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# The Photochemical and Antioxidant Defence Strategies of Two Maize Genotypes Exposed to Zinc Toxicity at the Seedling Stage

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ABSTRACT
The main objective of the current study was to elucidate photochemical and antioxidant strategies in two maize genotypes, namely DK626 and 3223 at the early seedling stage under zinc ($Zn^{2+}$) toxicity. The seedlings were grown in a controlled growth room at a temperature regime of 25±1 °C, with 40±5 % humidity, 16 h photoperiod and at 300 µmol $m^{-2}$ $s^{-1}$ light intensity for 8 days. Then, the seedlings were exposed to toxic zinc concentrations (2, 5 and 8 mM $ZnSO_4.7H_2O$) for 12 days. Both genotypes accumulated approximately the same amounts of Zn in leaves; however, the shoot and root lengths, and biomass decreased further in DK626 compared to 3223. The malondialdehyde content in the leaves increased gradually depending on the Zn concentrations, and the deterioration of the membrane structure was greater in DK626 compared to 3223 at highly toxic Zn levels. A reduction in photochemical activity was accompanied by non-photochemical quenching and excess energy was removed from the reaction centers by fluorescence and non-radiative inactivation in genotypes under Zn toxicity. The chlorophyll and carotenoid contents were significantly decreased, and the anthocyanin accumulation was increased with increasing Zn levels, especially in DK626. In addition, the activities of antioxidant enzymes and isoenzymes were induced at different levels in genotypes depending on the Zn toxicity level. The seedlings exposed to toxic Zn concentrations had achieved to sustain their growth by regulating their photosynthetic efficiency and their antioxidant defence system. Consequently, these genotypes could potentially be successfully used for the phytoremediation of Zn-contaminated areas. However, further studies are required to screen all growth stages for Zn tolerance capacity before making a more informed decision regarding the phytoremediation potentials of these two genotypes.

Keywords: Antioxidant defence system, Chlorophyll *a* fluorescence induction, Growth characteristics, Maize (*Zea mays* L.), Photochemical activity, Zinc toxicity

## 1. Introduction

Heavy metal accumulation in terrestrial and aquatic environments disrupts the normal functioning of ecosystems by causing strong toxicological effects on all living forms, including microorganisms, plants, animals and humans (Anwaar et al. 2015; Paunov et al. 2018; Alsafran et al. 2023; Sharma et al. 2023). Due to industrial and agricultural activities, such as mining, electroplating, leather tanning, traffic, use of sewage sludge or agrochemicals and fertilizers (stable manure) obtained from animals fed with feed containing heavy metals has become a global environmental crisis (Glińska et al. 2016; Chen et al. 2017). Elements such as cadmium (Cd), aluminum (Al), copper (Cu), arsenic (As), manganese (Mn), iron (Fe), mercury (Hg), nickel (Ni), zinc (Zn), cobalt (Co) and lead (Pb) are among these heavy metals. However, some of these metals (Cu, Fe, Mn and Zn) are classified as essential nutrients for the many structural and biochemical functions of plants (Karahan et al. 2020; Dobrikova et al. 2022; Alsafran et al. 2023).

Zinc ($Zn^{2+}$) is the second transition metal after Fe according to its abundance in plants. The uptake of Zn by plants from the soil depends on its concentration, soil clay fraction and soil pH (Andrejić et al. 2018; Antoniadis et al. 2018). Zn takes charge of many metabolic processes including the maintenance of the integrity of cell walls and membranes, regulation of the activity of many enzymes (isomerases, hydrolases, oxidoreductases, transferases, carbonic anhydrase, etc.), photosynthesis, carbohydrate, lipids and nucleic acid metabolism, regulation of the activities of hormones, pigment synthesis, gene expression and regulation, protein synthesis, and defence against stressors (Díaz-Pontones et al. 2021; Dobrikova et al. 2022). However, if the physiological range is exceeded, zinc concentrations rise above tolerable levels and a toxic effect occurs. Meanwhile, acidic soil pH increases the Zn solubility and uptake by plants (Chaney 1993; Kaur & Garg 2021; Natasha et al. 2022). High Zn concentrations in soil,

leading to the accumulation of approximately 400-500 mg $kg^{-1}$ DW Zn in the leaf tissues (Chaney 1993; Marschner 1995), can induce disruptions in the morphological, physiological and biochemical processes, limiting plant growth and development. Major indications of Zn toxicity include decrease in yield, reduction in leaf area, a lower biomass accumulation, formation of chlorosis and necrotic areas on leaves, root damages, reduced germination and stunted growth (Anwaar et al. 2015; Chen et al. 2017; Dobrikova et al. 2021). Zn toxicity is also associated with water imbalance, altered uptake and translocation of nutrients (such as P, Fe, and Mg deficiency) and change in membrane permeability (Ramakrishna & Rao 2015; Petrovic & Krivokapic 2020; Seregin et al. 2023). Moreover, Zn toxicity can restrict photosynthesis at a range of different structural-functional levels by reducing pigmentation, transpiration rate and stomatal and mesophyll conductivity, increasing respiration, reducing the activity of Calvin cycle enzymes and/or limiting photochemical reactions (Andrejić et al. 2018; Szopiński et al. 2019). Studies have shown that the photosynthetic responses of plants exposed to Zn toxicity vary depending on the plant species, Zn concentration or genetic variance (Paunov et al. 2018; Szopiński et al. 2019; Fatemi et al. 2020). In addition, the imbalance between absorption and consumption of light energy in the photosynthetic pathway by Zn toxicity triggers the overproduction of reactive oxygen species [ROS - hydroxyl radical ($OH^{\cdot}$), superoxide radical ($O_2^{\bullet-}$), and hydrogen peroxide ($H_2O_2$)]. Excessive ROS production disrupts redox homeostasis in cells and eventually causes oxidative stress in the plant (Chen et al. 2017). To cope with the destruction caused by ROS, plants have complex antioxidant protection systems consisting of enzymatic [superoxide dismutase (SOD), guaiacol peroxidase (POD), ascorbate–glutathione cycle enzyme (ascorbate peroxidase-APX and glutathione reductase-GR), etc.] and non-enzymatic (ascorbate, glutathione, anthocyanins, flavonoids, carotenoids, etc.) components (Miller et al. 2008; Yin et al. 2018).

