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Assessment of photochemical performance of photosystem II in *Limbarda crithmoides* and *Helianthus annuus* under Pb stress using chlorophyll *a* fluorescence kinetics

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ABSTRACT

The effectiveness of chlorophyll *a* fluorescence as a quick tool to detect the effect of Pb–stress on *Limbarda crithmoides* and *Helianthus annuus* species, when exposed to increasing Pb concentrations (0–500 µmol.L^{-1}) for 45 days, was evaluated. The chlorophyll level in plant leaves, as well as the fresh weight and Pb content in the shoots and roots in both plant species, were also determined. *L. crithmoides* did not show any significant variation in photochemical quenching parameters and energy fluxes in all Pb concentrations despite the change in OJIP plot of 100 µmol.L^{-1} Pb. In addition, a significant increase in chlorophyll *a* content was noted along with an increase in the biomass production. However, in *H. annuus,* Pb stress increased energy absorption, dissipation and trapping and decreased energy transport and electron transport rate. In addition, the OJIP curve showed a notable modification at 300 and 500 µmol.L^{-1} Pb, without significant changes in the chlorophyll contents and in the biomass production under all Pb concentrations. Results also revealed that Pb was accumulated in the shoots and roots of *L. crithmoides* and *H. annuus* and the accumulation was more notorious in the roots of the treated plants. Lead exhibited different influence on the photosystem II performance in both species, thus, the evaluation of chlorophyll fluorescence was a very efficacious tool to reflect the physiological status of *L. crithmoides* and *H. annuus* under Pb stress.

Keywords: Chlorophyll, energy fluxes, OJIP curve, photosystem II efficiency, fresh weight, Pb uptake.

INTRODUCTION: The extensive increase in urbanization, industrialization, and technological change engendered the contamination of the environment with several metal trace elements such as cadmium, copper, chromium, aluminium, mercury, lead (Pb), etc (Mussarat et al., 2007; Jia et al. 2018; Souri *et al.*, 2019). These non-biodegradable inorganic pollutants can be part of inorganic fertilizers and pesticides, used in agricultural lands, which leads to the increment of their concentrations in the soils (Nagajyoti et al., 2010; Tchounwou et al., 2012). Once metal trace elements are bioavailable, plants growing on polluted soils can be adversely influenced to experience metabolic alterations and irreversible damage. Lead, the second most toxic metal after arsenic, is known by its adverse effects on the morphological, physiological, and biochemical functions leading to severe inhibition of plant growth and development (Zulfiqar *et al.*, 2019). It has been reported that Pb exposure induces an over-generation of reactive oxygen species that will cause the deterioration of bio-fragment structures such as nucleic acids and proteins (Yadav, 2010). Furthermore, the attack of free radicals on the polyunsaturated fatty acid of membrane lipids induces lipid peroxidation that leads to an oxidative stress and hampers several biochemical and metabolic activities of cellular organelles, such as mitochondria, peroxisomes and chloroplast (Malecka et al., 2008; Sytar et al. 2013). Chlorophyll molecules are very sensitive to several abiotic stresses including metal stress; it was revealed that Pb caused a reduction in chlorophyll biosynthesis followed by an inhibition in photosynthesis process which resulted in leaf chlorosis in several plant species (Kumar and Prasad, 2015; Souri *et al.*, 2019). Additionally, metal stress can damage the oxygen-evolving complex, reduce the activities of photosystem II (PSII) and photosystem I (PSI), and inhibit the energy transfer towards PSII-reaction center (Subrahmanyam 2008; Drążkiewicz and Baszyński 2010; Sytar et al. 2013; Kumar and Prasad, 2015). Romanowska et al. (2006) stated that the photosynthetic rate was found highly reduced in pea plants under the effect of Pb; the authors reported that this decline occurred due to the impairment in the photosystems and chloroplast ATPase activities. Kumar and Prasad (2015) reported also that Pb reduced PSII quantum yield and PSI activity in *Talinum triangulare*. Chlorophyll *a* fluorescence kinetics has been widely used for the detection of the changes in the photosynthetic apparatus through the analysis of photochemical parameters that can precise the response of the plant to metal stress (Živčák et al. 2014; Kumar and Prasad, 2015; Huang et al. 2019; Faseela *et al.*, 2020).

