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EFFECT OF PB STRESS ON RELATIVE WATER CONTENT, PHOTOSYNTHETIC PIGMENTS, PB UPTAKE AND NUTRIENTS (CA, NA AND K) BALANCE IN BROAD BEAN (*VICIA FABA* L.) PLANT

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ABSTRACT

Lead is widely used in everyday life, it has important physicochemical properties, and it is a very useful chemical element, abundant but dangerous. In plants, it has no physiological role and is a toxic element. To study the effect of this pollutant physiologically and chemically in bean (*Vicia faba* L.) plants, different doses of lead in the form of [$Pb(NO_3)_2$] (0, 50, 100, 200, 500, 1000, 2000 ppm) were added to the culture substrate consisting of sand and compost. The results of this study showed a reduction in relative water content (RWC) in dry weight and chlorophyll pigment content. The results also showed a significant reduction in sodium (Na) in the roots and stems. The presence of lead caused significant physiological and chemical disturbances.

Keywords : *Vicia faba*, lead, chlorophyll, calcium, potassium, sodium.

Introduction

Lead (Pb) is a metal that has been used by humans for several thousand years (Gupta *et al*., 2020), its consumption continues to decrease and its main use is in batteries (Külahcı, 2020). Lead is present in the soil, but also in water, in the air, and in living organisms, it is the most common and toxic element affecting living organisms in the environment (Shahid *et al*., 2011). In fact, after arsenic, lead is considered the most hazardous pollutant (ATSDR, 2020). Due to its important physicochemical properties, lead is widely used in daily life, it is a very important, abundant but dangerous chemical element.

Lead is not very mobile in natural environments and tends to accumulate in the surface horizons of soils (Baize, 2010). Lead has no physiological role and remains toxic even at low doses (Beak *et al*., 2006). Lead is a non-biodegradable element, persists in the environment and can therefore be transmitted to plants and humans via soil, air, and water (Natasha *et al*., 2020).

The effects of lead on plants can vary depending on the duration of exposure, the intensity of the stress, the stage of development of the plant, the organs considered, and obviously the species and even the cultivar (Pourrut *et al*., 2011). In high doses, lead leads to the total inhibition of germination, reduces the development of the seedling and the rootlets (Islam*et al*., 2007; Dey *et al*., 2007; Gichner *et al*., 2008; Gopal and Rizvi, 2008), reduces the protein pool (Jana and Choudhuri, 1982; Mohan and Hosetti, 1997; Saxena *et al*., 2003; Mishra *et al*., 2006; Garcia *et al*., 2006; Piotrowska *et al*., 2009), and alters the qualitative composition of proteins (Beltagi, 2005). Lead can affect enzyme activity and causes dysfunction of a very large number of enzymes involved in various metabolic processes (Mitra *et al*., 2020). It leads to strong inhibition of photosynthesis, photosynthetic yield, and limitation of CO_2 assimilation rate (Xiong *et al*., 2006; Hu *et al*., 2007; Liu *et al*., 2008; Piotrowska *et al*., 2009; Singh *et al*., 2010; Cenkci *et al*., 2010).

Lead also affects plant mineral nutrition, disrupting nutrient uptake and transport (Chatterjee *et al*., 2004; Sharma and Dubey 2005; Gopal and Rizvi 2008). Genetically the toxic effects of lead on plants result in DNA damage (Pourrut *et al*., 2011; Shahid *et al*., 2011; Kumar *et al*., 2017) and chromosomal abnormalities (Rodriguez *et al*., 2013).

The choice of the plant is based on its interest from an agronomic and eco-toxicological point of view. Thus, the plant used the bean, an annual legume botanically known as *Vicia faba* L. (Hanelt and Mettin, 1989). The bean is used for food and feed (Singh and Bhatt, 2012). The global production of dried faba bean seeds for the year 2020 has been estimated at 5.67 million tons, with China (30.4%), Ethiopia (18.89%), and the United Kingdom (9.77%) being the major producers (TRIDGE, 2022). In addition to its agronomic interest, the bean is used in studies of the response to different stresses (Marcato-Romain *et al*., 2009).

This work consists to evaluate the impact of the presence of lead on the behavior and development of the bean. This is established by studying the application of different doses of lead in the form of $Pb[(NO_3)]_2$, in the culture substrate on the plants of the bean (*Vicia faba* L.). The reactions of the plants subjected to this stress are evaluated by physiological and chemical parameters.