Maize is one of the main cereal crops cultivated globally along with rice and wheat and is used as a raw material in the starch, syrup, industrial alcohol (ethanol for beer or whiskey) and bioplastic making industries as well as human and animal nutrition (Díaz-Pontones et al. 2021; Abedi et al. 2022). According to the 2021 data of FAOSTAT, maize is the most produced plant worldwide after sugarcane. Furthermore, due to its adaptability to many different environmental conditions, maize has a wide cultivation area from temperate to tropical regions (Saboor et al. 2021). As a consequence of its widespread use and enormous potential maize is sometimes referred to as the "miracle crop" and/or "queen of cereals" (Suganya et al. 2020). Unfortunately, maize kernels are naturally poor in zinc minerals, so maize is used as an indicator plant for the evaluation of Zn deficiency in soil (Ayyar & Appavoo 2017). Although most of the studies conducted with maize are related to the effects of Zn deficiency on plants and improving Zn deficiency, several studies have examined the effects of Zn toxicity on growth, photosynthesis, antioxidant system, yield and/or Zn content in different development stages as well as germination, stalk elongation and tassel formation, and reproductive stage (Alonso-Blázquez et al. 2015; Janeeshma et al. 2021). Despite relatively rich database on the effects of toxic Zn levels on plant metabolism, it is evident that there are still some unresolved issues. For example, the effect of toxic Zn levels on photochemical activity and antioxidant defence mechanisms at the cellular level is not sufficiently clear at the early seedling stage, when the organs necessary for maize to perform their vital functions begin to develop and mature. Considering the lack of information on this subject, the main objective of this study is to elucidate the photochemical and antioxidant defence strategies in the seedlings of two maize genotypes exposed to zinc toxicity. The relationship between Zn toxicity and tolerance of genotypes at the early seedling stage was comparatively investigated from the point of the membrane structure, photosynthetic pigment contents, functionality of photosystems and antioxidant defence mechanisms.

## 2. Material and Methods

### *2.1. Plant materials, growth and treatment conditions*

The seeds of the maize (*Zea mays* L. namely DK626 and 3223) genotypes were used in this study. In previous studies conducted by Ünalan (2006), it was determined that DK626 was tolerant and 3223 was sensitive to Zn toxicity at the germination stage. The seeds were germinated onto filter papers wetted with distilled water in germination boxes (20 x 13.5 x 8 cm) at 23±2 °C in dark conditions for 5 days. Subsequently, the seedlings of the genotypes were transferred to plastic pots (14 x 13 cm) filled with perlite and watered with Hewitt's nutrient solution (N, 168; P, 41; K, 156; Mg, 36; Ca, 160; S, 48; Fe, 2.8, Mn, 0.55; B, 0.54; Cu, 0.064; Zn, 0.065 and Mo, 0.048 mg $L^{-1}$) every other day. The plants were grown in a controlled growth room at a temperature regime of 25±1 °C, with 40±5% humidity, 16 h photoperiod and at 300 µmol $m^{-2}$ $s^{-1}$ light intensity. On the 8$^{th}$ day after transfer, zinc treatment was initiated for the next 12 days by applying Hewitt's nutrient solution containing 2, 5 and 8 mM $ZnSO_4.7H_2O$ to seedlings. At the end of the zinc treatment period, the seedlings were harvested for the morphological, physiological and biochemical analyses.

### *2.2. Growth parameters*

The shoot (the distance from perlite surface to the tip of the longest leaves) and root lengths (the longest seminal roots) of the maize seedlings were measured (cm $plant^{-1}$) for each zinc treatments. In addition, three plants were randomly taken from shoot and root for each treatment and their fresh weight (g $plant^{-1}$) was measured and then kept in an oven at 80 °C for 48 hours to determine the dry weight of these plants (g $plant^{-1}$).

*2.3. Zinc content*

The leaves of the harvested seedlings were washed in deionized water, then dried in oven at 80 °C for 48 hours and subsequently mill ground to powder. The tissues powder was burned in a muffle furnace at 550 °C for 4 h. The ash was brought to a standard volume with 1 M $HNO_3$. The Zn content in the tissues was determined using flame atomic absorption spectrophotometer (Unicam, 929 AAS) and the Zn contents of the leaves was calculated (mg $kg^{-1}$ DW). Additionaly, the bioaccumulation factor (BF) were calculated according to Roccotiello et al. (2010).

The bioaccumulation factor (BF): metal $content_{shoot}$/metal $content_{soil}$ (1)

*2.4. Chlorophyll a fluorescence measurements*

Chlorophyll *a* fluorescence measurements were performed with a portable, modulated fluorescence monitoring system (FMS2; Hansatech Ltd., Norfolk UK) on randomly selected leaves of the maize cultivars (6 replicates). Following at least 30 min of dark adaptation, the minimum chlorophyll a fluorescence ($F_O$) was determined using a measuring beam of 0.2 μmol $m^{-2}$ $s^{-1}$ intensity. A saturation pulse (1 s of white light of 7500 μmol $m^{-2}$ $s^{-1}$ intensity) was used to obtain the maximum fluorescence ($F_M$) after a dark-adapted state was reached. The maximal quantum efficiencies of PSII of dark-adapted plants ($F_V/F_M$ and its more sensitive $F_V/F_O$) were calculated using Equation 2. $F_V$ is known to be the variable fluorescence (Equation 3). Light-induced changes in chlorophyll a fluorescence following actinic illumination (300 μmol m–2 s–1) were recorded prior to the measurement of $F'_O$ (minimum chlorophyll a fluorescence in light-saturated state) and $F'_M$ (maximum fluorescence in light-saturated state. The quantum efficiency of PSII open centers in the light-adapted state, referred to as ΦPSII (Equation 4), was determined from $F'_M$ and $F_S$ (steady-state fluorescence in the light-saturated state) values. The quantum efficiency of the excitation energy trapping of PSII (Equation 5) was calculated according to Genty et al. (1989). After the actinic light was shut off, the minimum fluorescence in the light-adapted state ($F'_O$) was determined by illuminating the leaves with far-red light (7 μmol $m^{-2}$ $s^{-1}$). The photochemical quenching (Equation 6) and the nonphotochemical quenching (Equation 7) were calculated according to Genty et al. (1989). The electron transport rate (ETR) was determined by multiplying the quantum efficiency by incident photon flux density and an average factor of 0.84 for leaf absorbance and dividing by a factor of 2 to account for the sharing of absorbed photons between the two photosystems (PSI and PSII, Equation 8) (Genty et al. 1989). Additionally, the quantum yields of chlorophyll photophysical decay of light-adapted leaves referred to as $\Phi_C$ (Equation 9) were calculated (Guadagno et al. 2010; Calvo et al. 2017). Based on these, the energy partitioning in the PSII complex occurs in three parts; the photochemical processes, nonphotochemical quenching (heat dissipation) and chlorophyll photophysical decay. These three mechanisms are competitive and their sum is equal to 1 (Equation 10).

$$F_V/F_M = (F_M - F_O) / F_M \quad (2)$$

$$F_V = F_M - F_O \quad (3)$$

$$\Phi PSII = (F'_M - F_S) / F'_M \quad (4)$$

$$F_V'/F_M' = (F_M' - F_O') / F_M' \quad (5)$$

$$qP = (F'_M - F_S) / (F'_M - F'_O) \quad (6)$$

$$NPQ = F_M - F'_M / F'_M \quad (7)$$

$$ETR = (F'_M - F_S) / (F'_M) \times PAR \times 0.84 \times 0.5 \quad (8)$$

$$\Phi_C = F_S/F_M \quad (9)$$

$$\Phi PSII + NPQ + \Phi_C = 1 \quad (10)$$

*2.5. Pigment analysis*

The photosynthetic pigment [chlorophyll (Chl *a*, *b*) and carotenoids (Car *x*, *c*)] contents were determined from the leaf sample and calculated as mg g $FW^{-1}$ according to Lichtenthaler (1987). The absorbance values of the solutions were recorded at the 470, 644.8 and 661.6 nm and the pigment contents were calculated using the following equation:

Chl *a* = (11.24 x A661.6) – (2.04 x A644.8) (11)