OBJECTIVES: The current study examines chlorophyll *a* fluorescence as a specific indicator to evaluate the effect of increasing Pb concentrations on photosystem II activity and investigate the change in photosynthesis performance in *Limbarda crithmoides* and *Helianthus annuus* species. To better understand the plant's response to the metal stress, the chlorophyll content in leaves, the biomass production and the accumulation potential of Pb in the shoots and roots of the two plants were also evaluated.

MATERIALS AND METHODS: Growth and treatment experiments: Plants of *Limbarda crithmoides* and *Helianthus annuus* were grown in an inert substrate consisting of perlite and gravel (2:1 v/v) in a greenhouse under semi–controlled conditions (natural photoperiod, a mean temperature of 25±5°C, and a relative humidity between 60% and 90%). Seedlings were regularly irrigated with Hewitt nutritive solution (Hewitt, 1966). For each species, five groups of 10 grown seedlings were treated with nutritive solution supplied with Pb at 0, 100, 200, 300 and 500 µmol.L^{-1}, three times a week, for 45 days. At the end of the treatment period, plants were harvested and divided into shoot and root parts. The plant organs were immediately weighed to determine the fresh biomass production.

Chlorophyll fluorescence: Chlorophyll fluorescence parameters including the maximum quantum efficiency of photosystem II (PSII) (Fv/Fm), the photochemical efficiency of PSII (Fv'/Fm'), the electron transport rate (ETR), and the absorbed (ABS), trapped (TRo), dissipated (DIo), and transported (ETo) energy fluxes per reaction centre (RC), were measured by a pulse amplitude modulation chlorophyll FluoroPen FP100-MAX (Photon systems Instruments, Dràsov, Czech Republic). The measurements were performed on dark adapted old leaves of *L. crithmoides* and *H. annuus* after 45 days of Pb exposure (Sghaier et al. 2015; Dridi *et al.*, 2022).

Chlorophyll extraction: Chlorophyll contents (Chl *a* and Chl *b*) were determined after the extraction of the pigments (Bankaji *et al.*, 2016). Briefly, pigments were extracted using 250 mg of fresh leaves tissue and 10 mL of ethanol 80% (v/v). The absorbance of the obtained extracts was read at 645 and 663 nm and Chl *a* and *b* levels were calculated (Ayvaz *et al.*, 2012).

The Pb extraction: The quantification of Pb in the shoots and roots of *L. crithmoides* and *H. annuus* plants was performed by atomic absorption spectrometry (AAS; Perkin Elmer PinAAcle 900T, Waltham, MA, USA), after extraction Pb (Labidi *et al.*, 2021; Sleimi et al., 2022; Bankaji et al.; 2023).

Statistical analysis: Data were presented as average values ± standard deviation of 10 replicates among each applied Pb concentration. Statistical analyses were carried out according to one way ANOVA and Tukey honest significant difference (HSD) tests, using Statistica 8 software, and results were significantly different at $p < 0.05$. Principal component analysis (PCA) was also performed by Statistica to assess the correlations between Pb and chlorophyll a fluorescence parameters.

RESULTS AND DISCUSSION: It has been well documented that

Pb generally inhibits the growth and reduces the biomass production of several plants, even at very low concentrations (Elzbieta and Miroslawa, 2005; Sinha et al. 2006; Huang *et al.*, 2019). However, findings of the current study revealed that the fresh weight (FW) of *L. crithmoides* shoots were significantly higher in all Pb doses when compared to the control ($p < 0.05$; figure 1a) whilst, FW content of the roots in 100–300 µmol L^{-1} Pb did not significantly differ when compared to untreated plants ($p > 0.05$). In fact, a significant increase was only detected in *L. crithmoides* roots of 500 µmol.L^{-1} Pb (approx. 60% increase was recorded when compared to the control; figure 1b). In contrast, *H. annuus* plants have not been affected by the increased Pb concentrations in the growth medium (no statistically differences were noted in the FW of shoots and roots of treated and untreated plants, $p < 0.05$; figure 1c,d).