Materials and Methods

Plant material and experimental conditions

The plants used in this experiment are faba-bean plants *Vicia faba* L. major of the cultivar (super aguadulce).

The disinfected seeds were germinated for one week and then transplanted in a culture substrate composed of compost and sand 1V/3V. The plants were kept in the greenhouse until the end of the experiment.

Lead was added to the substrate after 5 days of transplanting the bean seedlings as lead nitrate $Pb[(NO_3)]_2$. The treatment levels selected were D1 = 50 ppm; D2 =100 ppm; D3 = 200 ppm; D4 = 500 ppm; D5 = 1000 ppm; D6 = 2000 ppm and the control D0 = 0 ppm.

Plant analysis

Physiological parameters

30 days after the application of stress the aerial (ADW) and root dry weight (RDW) of the plants is established after removal of the plants and each part is dried at 120°C for 24 hours. The relative water content (RWC) is determined by the equation of Barrs and Weatheley (1968):

$$RWC(\%) = [(FW - DW)/(TW - DW)] \times 100$$

TW: turgescent weight (g); FW: fresh weight (g); DW: dry weight (g)

Chlorophyll pigments

Chlorophyll pigment concentrations (chlorophyll a, chlorophyll b, and carotenoids) are determined by spectrometry (Wang *et al.*, 2010) and contents are expressed in mg/g fresh matter using the following formulas:

$$Chlr.a = 12.25 \times A663 - 2.79 \times A645 \text{(chlorophyll a)}$$

$$Chlr.b = 21.50 \times A645 - 5.10 \times A663 \text{ (chlorophyll b)}$$

$$CRTN = (1000 \times A470 - 1.82 \times Chlr.a - 85.02 \times Chlr.b)/198 \text{ (carotenoids)}.$$

Mineral elements

The concentrations of mineral elements calcium (Ca), potassium (K), and sodium (Na) are determined by spectrometry according to the method described by Martin-Prevel *et al.* (1984) after the destruction of the organic matter by calcination and reading with a flame spectrophotometer.

The determination of lead content is performed by atomic absorption spectrometry (AAS) after wet digestion with nitric acid and perchloric acid according to the method described by Vogel-Milkus *et al.* (2005).

Statistical analysis

The results are presented as means ± standard deviation (Mean±SD). All the data obtained were statistically studied using a one-way (ANOVA), and a simple linear regression with the presentation of R^2. Means are compared according to Dunnett's test at $p < 0.05$.

Results and Discussion

Table 1 : Results of the effect of lead on the tested physiological parameters

		Control	D1	D2	D3	D4	D5	D6	P value
chlorophyll a (mg/g)	Mean	1.20	1.00^{ns}	0.69^{**}	0.48^{****}	0.47^{****}	0.38^{****}	0.33^{****}	**p<0.0001**
	± SD	0.13	0.26	0.17	0.12	0.14	0.13	0.11	
chlorophyll b (mg/g)	Mean	0.83	0.59^{**}	0.52^{***}	0.31^{****}	0.28^{****}	0.17^{****}	0.17^{****}	**p<0.0001**
	± SD	0.12	0.14	0.09	0.04	0.09	0.02	0.08	
carotenoids (mg/g)	Mean	0.56	0.53^{ns}	0.51^{ns}	0.52^{ns}	0.47^{ns}	0.46^{ns}	0.42^{*}	p<0.0654
	± SD	0.08	0.04	0.01	0.08	0.09	0.03	0.06	
ADW (g)	Mean	11.30	7.66^{**}	6.94^{***}	6.52^{***}	6.11^{****}	5.39^{****}	3.75^{****}	**p<0.0001**
	± SD	1.62	0.82	1.60	1.44	1.84	0.47	0.60	
RDW (g)	Mean	4.13	3.41^{ns}	3.08^{ns}	2.63^{**}	1.68^{****}	1.45^{****}	0.96^{****}	**p<0.0001**
	± SD	1.01	0.53	0.29	0.49	0.30	0.48	0.19	
RWC (%)	Mean	85.56	77.56^{ns}	75.71^{ns}	72.30^{*}	71.37^{**}	73.48^{*}	68.64^{**}	**p<0.0103**
	± SD	6.17	7.15	4.50	3.72	4.18	5.13	7.56	
Pb (mg/g)	Mean	0.00	0.05^{****}	0.08^{****}	0.11^{****}	0.23^{****}	0.36^{****}	0.71^{****}	**p<0.0001**
	± SD	0.00	0.00	0.00	0.00	0.00	0.00	0.02	