Chl *b* = (20.13 x A644.8) – (4.19 x A661.6) (12)

Total Chl = (7.05 x A661.6) – (18.09 x A644.8) (13)

$$Car = [(1000 \times A470) - (1.9 \times Chl\ a) - (63.14 \times Chl\ b)] / 214 \quad (14)$$

In addition, the Chl *a*/*b* ratio was calculated from the Chl data. The anthocyanin content (mg g $FW^{-1}$) was determined according to the method of Mancinelli et al. (1975). The absorbances were recorded at the 530 and 657 nm and the content was calculated using the following equation:

$$\text{Anthocyanin content} = A530 - (A657/3) \quad (15)$$

*2.6. Antioxidant enzyme and isoenzyme activities*

Fresh leaf samples (0.5 g and 3 replicates) were ground with liquid nitrogen and the soluble protein was extracted in the antioxidant enzyme related extraction buffer. Protein concentrations from leaf extracts were determined according to (Bradford 1976). Fine powder was homogenized in 1 mL of buffer containing 9 mM Tris–HCl buffer (pH 6.8) and 13.6% glycerol and SOD (EC 1.15.1.1) enzyme and isoenzyme activities were determined (Laemmli 1970; Beauchamp & Fridovich 1971; Beyer & Fridovich 1987). Guaiacol POD (EC 1.11.1.7) enzyme activity was based on the determination of guaiacol oxidation ($\varepsilon$ = 26.6 mM $cm^{-1}$) at 470 nm by $H_2O_2$ (Pütter 1974). In addition, POD isoenzyme was assayed according to Rao et al. (1995). APX (EC1.11.1.11) enzyme and isoenzyme activities were assayed according to the method of Wang et al. (1991) and the staining of non-denatured polyacrylamide gels was performed according to Mittler & Zilinskas (1993). GR (EC 1.6.4.2) enzyme activity was determined by following the decrease in absorbance at 340 nm due to NADPH oxidation (Sgherri et al. 1994) and GR isoenzyme was determined to make the bands in non-denatured polyacrylamide gels apparent according to the method of Rao et al. (1995). SOD, POD, APX and GR isozyme bands were visualized and analyzed using the Bio-Profil V99 software program of the Vilber Lourmart imaging system (Marne la Vallee, France).

*2.7. Statistical analysis*

The experiments were performed in a completely randomized design by three replicates. Differences among the treatments as well as between the genotypes were analyzed using the SPSS 20.0 (Chicago, IL, USA). Statistical variance analysis of the data was performed using ANOVA and compared with the least significant differences (LSD) at the 5% level.

## 3. Result and Discussion

Zinc (Zn) plays a crucial role in various biological functions for all living organisms; however, plants employ different strategies to cope with the uptake, transport, and retention of high Zn concentrations, which can lead to toxicity (Anwaar et al. 2015; Petrovic & Krivokapic 2020; Kaur & Garg 2021; Stanton et al. 2022). In this study, it was investigated that the effects of Zn toxicity on the morphological structure, photochemical efficiency and antioxidant defence systems of maize genotypes. The bioaccumulation factor (BF) is accepted to be one of the important indexes for phytoremediation that imply the potential of plants to regulate the uptake, transport and accumulation of metals in their aerial parts (Rai et al. 2019; Wieczorek et al. 2023). Elevated toxic Zn concentration caused the decrease in BF of both maize genotypes (Figure 1). Although the amount of Zn required for optimum growth is universally assumed to be in the range of 30 to 200 µg $g^{-1}$ DW within plants, some species can accumulate zinc of more than 10.000 µg $g^{-1}$ DW without showing any indication of toxicity (Sofo et al. 2013; Sperdouli et al. 2022). By contrast, Zn is accepted to be toxic for plants when the tissue concentration is above 400 µg $g^{-1}$ DW (Sofo et al. 2013). The amount of Zn in the control leaves of DK626 and 3223 genotypes were measured as 342 and 347 µg $g^{-1}$ DW, respectively, and these values were below the threshold considered to be toxic (Figure 1). In all Zn treatments, both genotypes accumulated Zn significantly and similarly in their tissues, and this accumulation generally increased in parallel with the increase in application levels (41, 123 and 119-fold in DK626, and 47, 119 and 128-fold in 3223 at the 2, 5 and 8 mM compared to the control, respectively). This could be indicative that the leaves were approaching the saturation point in Zn uptake and accumulation under high toxicity conditions. When the results above are evaluated together, maize genotypes have exhibited almost similar tolerance to Zn toxicity, and these genotypes could be used as accumulator crops for contaminated soil with toxic zinc metal due to both genotypes having a bioaccumulation factor (BF) > 1. Wieczorek et al. (2023) have reported that if the BF values of plants are greater than 1 under zinc toxicity, it can be considered as a bioaccumulator plant.

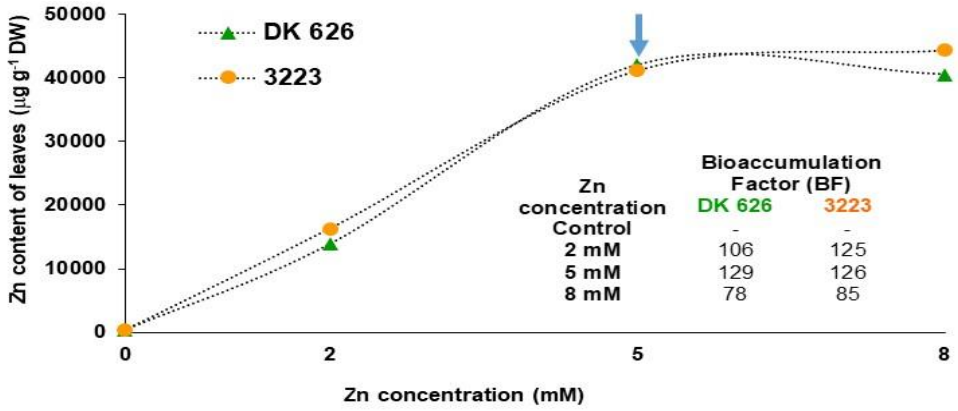


**Figure 1 - Zinc content (g kg$^{-1}$ DW) in leaf tissue of maize genotypes**

Zn accumulation in leaves caused growth restrictions by altering the ion balance and disrupting metabolic processes (Tiecher et al. 2017; M'Rah et al. 2023). The root and shoot lengths of maize genotypes were significantly declined in especially 5 and 8 mM Zn treatments (more than 8%) (Figure 2). In addition, a gradual reduction in biomass accompanied these alterations, and the fresh and dry weights of shoot and root decreased under all Zn treatments in DK626 (24-60% and 16-70% compared to control, respectively), whereas the decreases in biomass were more pronounced under the 5 and 8 mM Zn concentration in 3223 (21-54% and 35-67% compared to control, respectively) (Figures 2 and 3). Declines in biomass production and lengths are general responses of Zn toxicity and, therefore, these parameters are frequently a reliable indicator of the plant's susceptibility to stress (Glińska et al. 2016). Moreover, the root/shoot dry weight ratio and total biomass values indicated that root and shoot growth were similarly affected in DK626 under Zn toxicity, with the exception of the 2 mM Zn concentration, while the root growth was more inhibited in 3223 only at the highest toxicity (8 mM) (Figure 3). Many researchers have stated that roots are in direct contact with zinc toxicity, so the toxicity is first perceived by the roots, which triggers a reduction in cell division and a change in hormone balance and a retardion of minerals and water acquisition, thus limiting root growth (Glińska et al. 2016; Kaur & Garg 2021). In addition to these, all growth parameters values decreased more dramatically in DK626 compared to 3223, revealing that DK626 was slightly sensitive to zinc toxicity.