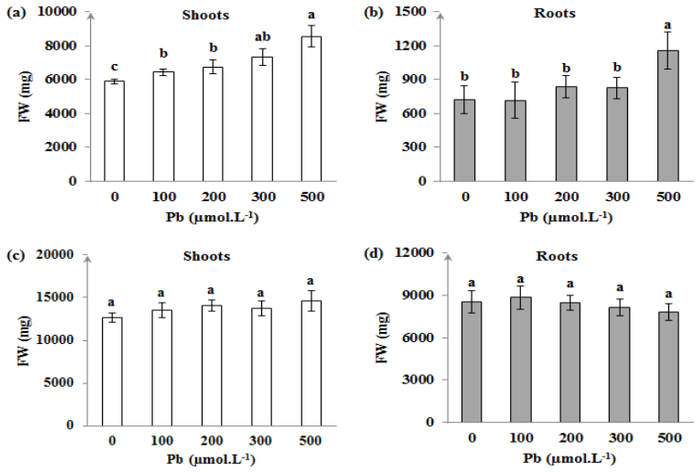

Figure 1: Fresh biomass production of the shoots and roots of *Limbarda crithmoides* (a, b) and *Helianthus annuus* (c,dd) plants after 45 days of Pb treatment. Values are means ± standard deviation (n = 10) and bars with different lower-case letters are significantly different at $p < 0.05$. FW: fresh weight, Pb: lead.

Throughout the trial, morphological observations of the plants were made and no differences in the greenness of the plant's leaves, between the treated plants and the control, were noticed. At the end of the experiments, no chlorosis signs were observed in the Pb treated plants of *L. crithmoides* (figure 2) and *H. annuus* (figure 3), for all Pb doses.

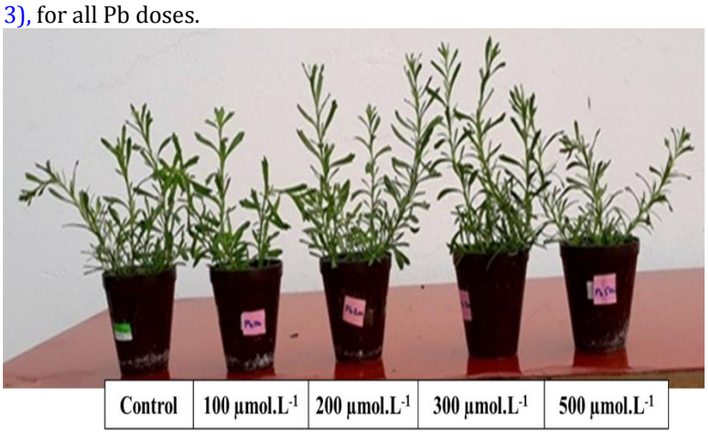

Figure 2: Representative photo of *Limbarda crithmoides* status after 45 days of exposure to different Pb concentrations (0, 100, 200, 300 and 500 µmol.L^{-1}).

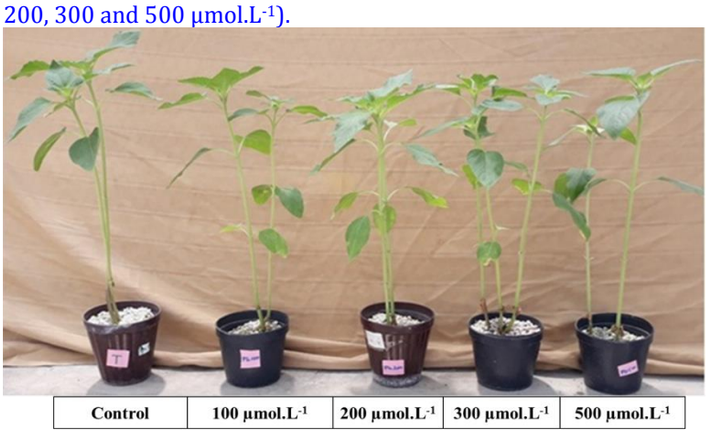

Figure 3: Representative photo of *Helianthus annuus* status after 45 days of the exposure to different Pb concentrations (0, 100, 200, 300 and 500 µmol.L^{-1}).