(^{ns}) not significant ; (*,**,***,****) Significant (Dunnett test)

(p) p value not significant ; (**p**) p value significant (one-way ANOVA test)

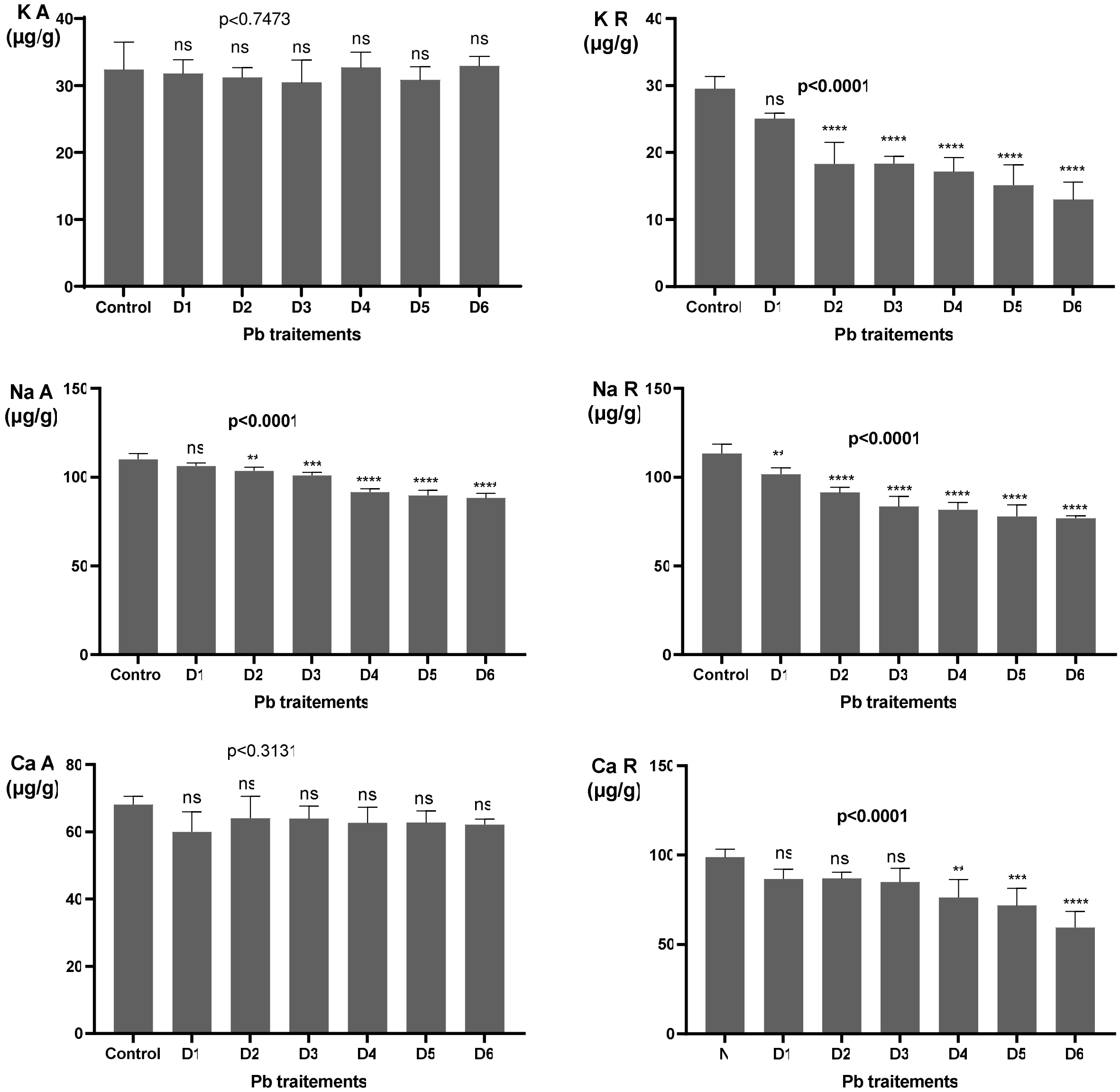

Fig. 1: Evolution of K, Na and Ca content in the aerial (A) and root (R) parts of the bean.
(^{ns}) not significant; (*,**,***,****); Significant (Dunnett test)
(p) p-value not significant; (**p**) p-value significant (one-way ANOVA test)

Dry weight

The results obtained show that the increase of the lead dose applied in the substrate is accompanied by a decrease in the dry weight (Table 1), these decreases affect both parts of the plant (root and aerial). In the roots, the weight reductions are more than 17% at 50 ppm dose (D1) and reach more than 76% at D6 dose (2000 ppm). The same trend is observed for the dry weight of the aerial part where the reductions are more than 32% at D1 dose (50 ppm), and more than 66% at D6 dose (2000 ppm) in comparison with the controls (Table 1).

