131 incoming radiation while reflecting very little, thus warms the soil.^[16] The white
132 polyethylene covers (T2) induced a cooler soil temperature than did black covers (T4)
133 because the former reflected most wavelengths than transparent mulches (T1) do not
134 cause soil warming, presenting mean temperatures of 18–20°C whereas the white & black
135 coextruded covers generated higher mean RZT (27°C in T3).

136 Table 1 also presents the results of the biomass (in a dry-weight basis) for the
137 different organs of the potato plants. The dry mass was significantly affected by the RZT
138 and showing for the roots, leaves and tubers, the highest values in T3 (27°C), and the
139 lowest in T1 (20°C), the latter being lower than in T0 (16°C). On the contrary, in the

140 stems, T1 reached the highest dry weight while T3 showed the lowest. Similarly to the
141 results of Klock et al.,^[17] with tomato plants, the increase in total biomass was obtained
142 in plants within the root-zone temperature ranging of 23–27°C in roots, tubers and
143 leaflets, while outside this range (T0, T1 and T4), the dry weight fell with a lower dry
144 weight accumulation (Table 1).

145 In relation to the effect of the different root-zone temperatures on Se in roots,
146 T3 reached the highest concentration surpassing T0 by 51%, while the lowest value was
147 found in T1 (Table 2). In tubers, the Se concentrations were below the limit of the
148 detection of the employed technique, whereas in stems T4 presented the highest Se
149 concentration (9% higher than in T0), and the lowest in T2. Finally, the highest and the
150 lowest Se concentrations in leaflets were recorded in T3 (60% higher than in T0) and T1
151 (22% lower than T0), respectively.

152 Root-zone temperature strongly influences the uptake of elements.^[1,18] In our
153 experiments, the RZT treatments significantly increased the Se in the roots and leaflets
154 in T3 and T4 with higher root temperatures (Table 2) increasing the root absorption and
155 its transport to the aerial part. The failure to detect Se in the tubers was possibly due to
156 the minor translocation of this element via the phloem,^[19] since edible plant parts
157 (tubers) contain much less Se than do the inedible parts.^[20]

158 Non-significant differences were found between the treatments for SO_4^{2-}
159 concentration in roots (Table 3), whereas in tubers, the highest concentration was found
160 in T1 (exceeding T0 by 56%) and the lowest in T2 and T3 (33% and 35% lower than T0,
161 respectively). In stems, higher SO_4^{2-} concentration were found in T0 and T1 whereas T2,
162 T3 and T4 were significantly lower. Finally, T1 gave highest leaflets SO_4^{2-} concentration
163 (15% higher than in T0), and the lowest in T3 (10% less than in T0).

164 Since SO_4^{2-} is absorbed in low quantities,^[5] the different root zone temperatures
165 did not significantly affect the SO_4^{2-} concentration in the roots (Table 3). However, in
166 tubers, the RZT in T1 induced higher redistribution of this elements and the
167 temperatures generated by T2 and T3 reduced the SO_4^{2-} concentration significantly,
168 possibly due to a high reduction and assimilation in the aerial part in organic compounds,
169 giving the low concentration in the tubers. With regard to the aerial part (Table 3), both
170 in stems and leaflets, the SO_4^{2-} concentrations in T2 and T3 were the lowest. The
171 reduction of SO_4^{2-} under this conditions was higher and the SO_4^{2-} which are highly mobile
172 in the xylem would be assimilated in the leaflets, while in T0 and T1, occurred high levels
173 of SO_4^{2-} possibly for a decrease in assimilation.^[4]

174 The organic-S concentrations reflects S fraction in organic structures and
175 represents its assimilation, varying similarly to the total-S.^[21] In roots, the highest
176 organic-S concentration was also recorded in T3 (26% higher than T0; Table 4) and the
177 lowest values were recorded in T0 and T1. In tubers, except T1, which gave the lowest
178 concentration (18% lower than T0), the rest of treatments did not statistically differ from
179 each other. In stems, non-significant differences were found between treatments.
180 Finally, in leaflets, T3 also gave the highest concentration (23% higher than T0), and no
181 significant differences were found between the rest of treatments.

182 With respect to this organic form of the S in the roots (Table 4), the temperatures
183 induced by T2, T3 and T4 favoured a higher assimilation of SO_4^{2-} , in comparison with the
184 lower root temperatures of T0 and T1.^[22] In the tubers, except for the T1 treatment, with
185 low concentrations of organic S, the temperatures generated by other treatments did
186 not influence its redistribution. For the aerial part (Table 4), we observed that the

187 concentration of organic-S in the stems was not significantly affected by the root zone
188 temperatures, while in the leaflets T3 induced maximum concentration of this S forms,
189 implying a higher SO_4^{2-} reduction and a high transport rate of SO_4^{2-} from the roots,^[5]
190 while the lower temperatures induced by T0 and T1, and the too high temperatures of
191 the T4 had a negative influence on these processes.

192 The total-S in roots (Table 5), presented in T3 the highest concentration surpassing
193 T0 by 22% and the lower values were found in T0 and T1, while in tubers, non-significant
194 differences were found between treatments. For stems, the highest total-S
195 concentrations were found in T0 and T4 and the lowest in intermediate treatments. In
196 leaflets, T3 gave an increase of 19% with respect to T0.