Overall, H. *annuus* obtained results were in line with Yang *et al.* (2015) results where a concentration of 500 mg.kg^{-1} of Pb did not significantly vary the biomass production of the shoots and roots in *Robinia pseudoacacia* plants. Similar results were found in *Arabis paniculata* plants treated with increased Pb concentrations going from 24 to 193 µmol.L^{-1} without showing any phytotoxicity signs (Tang *et al.*, 2009). On the other hand, and like in our *L. crithmoides* results, Małkowski *et al.* (2020) trials revealed a 27% increase in the growth of the shoots of maize when treated with 5 µmol.L^{-1} Pb for four days. Moreover, various Pb concentrations (from 10 up to 1000 µmol.L^{-1}) induced a significant stimulation in the length and in the FW of maize plants (Figlioli *et al.*, 2019). Several studies reported the stimulatory effect of Pb on plant growth and biomass production, and this has been explained by the fact that metal stress induced by low Pb concentrations may engender hormetic mechanisms (Wang et al., 2010; Szuba *et al.*, 2017). However, some plant species have experienced the stimulatory effect even at increased concentrations of metals (for example up to 2000 mg.kg^{-1} for Pb) that overpass the toxic threshold for common plants (Tang *et al.*, 2009). Recent studies proved that the stimulation of plant growth during hormesis might be related to a low level of oxidative stress and the generated reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), were involved in the hormetic effect (Jia et al., 2013; Luna-López et al., 2014; Shahid et al. 2019). It is well known that the generation of very high level of ROS leads to the cell death of the plant. However, low and sub-toxic concentrations of these molecules can regulate cell cycle and genomic integrity and also improve plant tolerance to abiotic stress (Achary and Panda, 2010; Jalal et al., 2021). For example, H_2O_2 can promote the stimulation of the biosynthesis of auxin, the major growth regulator, by inducing the expression of the genes VvAMI1 and VvYUC3, which increases auxin content in the plant (Pérez et al., 2021). Małkowski *et al.* (2020) observed a hormetic effect on growth of maize seedlings at 5 µmol.L^{-1} Pb with an increased content of indole-3- acetic acid (IAA) in the maize shoots. Therefore, the authors suggested that the increment in auxin production in response to sub-toxic level of Pb plays a key role in the phenomenon of hormesis in plants. On the other hand, H_2O_2 can act as a signalling molecule to promote cell walls malleability and allow inward water transport to induce cell expansion (Jalal *et al.*, 2021); this fact might also explain the increase of plant FW under the effect of metal stress.

Chlorophyll content: Results of the chlorophyll contents and measured fluorescence variables in leaves of *L. crithmoides* and *H. annuus* after 45 days of Pb exposure are presented in Table 1. As can be seen, *L. crithmoides* Chl *a* content significantly increased with the increase of the Pb concentration in the nutritive solution, as compared to the control ($p < 0.05$; a positive correlation between Chl *a* and Pb was shown in figure 4A), while *H. annuus* showed a non-significant decrease in Chl *a* level under the same conditions ($p > 0.05$).

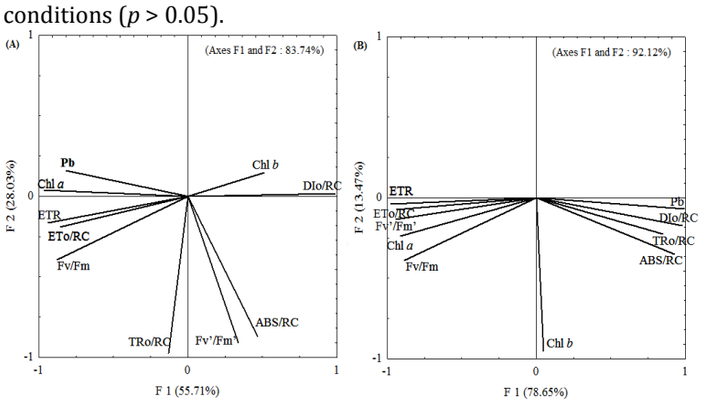

Figure 4: Principal component analysis (PCA) of the chlorophyll content and chlorophyll fluorescence parameters under the effect of increased Pb concentrations in *Limbarda crithmoides* (A) and *Helianthus annuus* (B) leaves. Pb: lead, Chl: chlorophyll, Fv/Fm: maximum quantum efficiency of photosystem II, Fv'/Fm': photochemical efficiency of Photosystem II, ETR: electron transport rate, ABS/RC: absorption flux, TRo/RC: trapped energy flux, ETo/RC electron transport flux, DIo/RC: dissipation flux, RC: active reaction center. Axes present Factor 1 and Factor 2, where Factor 1 (F1) explained the variance of 51.71% and 78.65% in *Limbarda crithmoides* and *Helianthus annuus* leaves, respectively and Factor 2 (F2) explained 28.03% and 13.74% of variance in *Limbarda crithmoides* and *Helianthus annuus*, respectively.

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Table 1: Chlorophyll contents and measured fluorescence variables in leaves of *Limbarda crithmoides* and *Helianthus annuus* after 45 days of Pb exposure. Pb: lead, Chl: chlorophyll, Fv/Fm: maximum quantum efficiency of photosystem II, Fv'/Fm': photochemical efficiency of photosystem II, ETR: electron transport rate, ABS/RC: absorption flux, TRo/RC: trapped energy flux, ETo/RC electron transport flux, DIo/RC: dissipation flux, RC: active reaction center.