These results are confirmed by those found by Islam *et al.* (2008); Kopittke *et al*, (2007); Gupta *et al*, (2010) who reported a very significant decrease in the growth of the underground and aerial parts of plants even at low doses. The obtained results are in agreement with those found by Mroczek-Zdyrska *et al.* (2017) with very significant ($p<0.05$) reductions in fresh and dry weight of roots, in *Vicia faba* plants treated with 50 µM Pb. Islam *et al.* (2008) reported a reduction in fresh weight up to more than 50%, compared to the control, with significant inhibition of plant development. Root growth is more influenced by Pb toxicity than aerial parts (Zhou *et al.*, 2016). The inhibition of plant growth by decreasing biomass could be from the disruption of cell division (Rucińska *et al.*, 2004; Kozhevnikova *et al.*, 2009).

Relative Water Content (RWC)

The observation of the RWC (Tab.1) shows that increasing the Pb dose causes a decrease in RWC in the bean plants. These reductions are significant from the 200 ppm dose (D3) with more than 15%, and more than 19% reduction at the 2000 ppm dose (D6). The analysis of variance (Tab.1) records a significant effect ($p<0.0103$) of the Pb dose added to the substrate on RWC. The dependence between lead stress and RWC is highly significant (Fig. 2) with R^2= 0.2351.

Disruptions in the water status of plants exposed to different concentrations of lead are reported in various crops (Sharma and Dubey, 2005). On *Helianthus annuus* L. plants (Kastori *et al.*, 2008), on wheat (Alsokari and Aldesuquy, 2011), and on faba bean (Pourrut, 2008). The reduction in RWC is attributed to reduced leaf area in *Glycine max* L.

plants (Elzbieta and Miroslawa, 2005), and attributed to the limitation in transpiration for to reduce Pb translocation from roots to aerial parts (Wang *et al*., 2013).

Photosynthetic pigment content

The results obtained (Tab.1) show that the chlorophyll a content strongly decreases with the increase of the lead dose added in the substrate. The regression of the chlorophyll a content is highly significant from the 100 ppm dose (D2) where it is more than 42%, this regression reaches more than 72% at the 2000 ppm dose (Table 1). The results obtained (Table 1) show that the fluctuations of chlorophyll a are highly dependent on the variation of the lead dose ($p < 0.0001^{****}$). The observations of the results (Table 1) also show that the increase of the lead dose in the culture substrate causes a very important regression of the chlorophyll b content, these regressions are very significant starting from the 50 ppm dose (D1) where we recorded a decrease compared to the control of about 28.85% and reach at the 2000 ppm dose (D6) more than 79% (Table 1). The fluctuations in chlorophyll b content (Table 1) are dependent on the variation of the lead dose factor ($p<0.0001^{****}$). For Carotenoids, the results recorded (Table 1), shows a slight decline with the increase of the lead dose added in the substrate, this decrease remains insignificant until the 2000 ppm dose (D6) with a reduction of about 25.14% compared to the values recorded for the controls (Table 1). The variance of this parameter as a function of the presence of lead in the medium (Table 1) remains non-significant ($p<0.0654^{ns}$).

The inhibition of photosynthesis and reduction of photosynthetic yield in the presence of lead stress observed is confirmed by several authors (Xiong *et al*., 2006; Hu *et al*., 2007; Liu *et al*., 2008; Piotrowska *et al*., 2009; Singh *et al*., 2010; Cenkci *et al*., 2010; Azooz *et al*., 2011). The decreases in chlorophyll pigment observed in bean plants in this study have been reported by several works performed on the same plant (Hedaya, 2008; Pourrut, 2008; Wang *et al*., 2010), or on other species (Gajic'*et al*., 2009, Ashraf *et al*., 2017). The decrease in chlorophyll levels was as high as +77% (at 40 mg/L after 30 days of treatment) compared to the control in *Lemna polyrrhiza* L. (John *et al*., 2008). Ashraf *et al*, (2017) showed that Pb stress significantly ($p<0.05$) inhibits photosynthetic pigments (Chlorophyll a , b, and carotenoids) in rice plants (+87% reduction at 1200 ppm dose). In *Triticum aestivum*, the presence of lead at different doses (1-4 mM) in the culture medium caused very significant reductions in photosynthetic pigments (Yang *et al*., 2010).