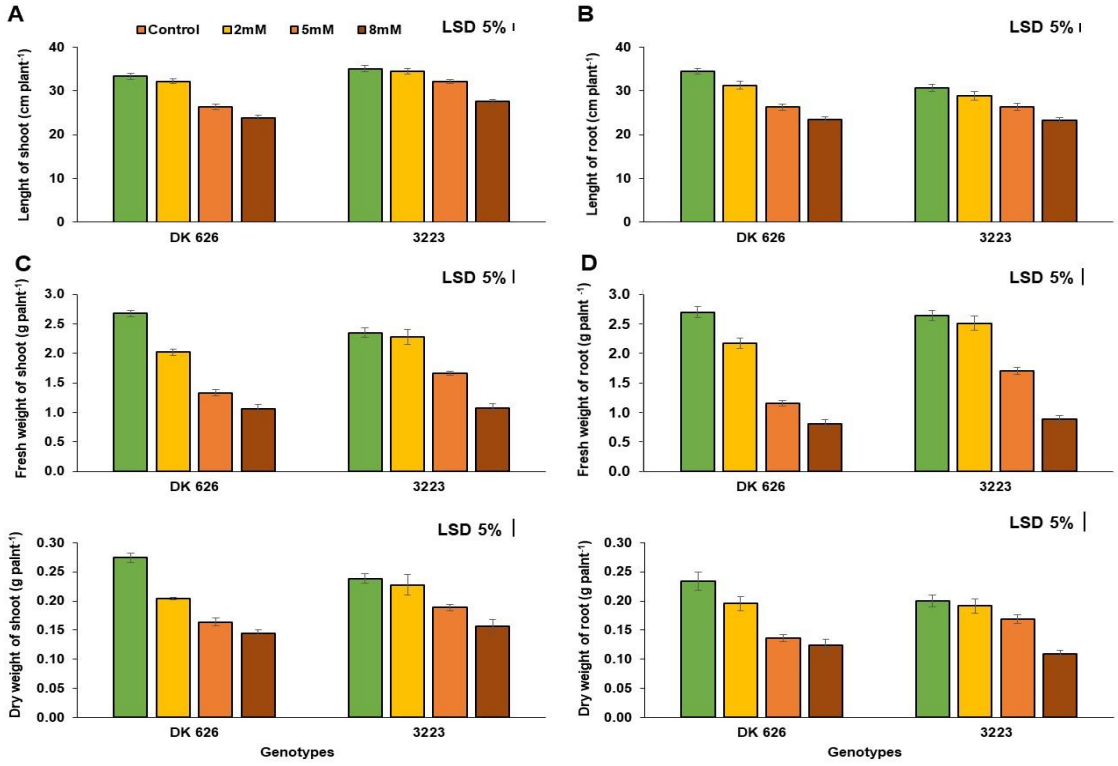


**Figure 2- Length of shoot (A) and root (B), fresh weight of shoot (C) and root (D), and dry weight of shoot (E) and root (F) of maize genotypes exposed to Zn toxicity. The values are presented as the mean ± standard error (SE), n=15 for length of shoot and root and n=5 for fresh and dry weight of shoot and root. The bars and different letters indicate significant differences between treatments and cultivars at P<0.05 according to the LSD test**

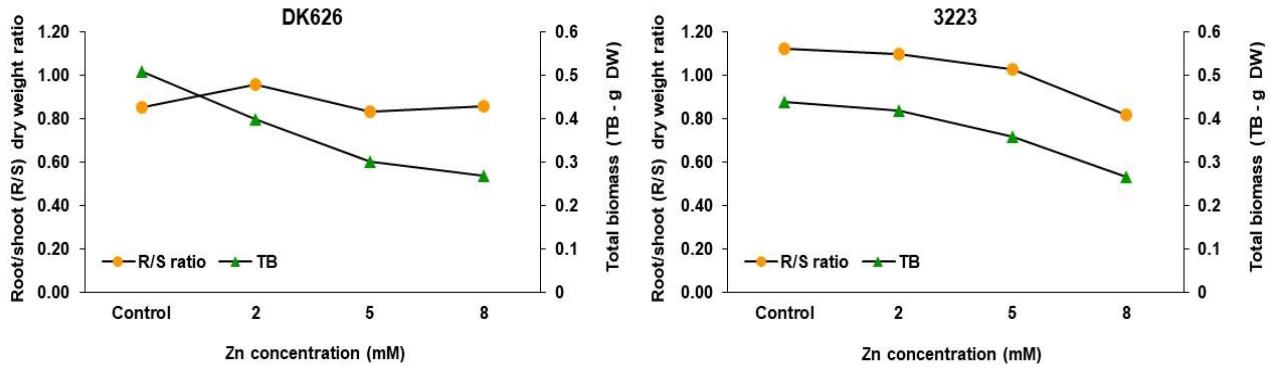


**Figure 3 - The effects of Zn toxicity on root/shoot dry weight ratio and total biomass accumulation in maize genotypes. Values shown are mean ± SE**

Oxidative stress, which occurs due to uncontrolled and excessive production of reactive oxygen species (ROS), is among the main factors that impair metabolic function by affecting cellular structures in plants exposed to heavy metal stress conditions. The non-quenched ROS trigger peroxidation in cellular membranes, resulting in an increase in malondialdehyde (MDA), an indicator of lipid peroxidation (Ramakrishna & Rao 2015; Dobrikova et al. 2021; Kaur & Garg 2021). MDA content increased 3.0 and 6.3- fold in DK626 at 5 and 8 mM Zn concentrations, while it elevated gradually 1.4 to 3.1-fold in 3223 at all Zn treatments (Table 1). It has been reported that under toxic Zn conditions, ultrastructural alterations of cellular organelles such as chloroplasts and mitochondria occur due to the disruption of the membrane structures of these organelles (Mukhopadhyay et al. 2013). In addition, Jayasri & Suthindhiran (2017) have suggested that heavy metals such as Zn and Pb could demolish the structure and function of chloroplast by attaching to sulfhydryl (-SH) group of the chloroplast proteins and could limit the chlorophyll biosynthesis by targeting Fe and Mg. The increase in MDA content, which indicates degradation in the membrane structures, could have limited plant biomass production in maize genotypes by destroying the structure of chlorophyll pigments and photosynthetic apparatus (Figures 4 and 5).