Limbarda crithmoides									
Pb (µmol.L^{-1})	**Chl a**	**Chl b**	**Fv/Fm**	**Fv'/Fm'**	**ETR**	**ABS/RC**	**TRo/RC**	**ETo/RC**	**DIo/RC**
0	0.42^{c} ± 0.02	0.26^{a} ± 0.02	0.62^{a} ± 0.02	0.70^{a} ± 0.04	62.50^{a} ± 4.51	3.02^{a} ± 0.08	1.88^{a} ± 0.10	0.26^{a} ± 0.02	1.14^{a} ± 0.07
100	0.50^{b} ± 0.03	0.27^{a} ± 0.01	0.64^{a} ± 0.01	0.74^{a} ± 0.53	62.48^{a} ± 4.27	3.15^{a} ± 0.12	2.02^{a} ± 0.05	0.27^{a} ± 0.03	1.13^{a} ± 0.08
200	0.57^{ab} ± 0.04	0.22^{a} ± 0.02	0.64^{a} ± 0.01	0.72^{a} ± 0.06	70.00^{a} ± 3.37	3.04^{a} ± 0.11	1.97^{a} ± 0.04	0.33^{a} ± 0.02	1.07^{a} ± 0.08
300	0.56^{ab} ± 0.01	0.24^{a} ± 0.00	0.64^{a} ± 0.01	0.70^{a} ± 0.07	66.01^{a} ± 2.00	3.04^{a} ± 0.08	1.96^{a} ± 0.03	0.28^{a} ± 0.02	1.08^{a} ± 0.06
500	0.61^{a} ± 0.02	0.27^{a} ± 0.02	0.64^{a} ± 0.02	0.69^{a} ± 0.03	68.50^{a} ± 2.36	3.00^{a} ± 0.15	1.93^{a} ± 0.04	0.30^{a} ± 0.02	1.07^{a} ± 0.11
Helianthus annuus									
Pb (µmol.L^{-1})	**Chl a**	**Chl b**	**Fv/Fm**	**Fv'/Fm'**	**ETR**	**ABS/RC**	**TRo/RC**	**ETo/RC**	**DIo/RC**
0	0.62^{a} ± 0.02	0.59^{a} ± 0.07	0.71^{a} ± 0.03	0.76^{a} ± 0.03	85.00^{a} ± 3.79	2.07^{c}± 0.05	1.48^{b} ± 0.02	0.41^{a} ± 0.03	0.59^{d} ± 0.03
100	0.58^{a} ± 0.02	0.53^{a} ± 0.06	0.68^{a} ± 0.01	0.75^{a} ± 0.02	77.50^{a} ± 3.30	2.11^{bc} ± 0.07	1.44^{b} ± 0.03	0.38^{a} ± 0.03	0.67^{cd} ± 0.04
200	0.56^{a} ± 0.04	0.60^{a} ± 0.04	0.67^{a} ± 0.02	0.73^{a} ± 0.03	74.50^{ba} ± 5.50	2.32^{ba} ± 0.11	1.47^{ab} ± 0.06	0.36^{a} ± 0.04	0.85^{b} ± 0.06
300	0.56^{a} ± 0.03	0.53^{a} ± 0.05	0.69^{a} ± 0.01	0.72^{a} ± 0.01	74.00^{ba} ± 6.48	2.26^{a} ± 0.04	1.57^{a} ± 0.04	0.24^{b} ± 0.01	0.69^{c} ± 0.02
500	0.55^{a} ± 0.03	0.59^{a} ± 0.04	0.66^{a} ± 0.01	0.72^{a} ± 0.05	67.00^{b} ± 5.20	2.65^{a} ± 0.04	1.65^{a} ± 0.02	0.20^{b} ± 0.01	1.00^{a} ± 0.04