197 Therefore, the different root-zone temperatures significantly influenced the total-
198 S concentration (Table 5), with lower concentrations of total-S in the roots in T0 and T1
199 as a result of the lower root-zone temperatures,^[23] affecting significantly the S uptake
200 and assimilation as well as growth, in comparison with the treatments of high
201 temperatures, mainly the T3 that favoured a higher assimilation and concentration of
202 this macronutrient. In the tubers, the root-zone temperature did not affect the total-S
203 concentrations and for the stems, the highest levels were given in the treatments with
204 the highest (T4) and the lowest root temperatures (T0), since the root-zone temperature
205 directly affect the total-S concentration, because the effect was exerted on the
206 concentrations of SO_4^{2-} and organic-S, while the range of more appropriate
207 temperatures, T2 and T3, reduced the concentration of total S in the stems. In the
208 leaflets (Table 5), in order to favour the synthesis of amino acids as tolerance mechanism
209 to toxic elements, would interest a higher growth and S concentrations,^[24] since a high
210 level of organic-S was observed in T3 (treatment with a high concentration of Se), a
211 higher SO_4^{2-} assimilation was favoured in the leaflets.

212 For plants, Se toxicity results primarily from the interference with the sulfur
213 metabolism,^[7] and most agricultural crops have low Se tolerance (<50mgSekg⁻¹ d.w.). In
214 relation to such phytotoxicity, according to Pais and Jones,^[24] the normal Se content is
215 0.02mgkg⁻¹ leaf d.w., while in our experiments the plants accumulated quantities
216 exceeding 0.4–0.5mgkg⁻¹ without any toxicity symptoms. Ulrich,^[25] suggested that the
217 normal concentrations of S and SO_4^{2-} are between 0.8–3.0mgg⁻¹ leaf d.w., and between
218 0.25–1mgg⁻¹ leaf d.w., respectively. Our potato plants presented higher concentrations
219 in leaflets (25–30mg g⁻¹S d.w.; 3–4mgg⁻¹ SO_4^{2-} d.w.) probably as a tolerance mechanism
220 of these plants to the high S status.

221 According to Salt and Krämer,^[26] a plant is a hyperaccumulator if the ratio
222 (concentration of the metal in the aerial part):(concentration of metal in the root part)
223 exceeds 1. According to this, for Se, the ratio exceeded 1 and implies a potential for
224 hyperaccumulation.^[27] Although the Se levels is below 0.1% d.w. in the aerial part, these
225 potato plants provides an advantage in phytoremediation against the techniques based
226 on engineering, which are costly and also cause pollution.^[11] Therefore, there is a need
227 to improve the possibilities of accumulation of elements in potato crops and/or in other
228 species, using the mulch technique to ensure phytoextraction by manipulation of the
229 root-zone temperatures.

230

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Table 1. Effect of Mulch Treatments on Root-Zone Temperature (RZT) and on Biomass (Dry Weight) of Potato Organs

Treatments	RZT	Roots	Tubers	Stems	Leaflets
	°C	Biomass g Plant ¹			
T0	16 e ^a	1.75 bc	19.94 c	1.82 b	2.48 b
T1	20 d	1.04 c	10.89 d	2.19 a	1.93 c
T2	23 c	1.97 b	22.42 b	1.85 b	2.59 b
T3	27 b	2.34 a	26.93 a	1.70 c	2.84 a
T4	30 a	1.63 bc	20.70 c	2.09 ab	2.51 b

^aMean values followed by the same letter within a column were not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

Table 2. Effect of Root-Zone Temperatures on Total Se Concentration in Potato Organs

Treatments	Roots	Tubers	Stems	Leaflets
	Mgg ⁻¹ d.w.			
T0	209 cd ^a	- ^y	448 b	263 c
T1	115 d	-	465 ab	206 d
T2	225 c	-	154 d	322 b
T3	315 a	-	195 c	423 a
T4	260 b	-	489 a	307 bc

^aMean values followed by the same letter within a column were not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test. ^yConcentration below detection limits.

Table 3. Effect of Root-Zone Temperatures on SO₄ Concentration in Potato Organs

Treatments	Roots	Tubers	Stems	Leaflets
	Mgg ⁻¹ d.w.			
T0	3.61 a ^a	2.45 b	4.05 a	3.38 ab
T1	3.73 a	3.84 a	4.31 a	3.89 a
T2	3.72 a	1.66 c	3.14 b	3.18 b
T3	3.65 a	1.61 c	3 b	3.07 b
T4	3.60 a	2.49 b	3.01 b	3.41 ab

^aMean values followed by the same letter within a column were not significantly different at p<0.05 according to Duncan's Multiple Range Test.

Table 4. Effect of Root-Zone Temperatures on Organic-S Concentration in Potato Organs

Treatments	Roots	Tubers	Stems	Leaflets
	mgg ¹ d.w.			
T0	20.98 b ^a	13.24 a	25.47 a	22.76 b
T1	20.49 b	10.82 b	22.11 a	21.28 b
T2	23.57 ab	13.96 a	23.11 a	23.53 b
T3	26.48 a	14.28 a	23.67 a	28.11 a
T4	23.47 ab	13.56 a	26.46 a	22.67 b

^aMean values followed by the same letter within a column were not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

Table 5. Effect of Root-Zone Temperatures on Total-S Concentration in Potato Organs

Treatments	Roots	Tubers	Stems	Leaflets
	mgg ⁻¹ d.w.			
T0	24.6 c ^a	15.7 a	29.5 a	26.1 b
T1	24.2 c	14.7 a	26.4 b	25.2 b
T2	27.2 b	15.6 a	26.3 b	26.7 b
T3	30.1 a	15.9 a	26.7 b	31.2 a
T4	27.1 b	16.1 a	29.5 a	26.1 b

^aMean values followed by the same letter within a column were not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.