**Table 1 - Effects of elevated toxic zinc concentration on malondialdehyde (MDA) content in the leaves of two maize cultivars. Each value represents the mean ± SE (n = 3)**

| *Cultivars* | *Zn concentration* | | | |
|---|---|---|---|---|
| | *Control* | *2 mM* | *5 mM* | *8 mM* |
| **DK 626** | 3.84 ± 0.03[a] | 3.66 ± 0.06 [a] | 11.38 ± 0.03 [b] | 23.12 ± 0.20 [c] |
| **3223** | 2.96 ± 0.10 [a] | 4.15 ± 0.09 [b] | 6.86 ± 0.01 [c] | 9.15 ± 0.09 [d] |
| LSD 5% | 0.39 | | | |

The novelty of this study is to address the lack of research data concerning the effects of zinc toxicity on photosynthetic functionality strategy of maize genotypes in the early seedling stage. For this purpose, the effect of Zn toxicity on energy fluxes of utilization and dissipation of light energy absorbed by PSII was evaluated by using the chlorophyll a fluorescence technique (Figure 4). The maximal quantum efficiency of PSII ($F_V/F_M$) slightly decreased in both maize genotypes at the 5 and 8 mM Zn treatments (Figure 4C). The alterations in $F_V/F_M$ ratio reflect disruptions in the photochemical activity of PSII (Küpper & Andresen 2016). The $F_V/F_O$ value, which provides information regarding the maximum quantum yield of PSII photochemistry (Lichtenthaler et al. 2005), was significantly decreased due to zinc toxicity compared to $F_V/F_M$ (Figure 4D). Lichtenthaler et al. (2005) have also reported that $F_V/F_O$ is more sensitive parameter than $F_V/F_M$. The decreases in $F_V/F_M$ and $F_V/F_O$ of genotypes were due to the increases in $F_O$ and decrease in $F_M$, particularly under 5 and 8 mM Zn treatments (Figures 4A and B). The decline in $F_M$ could indicate that the reduced $Q_A$ ratio increases and accordingly the electron transfer from $Q_A$ to the $Q_B$ slows down (Andrejić et al. 2018) and could also imply PSII photoinhibition (Sapeta et al. 2023). In addition, Paunov et al. (2018) suggested that the decrease in $F_M$ may be partially related to a decrease in the content of chlorophyll a. A similar relationship between Chl *a*+*b* content and Chl *a*/*b* ratio was found in this study (Figures 5A and C). The increase of $F_O$ could be a result of the decline in the transfer of excitation energy from the antenna complex to the reaction center of the PSII, but also to the direct effect of Zn on the reaction centers of PSII (Vaillant et al. 2005). These alterations in $F_O$ and $F_M$ of genotypes have indicated that there was damage to the acceptor and donor sides of PSII at high Zn concentrations. In a similar manner to $F_V/F_M$ and $F_V/F_O$ ratios, the value of $F'_V/F'_M$ diminished marginally in both maize genotypes at 5 and 8 mM Zn concentrations (Figure 4E). The decline in $F'_V/F'_M$ indicated that Zn toxicity also caused a deterioration in the efficiency of trapping the excitation energy by open PSII reaction centers. The observed decreases in $F_V/F_M$ and $F'_V/F'_M$ could be due to Zn-induced deterioration in the structure of PSII reaction centers or photoinhibition of PSII, which could trigger a decrease in the electron transfer rate from $Q_A$ to $Q_B$ and a subsequent reduce in electron flow from PSII to PSI (Paunov et al. 2018). Moreover, the ETR of both genotypes were also adversely affected by elevated Zn toxicity (especially at higher Zn levels) (Figure 4J). Other studies have reported that

photosynthetic electron transport was decreased in wheat, Arabidopsis and young bean by zinc toxicity (Paunov et al. 2018; Szopiński et al. 2019). It has been suggested that the detrimental effect of Zn on photosynthetic electron transport is due to the loss of membrane integrity, decreased permeability of biomembranes, and the disassembly of the thylakoid membrane due to the production of reactive oxygen species under stress (Balafrej et al. 2020). The ΦPSII value which expresses the efficiency of the energy used to drive photosynthesis slightly declined only in DK626 at 5 and 8 mM Zn concentrations (Figure 4F). Despite the changes in $F'_V/F'_M$ and ETR, photochemical quenching (qP) was not significantly affected in maize genotypes at any Zn concentrations (Figure 4G). The qP expresses the trapping photon energy that derives photosynthesis and also results from the activation of light-induced enzymes involved in carbon metabolism and the opening of stomata (Calvo et al. 2017). The stability of qP under Zn toxicity indicated that maize genotypes could protect a balance between the captured photon energy and the use of electrons passing through the photosynthetic electron transport chain (Çiçek & Çakirlar 2008). The excitation energy absorbed by plants is accomplished in the dissipation in three separate ways: it can be used in the photochemical pathway, it can be distributed in photophysical processes (fluorescence, inductive rezonans and radiationless transfer) or it can be dispersed by thermal deactivation (non-photochemical quenching) (Iriel et al. 2019). However, non-photochemical quenching (NPQ) markedly reduced at all Zn treatments in DK626 (16, 26 and 25% decrease at 2, 5 and 8 mM Zn concentrations, respectively) and only at 2 mM Zn concentration in 3223 (26%) (Figure 4H). The findings reveal that the excess excitation energy not used in the photochemical pathway was dissipated by using photophysical processes instead of the photoprotective pathway NPQ in maize genotypes, especially in DK626, under Zn toxicity. The $\Phi_C$, expresses the quantum yield of chlorophyll photophysical decay, displays the efficiency of photophysical processes, and is associated with radiative degradation of chlorophyll (Guadagno et al. 2010). The $\Phi_C$ increased significantly at 5 and 8 mM Zn treatments in DK626 (17 and 14%, respectively), and only at the highest Zn concentration in 3223 (8%) (Figure 4I). These indicated that Zn toxicity induced a greater fraction of the excession energy to be lost from the reaction centers as fluorescence and non-radiative inactivation of reaction centres (Cordon et al. 2018). Moreover, these three mechanisms are competitive in sharing the absorbed energy, and, therefore, any alteration in the quantum efficiency of one can typically induce changes in the efficiency of the others (Maxwell & Johnson 2000; Iriel et al. 2019). Consequently, all chlorophyll a fluorescence results have revealed that the effects of toxic Zn levels on photochemical activity were minor. Also, along with somewhat occurrence of photoinhibition in the photosynthetic apparatus due to zinc toxicity, the results obtained may suggest that an adaptation mechanism has been working and a balance has been established.

Regarding pigments, exposure to Zn toxicity dramatically decreased chlorophyll and carotenoid concentrations in maize genotypes (Figures 5A and B). The Chl *a+b* content decreased by 35-71% and 23-56% with increasing Zn concentration in DK626 and 3223, respectively. The reduction of the chlorophyll content may be due to many possible and interrelated causes: obstruction of enzyme activities involved in chlorophyll biosynthesis such as δ-aminolevulinic acid dehydratase (ALA-D) and protochlorophyllide reductase as a result of interference to the functional sulfhydryl region of Zn and/or activation of the chlorophyll-degrading enzyme chlorophyllase as well as competition of Zn with Fe and Mg in the structure of the intermediate or final product during chlorophyll synthesis (Anwaar et al. 2015; Ramakrishna & Rao 2015; Kaur & Garg 2021) as well as lipid peroxidation, especially photosynthetic membrane damage.