However, Pb treatment had no impact on Chl *b* contents in both species when compared to the control (p > 0.05; table 1). According to previous studies, the level of photosynthetic pigments (Chl *a* and *b*) can increase or remain unaffected under the effect of Pb (Szuba *et al.*, 2017). For example, at Pb concentrations of 24–386 µmol.L^{-1}, no significant modification in Chl *a* and *b* contents in *Arabis paniculata* was detected (Tang *et al.*, 2009). Yang *et al.* (2015) reported that Chl *a* content remained the same as that of the control in *Robinia pseudoacacia* leaves when exposed to 500 mg.kg^{-1} Pb. Similar results were observed in maize plants when exposed to 10^{-5} and Pb 10^{-4} mol.L^{-1} Pb; however, this plant showed a significant increase in Chl *a* at 10^{3} µmol.L^{-1} Pb (Figlioli *et al.*, 2019). Figlioli *et al.* (2019) reported that the increase in chlorophyll biosynthesis in Pb treated plants was related to increased photosynthetic protein level, Rubisco (ribulose-1,5-bisphosphate carboxylase-oxygenase). In addition, the chloroplasts had a preserved ultrastructure after Pb exposure with no alteration in thylakoid organization (Figlioli *et al.*, 2019) which might be due to a limited Pb translocation to the aboveground parts (Mellem et al., 2009).

Chlorophyll a fluorescence: The evaluation of the quenching parameters in *L. crithmoides* plants treated with increasing Pb concentrations showed that Fv/Fm, Fv'/Fm', ETR and all of the energy fluxes (ABS/RC, TRo/RC, ETo/RC and DIo/RC) were not affected by Pb stress and remained stable as the control (p < 0.05; table 1). The analysis of the OJIP induction curve of *L. crithmoides* plants showed an increase in the J, I and P steps, only at 100 µmol.L^{-1} Pb, when compared to the control (figure 5A).

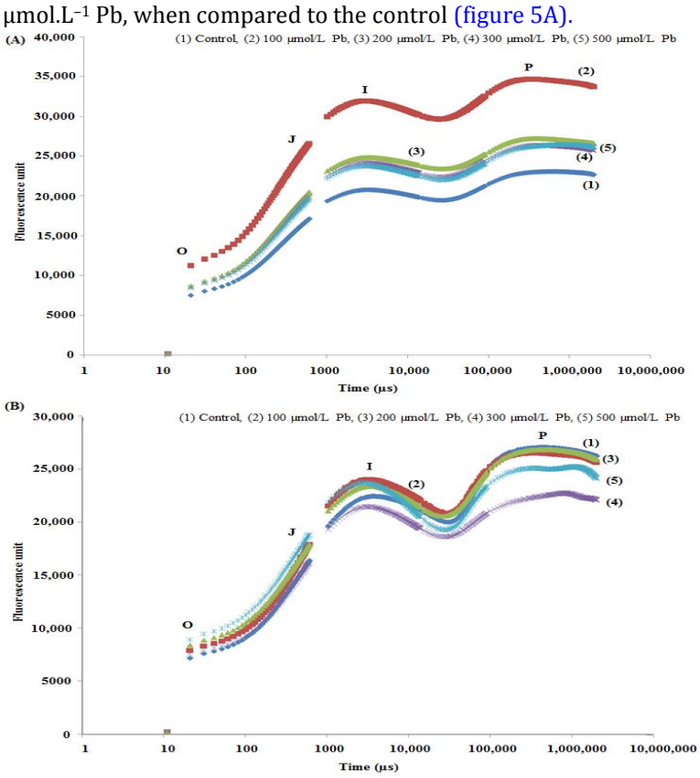

Figure 5: OJIP induction curves in dark-adapted leaves of *Limbarda crithmoides* (A) and *Helianthus annuus* (B) after 45 days of lead (Pb) treatment.