Chemical parameters

The recorded results of potassium content in aerial parts (Fig. 1) show a tendency to decrease slightly at dose D1 (1.89%), at dose D2 (3.61%), at dose D3 (5.92%), and at dose D5 (4.9%). The results also show an increase in potassium content in the aerial parts at dose D4 (0.93%) and at dose D6 (1.64%). For potassium in the roots, the results show a decrease with the increase in lead in the substrate (Fig. 1), this decrease is very significant from dose D2 (100ppm) with a reduction of 38% and it reaches over 56% at dose D6 (2000 ppm).

The sodium content in both parts of the plant decreases with the dose of Pb in the substrate increases. In the aerial parts, this decrease is only significant from D2 (100 ppm) 5.9%, and it reaches 19.83% for D6 (2000 ppm) (Fig. 1). In the root parts, the decrease in Na content is more important than that recorded for the aerial parts, we record (Fig. 1) for the dose D1 (50 ppm) a decrease is about 10.21% compared to the control, 26.33% for the dose D3 and more than 32% for the dose D6 (2000 ppm) (Fig.1).

The recorded results of calcium content (Fig. 1) show decreases of more than 6% for D2 and D3, more than 8% for D4 and D6, and decreases of more than 10% for D1 (50 ppm), but these decreases are non-significant $p<0.3131ns$ (Fig. 1). Calcium in roots also decreases with increasing Pb dose, the observed decrease is only significant from dose D4 (Fig. 1) with more than 22% reduction, more than 27% at dose D5 and more than 39% at dose D6 (Fig. 1).

Pb stress disrupts the relationship between the plant and nutrients and alters the internal ratios of plant mineral elements (Gopal and Rizvi, 2008), and reduces the mineral nutrition of plants (Dotaniya *et al.,* 2020). The passage of lead through the plant membranes affects the absorption mechanism in the roots and blocks the distribution of nutrients to different parts of the plant (Sharma and Dubey, 2005).

The results presented in this work (Fig. 2) show that Pb stress induces a significant correlation with sodium in the roots ($R^2=0.4315$) and in the aerial part ($R^2=0.6563$), Pb stress also induces a significant correlation for Ca in the roots ($R^2=0.6628$) and for potassium in the same parts ($R^2=0.4787$), the correlation in the aerial parts for these two elements is not significant.

Experiments performed on wheat plants treated with different concentrations (0, 1.5, 3 and 15 mM) of Pb showed a decrease in the concentration of some macro and micronutrients (Lamhamdi *et al*., 2013). The results obtained by Singh *et al.* (2015) showed reductions in the concentrations of calcium, sodium, and potassium in roots and aerial parts of maize plants exposed to Pb stress. Also, the reduction of potassium concentration was observed in root parts of faba bean plants exposed to mixed lead and salt stress (Azzouz and Bouziani, 2022). It was also found that Pb treatment resulted in decreased concentrations of zinc, iron, manganese, copper, calcium, phosphorus, and magnesium in *Oryza sativa* (Chatterjee *et al*., 2004), *Medicago sativa* (Lopez *et al*., 2007), and *Raphanus sativus* (Gopal and Rizvi, 2008).

Lead accumulation

The observation of the results of the accumulation of lead in plants (Table 1) shows that the increase in the Pb doses applied in the substrate is accompanied by a significant increase of the lead content in the treated plants, this accumulation reaches its maximum at the dose D6 (2000 ppm) and registers more than 0.7 mg/g. The analysis of variance (Table 1) shows that the variations of the lead dose factor exert a very highly significant effect ($p<0.0001$) on the accumulation of lead in the plants,

The results of this work are in agreement with those obtained by Hedaya (2008) which show that the lead level in the plant increases with the concentration of lead in the medium. The work of Nadgórska-Socha *et al.* (2013) performed on faba bean showed a highly significant correlation between the presence of lead in the soil and its content in the plant organs, r=0.99 in the stem, and r=0.97 in the leaves. A highly significant correlation (r=0.99*) was also reported in faba bean (Bouziani *et al*., 2020). Lead accumulation in the leaves of faba bean is also reported by Wang *et al*. (2007) with significant correlation r=0.97.