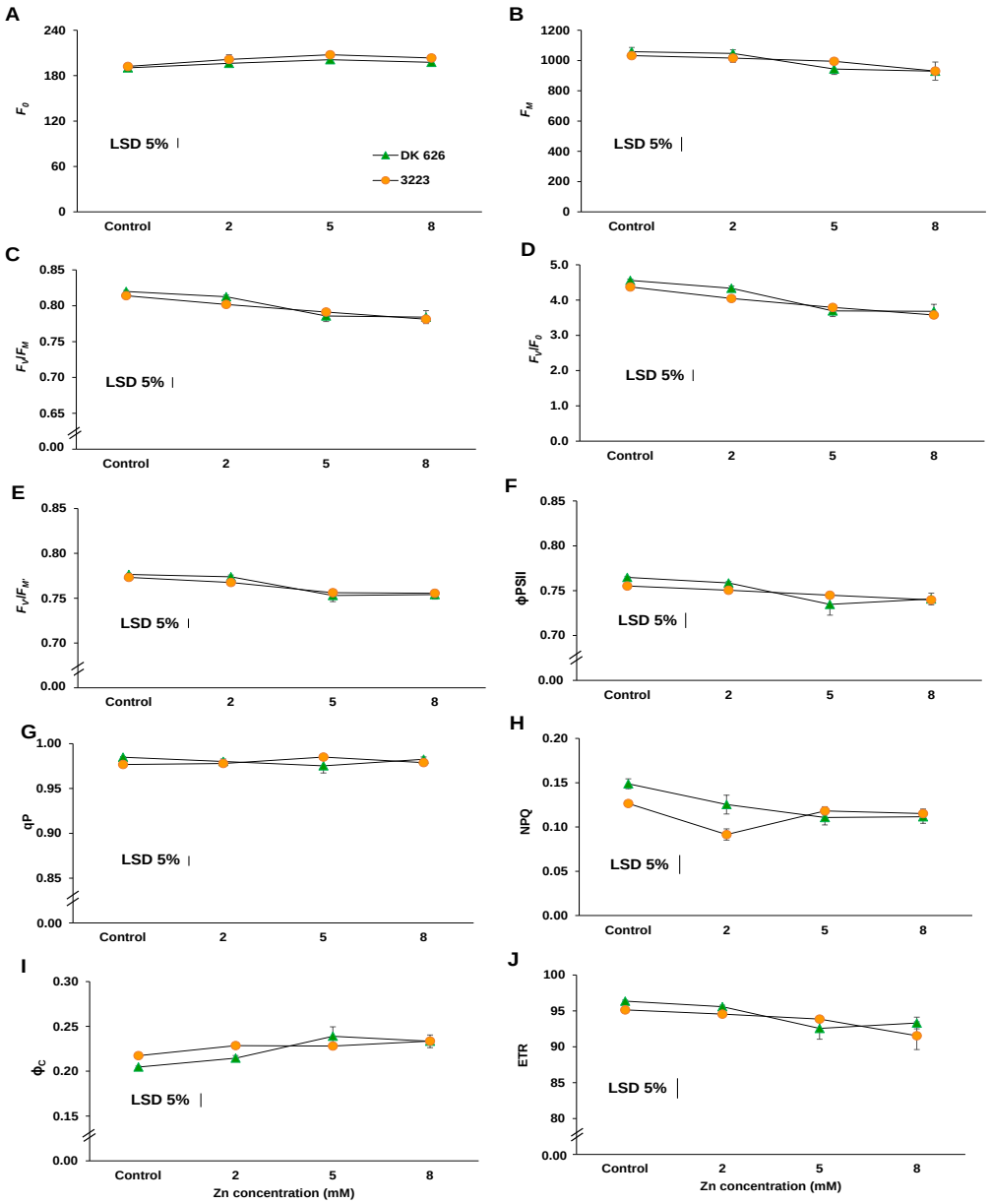


**Figure 4 - $F_O$, minimum fluorescence (A); $F_M$, maximum fluorescence (B); $F_V/F_M$ and $F_V/F_0$, maximum quantum yields of PSII (C and D); $F_V'/F_M'$, quantum efficiency of excitation energy trapping of PSII of light-adapted leaves (E); ΦPSII, efficiency of open reaction centre of light adapted state (F); qP, photochemical quenching (G); NPQ, nonphotochemical quenching (H); $\Phi_C$, quantum yields of chlorophyll photophysical decay of light-adapted leaves (I) and ETR, electron transport rate (J) of maize genotypes exposed Zn toxicity. The error bars represent the standard error (±SE) for six replicates**

Zn toxicity caused a decrease in the Chl *a*/*b* ratio as well as reducing pigment content, and the reduction was remarkable in DK626 at 5 and 8 mM Zn treatments (17 and 25%, respectively) (Figure 5C). The decline indicated that Chl *a* had a higher sensitivity to Zn exposure than Chl *b*. Moreover, the Chl *a*/*b* ratio in an indicator that reveals the status of light harvesting complexes (LHC) and reaction center of PSs, because LHC contains Chl *a* and Chl *b*, whereas the reaction centers only contain Chl *a* (Çiçek & Çakirlar 2008). In addition, the Car content decreased by 37-71% in DK626 and 23-55% in 3223 under Zn toxicity. The ratio of Chl (*a*+*b*) / Car (*x*+*c*) was also decreased by zinc toxicity, indicating the Chl content declined more than the Car content (Figure 5D). Several studies show that excess Zn could induce the decrease in carotenoid content (Ramakrishna & Rao 2015; Paunov et al. 2018). In addition to chlorophylls and carotenoids, leaf colors in plants are associated with a change in the relative content of other pigments including anthocyanin (Pan et al. 2020). The anthocyanin content increased by 73 and 82% in DK626 at the 5 and 8 mM Zn concentrations, respectively, and 41% at 8 mM Zn treatments in 3223 compared to controls (Figure 5E). It has been reported that Zn-induced anthocyanin synthesis and accumulation may play a role in protecting cell macromolecules from oxidative stress and/or increasing metal chelation in plants (Sofo et al. 2018; Szopiński et al. 2019; Kaur & Garg 2021). Moustaka et al. (2018) have claimed that the biosynthesis of anthocyanin was strongly related to the redox state of plastoquinone pool the in red leaves of poinsettia under high light intensity conditions and this accumulation could serve a protective role, limiting the production of ROS.