The change in the OJIP plot in the lowest Pb concentrations (100 µmol.L^{-1}) was in line with results found in the same species under the effect of 200 µmol.L^{-1} Ba (Dridi *et al.*, 2022). This possibly indicates that damage in the oxygen evolving complex (OEC) might start to appear at this Pb concentration (Małkowski *et al.*, 2020). Overall, despite this notable variation in OJIP plots, *L. crithmoides* presented a normal performance of the PSII in all Pb concentrations that was comparable to the control. Similar findings were reported in maize plants cultivated in the presence of Pb (Figlioli *et al.*, 2019). In contrast, *H. annuus* plants showed a non-significant decrease in Fv/Fm and Fv'/Fm' in all Pb concentrations (p > 0.05); ETR also decreased under the effect of Pb, but the decline was statistically significant only at 500 µmol.L^{-1} Pb (p < 0.05; table 1). Regarding the energy fluxes variations in *H. annuus*, the absorbed and dissipated energies increased significantly in the 200, 300 and 500 µmol.L–1 Pb concentrations (table 1). In addition, the highest Pb concentrations (300 and 500 µmol.L^{-1}) induced a significant increase in trapped energy (p < 0.05) while a significant decrease in transported energy was also observed (p < 0.05; table 1). The PCA analysis (figure 4B) revealed that Pb was positively correlated to ABS/RC, TRo/RC and DIo/RC and negatively correlated to Fv/Fm, Fv'/Fm', ETR and ETo/RC. Similar results were reported by Dridi *et al.* (2022) where *H. annuus* treated with elevated barium concentrations (100–500 µmol.L^{-1}) showed a non-significant variation in Fv/Fm and Fv'/Fm', but a significant increase in ABS/RC and DIo/RC and a significant decrease in ETo/RC was observed at 300 and 500 µmol.L^{-1} Ba. It is well known that the quantum efficiency of PSII is a very sensitive indicator that can describe the capacity of photosynthetic apparatus to perform photosynthesis under metal stress (Mlinarić et al., 2017). Thus, the slight decrease in Fv/Fm and Fv'/Fm' in *H. annuus* might indicate the initiation of PSII damage under the effect of Pb (Huang *et al.*, 2019). Several studies have reported the alteration of ETR in photosystem II and photosystem I under Pb or other metal toxicity (Subrahmanyam 2008; Ekmekçi et al., 2008). The decrease in ETR in *H. annuus* in Pb treated plants was in accordance with Kumar and Prasad (2015) who demonstrated that a concentration of 1.25 mmol.L^{-1} Pb induced a significant decrease in ETR in PSII reaction center of *Talinum triangulare* plants and that the downregulation of ETR in PSII might be due to the prevention of over-reduction of plastoquinone (QA), to reduce the load on electron transport chain. Furthermore, the alteration of electron transport efficiency during photosynthesis is explained by the fact that metal ions can induce the decrease of cytochromes b6f, plastocyanin, and ferredoxin levels in the chloroplast (Souri *et al.*, 2019). Qufei and Fashui (2009) reported that the accumulation of Pb in PSII can damage PSII structure, inhibit energy transfer among amino acids in the PSII protein–pigment complex, and decrease energy transport from tyrosine residue to chlorophyll; this effect was observed in *Spirodela polyrrhiza* Pb treated plants. Indeed, ETo/RC declined in *H. annuus* plants under Pb exposure, and this can be also explained by a decrease in the re-oxidation of QA– through electron transport in an active reaction center (RC) due to the availability of high number of active RCs (Singh *et al.*, 2022). The significant increment in ABS/RC and TRo/RC in *H. annuus* might indicate that some parts of the reaction centers in PSII are inactivated due to the inactivation of the oxygen-evolving

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complexes (OEC) or due to the transformation of active RCs to silent ones (Strasser et al., 2004; Zhang et al., 2016), which make the RCs of PSII become more vulnerable to damage under the influence of metal stress (Liu et al., 2021). According to these findings, the increase in ABS/RC and the decrease in ETo/RC reflect the disturbance of the balance between the absorbed photosynthetic light and the used energy. Therefore, the imbalance between light absorption and consumption will induce the over-excitation of PSII reaction centers (Singh *et al.*, 2022). Consequently, DIo/RC increased in *H. annuus* grown in presence of Pb. Indeed, plants dissipate the excess energy as heat to avoid the photo-oxidation damage of the photosynthetic apparatus by ROS generated of the excess excitation energy (that is not used in photosynthesis) (Zhang *et al.*, 2016). Additionally, *H. annuus* showed an evident decrease in P steps of the OJIP curves of 300 and 500 µmol.L^{-1} Pb when compared to the control (figure 5B). The results are in accordance with the discussion presented above. In addition, this decline could be due to the inhibition in ferredoxin-NADP^{+} reductase (FNR) activity affected by Pb stress (Małkowski *et al.*, 2020).

Lead accumulation: The alteration of several mechanisms responsible for the variation in PSII performance could be due to the accumulated proportions of Pb in different plant parts, specifically in the aerial plant parts. Under Pb treatment, *L. crithmoides* and *H. annuus* mainly accumulated Pb in their roots (figure 6).