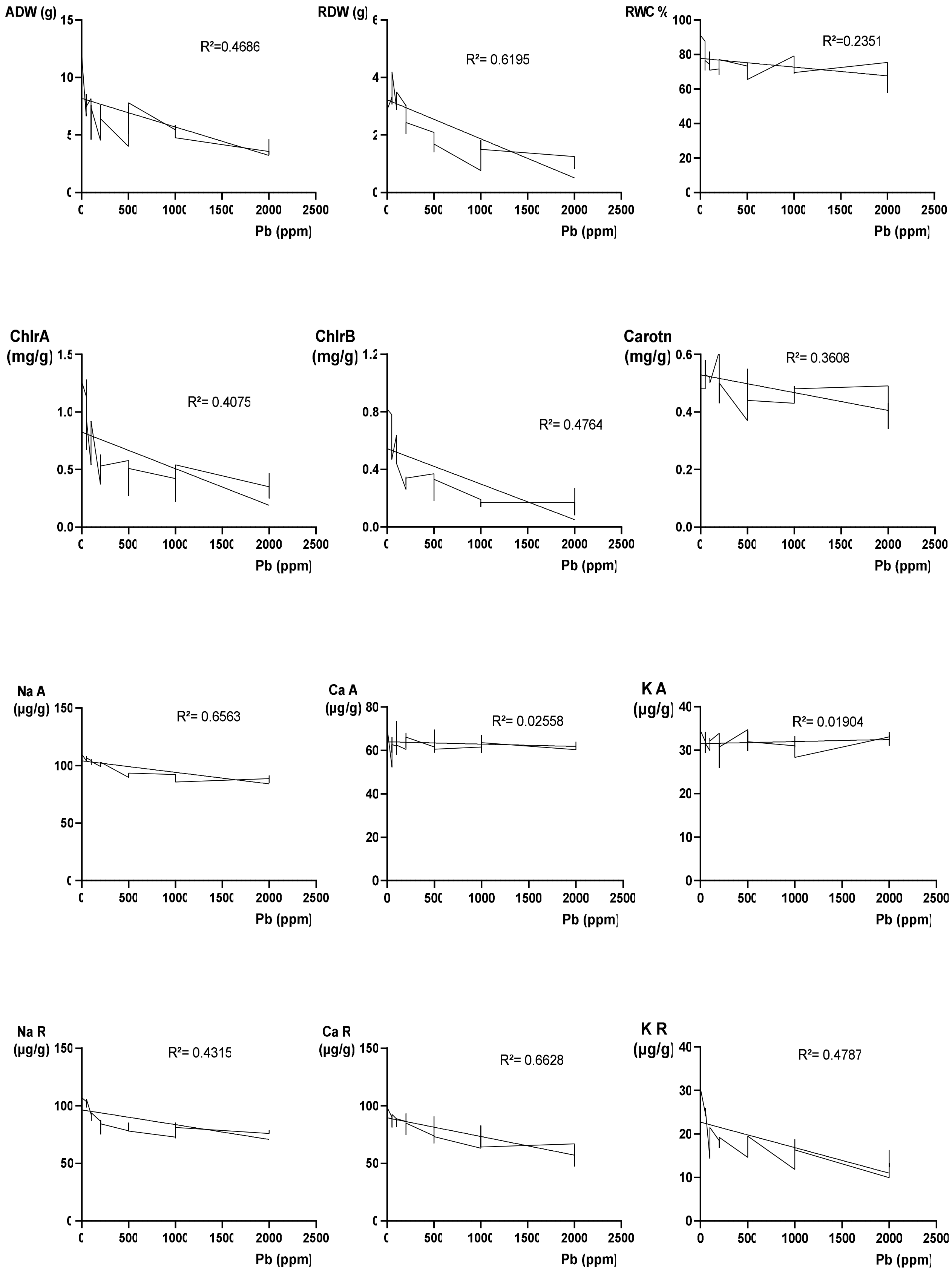

Fig. 2: Simple linear regression of the effect of Pb doses (ppm) on the physiological and chemical parameters tested, (R^2) R squared.

Conclusion

Under lead stress conditions, the present work showed the following:

- A significant decrease in relative water content (RWC), dry weight, and chlorophyll pigments,
- A significant reduction of Na content in the roots and aerial part and of K and Ca in the roots.
- The root parts in direct contact with Pb are the most affected by this stress.

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