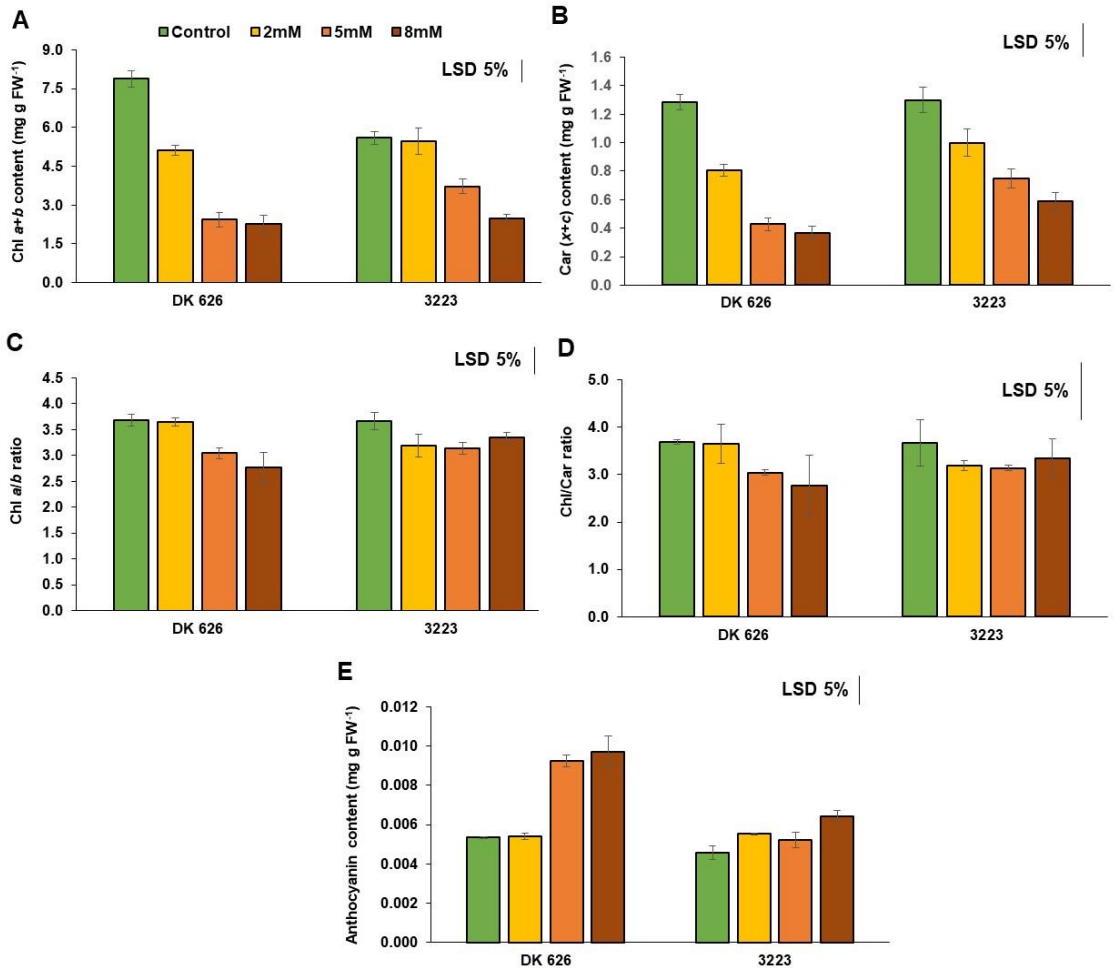


**Figure 5 - Chlorophyll (Chl) *a* +*b* (A) and carotenoid (B) contents, Chl *a*/*b* ratio (C), Chl / Car ratio (D) and anthocyanin content (E) in leaves of maize genotypes at elevated toxic Zn concentrations. The values are presented as the mean ± standard error (SE), n=5 for chlorophyll and carotenoid and n=3 for anthocyanin. The bars and different letters indicate significant differences between treatments and cultivars at P<0.05 according to the LSD test**

Zinc is a redox-inert element that cannot carry out monovalent oxidation-reduction reactions, but it can indirectly induce the generation of ROS such as $O_2^{\bullet-}$ and $H_2O_2$ (Díaz-Pontones et al. 2021). However, Zn also plays a role in scavenging ROS due to its role as a component of antioxidant enzymes (Dalcorso et al. 2014). In this way, excess zinc causes an imbalance between ROS and detoxification mechanism, and in this case, oxidative stress is triggered, which causes cellular damage (Ramakrishna & Rao 2015; Sofo et al. 2018). Oxidative stress is generally determined by the change in MDA content, a marker of lipid peroxidation (Sperdouli et al. 2021). In our study, the marked increase in Zn content and MDA contents, and the significant decrease in plant biomass and lengths at the highest Zn concentrations are an indication of triggering oxidative stress. The activities of four antioxidant enzymes and their isoenzymes in the cellular defence system of maize leaves under Zn toxicity were examined in this study: SOD, which catalyzes the conversion of $O_2^{\bullet-}$ radicals to $H_2O_2$ and $O_2$; subsequently; POD, APX and GR in detoxifying the formed $H_2O_2$ (Figure 6). Total SOD enzyme activity was increased in all Zn treatments compared to controls; however, it was statistically significant only at the highest Zn concentration in DK626 (2.2-fold) and at 5 mM Zn treatment in 3223 (1.8-fold) (Figure 6A). Also, three SOD isoforms were detected in leaves of both genotypes and the alteration in band intensity of the isoforms supported changes in total SOD isoenzyme activity (Figure 6A, right side). Total POD enzyme activity was increased only at 2 mM Zn concentration in DK626 and at 5 mM Zn treatment in 3223 (about 1.4 and 1.3-fold, respectively) compared to their controls (Figure 6B). As a comparison, four POD isoforms in maize leaves, with one different band in each genotype, were separated with non-denaturing PAGE. The band densities of POD isoforms enhanced at the same levels of Zn treatment that the total enzyme activities were increased. APX activity remained unchanged in the DK626 genotype (except 8mM), whereas it significantly increased in the 3223, especially under the highest zinc toxicity (Figure 6C). In addition, the alteration of GR activity in DK626 exposed to Zn toxicity was found to be similar to that in APX activity, and GR activities were found somewhat increased in DK626 under all Zn treatments (Figure 6D). For this reason, we suggest that POD may have played a more active role in $H_2O_2$ scavenging in DK626 under excess zinc treatments. In 3223, total APX activity was significantly upregulated at 5 and 8 mM Zn treatments (about 1.41 and 1.75-fold, respectively) whereas increased GR activity was detected at 2 and 5 mM Zn concentrations (approx. 1.7 and 2-fold, respectively). Moreover, alteration in the number of bands and/or band intensity of the isoforms in the gel images of APX and GR enzymes in 3223 supported the changes in the total enzyme activity (Figures 6C and D). These findings indicate that the 3223 genotype used the ascorbate-glutathione cycle

to detoxify $H_2O_2$ and in this pathway catalyzes ascorbate (AsA) to form monodehydroascorbate (increased APX activity) while increasing recuperation of GSH from GSSG (increased GR activity). Various studies have exhibited that the increases in POD and Asada-Halliwell pathway enzymes (APX, GR etc.) activities in plants were detoxified damaging effects of metal toxicity (Ekmekçi et al. 2008; Mukhopadhyay et al. 2013; Tiecher et al. 2017; Baran & Ekmekçi 2022).