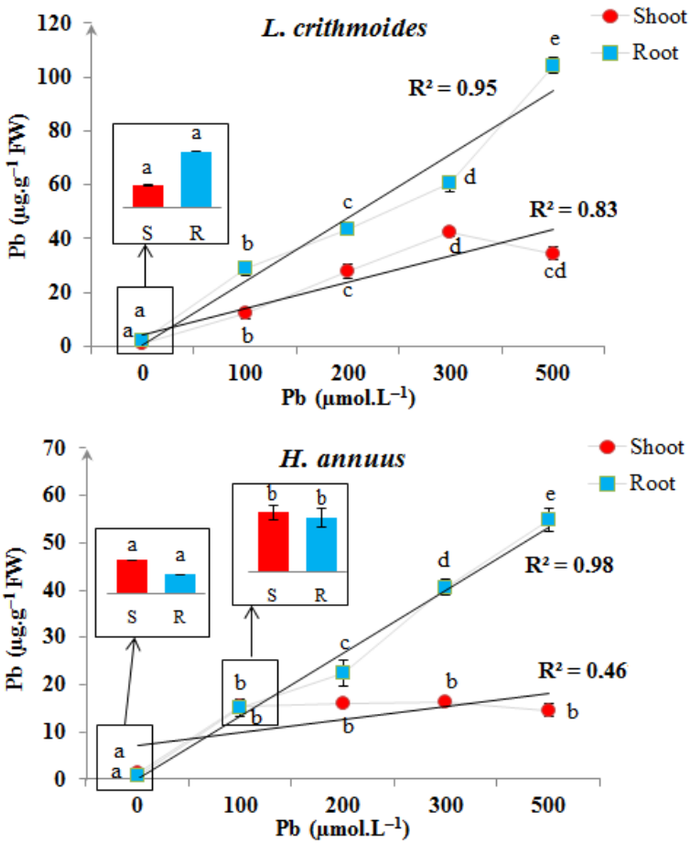

Figure 6: Lead (Pb) contents in the shoots (S) and roots (R) of *Limbarda crithmoides* and *Helianthus annuus* plants after 45 days of Pb treatment. Values are means ± standard deviation (n = 10). FW: fresh weight

The accumulation was higher by 2.4–, 1.5–, 1.4– and 3–fold in *L. crithmoides* and by 1–, 1.4–, 2.5– and 3.7–fold in *H. annuus* in roots than in shoots (at 100, 200, 300 and 500 µmol.L^{-1}Pb, respectively). In addition, the Pb contents in the roots of both species were correlated to the increase of Pb concentration in the growth medium. Results also showed that Pb contents were 2-fold higher in *L. crithmoides* shoots and roots than in those of *H. annuus*. Similar results were reported in *Koelreuteria paniculata* and *Zelkova schneideriana* plant species where the majority of Pb was accumulated in the roots than in the aerial parts after the Pb exposure (Huang *et al.*, 2019). As a non-essential element for plant growth and metabolism, Pb translocation from roots to the aboveground parts was generally controlled by the apoplastic barriers of the cortex in the roots (Ignatius et al., 2014; Huang *et al.*, 2019) in order to limit Pb to reach photosynthetic organs. This strategy might be adopted by plants to prevent damage of photosynthesis and thus the plant growth. According to our results, *L. crithmoides* has accumulated an elevated Pb proportion in the shoots compared to *H. annuus* without showing harmful effects on PSII activity and chlorophyll production. The harmless Pb effect on the photosynthetic apparatus may be explained by the ability of *L. crithmoides* to sequestrate Pb into the vacuoles as non-reactive ions (Singh et al., 2016).

CONCLUSION: To summarise, regarding the morphological features, both *L. crithmoides* and *H. annuus* plants were not negatively influenced by the addition of Pb. However, both species presented different physiological responses to Pb stress. In *L. crithmoides*, an obvious gradually improvement in the plant biomass production (especially shoots) and in chlorophyll *a* content was noticed when increasing Pb dose, along with a normal photosynthetic performance of PSII comparable to that of the control. Adversely, in *H. annuus,* the biomass production remained stable, compared to the control, despite a disturbance in the energy balance of the PSII and a modest non-significant decrease in chlorophyll *a* content and PSII quantum efficiency. Therefore, an exposure to high Pb concentrations (>500 µmol.L^{-1}) might be expected to damage photosynthetic functions in *H. annuus*. In addition, it is noteworthy that the two species accumulated most of the Pb in the roots which might be a strategy to protect photosynthetic organs and prevent growth damage. Nonetheless, *L. crithmoides* exhibited more resistance to the Pb treatment than *H. annuus.* The results obtained in the Chl *a* fluorescence evaluation showed that this technique was efficient to describe the photochemical status in *L. crithmoides* and *H. annuus* plants and can be used as a quick Pb-stress tool indicator.

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