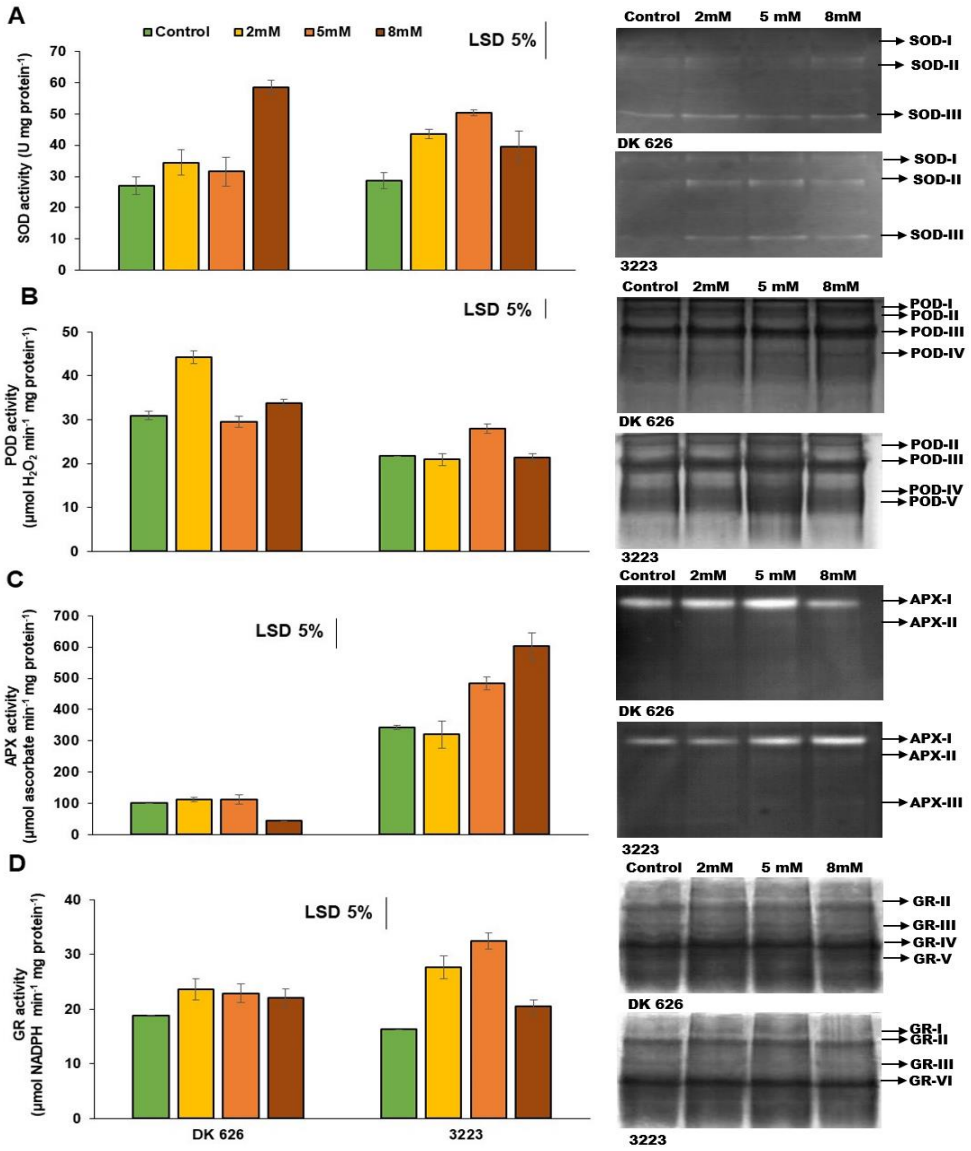


**Figure 6 - Alteration induced by Zn toxicity in the activities of total enzymes and isoenzymes in leaves of maize genotypes: SOD (A), POD (B), APX (C) and GR (D). The error bars represent the standard error (±SE) for three replicates**

## 4. Conclusions

The accumulation of excess Zn in leaves led to the restricted growth, loss of photosynthetic pigments and photochemical efficiency of the two maize genotypes. However, DK626 showed more membrane deterioration than 3223 with highly toxic Zn concentrations according to the MDA results. Furthermore, the decrease in photosynthetic pigment contents and the increase in anthocyanin contents reveal that DK 626 was more affected by Zn toxicity than the other genotype. However, by slightly increasing SOD and POD enzyme activities, DK626 overcome the damaging effects of oxidative stress induced by Zn toxicity compared to 3223. The genotypes achieved an adaptation to zinc toxicity by increasing the anthocyanin content, regulating antioxidant enzyme activities, and maintaining the functionality of the photosynthetic machineries.

In the previous study, the genotypes were identified as tolerant (DK626) and sensitive (3223) at the germination stage. However, the results of this study have shown that both genotypes the differentiation in tolerance responses to zinc toxicity between these genotypes disappeared during the early seedling stage. Therefore, tolerance levels to Zn toxicity of both genotypes were quite high and of a similar nature. It may be declared that both genotypes have exhibited tolerant responses to Zn toxicity, but with different strategies. Consequently, both genotypes of *Zea mays* L. might be suggested to be used for the phytoremediation of highly toxic Zn-contaminated areas. However, further studies are required to screen all growth stages for

Zn tolerance capacity before a more informed decision can be made regarding the phytoremediation potentials of these two genotypes.

**Data availability:** Data are available on request due to privacy or other restrictions.
**Author Contributions:** YE and DT took part in conceptualization, review and editing; YE and ŞÜO were involved in experimental data collection and analysis; ŞÇE and NÇ were involved in writing and the original draft.
**Conflict of Interest:** The authors declare that they have no conflicts of interest.
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## References

Abedi T, Gavanji S & Mojiri A (2022). Lead and zinc uptake and toxicity in maize and their management. Plants 11: 1922. https://doi.org/10.3390/plants11151922

Alonso-Blázquez N, García-Gómez C & Fernández MD (2015). Influence of Zn-contaminated soils in the antioxidative defence system of wheat (*Triticum aestivum*) and maize (*Zea mays*) at different exposure times: potential use as biomarkers. Ecotoxicology 24: 279-291. https://doi.org/10.1007/s10646-014-1376-6

Alsafran M, Saleem MH, Al Jabri H, Rizwan M & Usman K (2023). Principles and applicability of integrated remediation strategies for heavy metal removal/recovery from contaminated environments. *Journal of Plant Growth Regulation* 42: 3419-3440. https://doi.org/10.1007/s00344-022-10803-1

Andrejić G, Gajić G, Prica M, Dželetović Ž & Rakić T. (2018). Zinc accumulation, photosynthetic gas exchange, and chlorophyll *a* fluorescence in Zn-stressed *Miscanthus*×*giganteus* plants. Photosynthetica 56(4): 1249-1258. https://doi.org/10.1007/s11099-018-0827-3

Antoniadis V, Shaheen S M, Tsadilas C D, Selim M H & Rinklebe J (2018). Zinc sorption by different soils as affected by selective removal of carbonates and hydrous oxides. Applied Geochemistry 88: 49-58. https://doi.org/10.1016/j.apgeochem.2017.04.007

Anwaar S A, Ali S, Ali S, Ishaque W, Farid M, Farooq M A, Najeeb U, Abbas F & Sharif M (2015). Silicon (Si) alleviates cotton (*Gossypium hirsutum* L.) from zinc (Zn) toxicity stress by limiting Zn uptake and oxidative damage. Environmental Science and Pollution Research 22(5): 3441-3450. https://doi.org/10.1007/s11356-014-3938-9

Ayyar S & Appavoo S (2017). Effect of graded levels of Zn in combination with or without microbial inoculation on Zn transformation in soil, yield and nutrient uptake by maize for black soil. Environment & Ecology 35(1): 172-176

Balafrej H, Bogusz D, Triqui Z-E A, Guedira A, Bendaou N, Smouni A & Fahr M (2020). Zinc hyperaccumulation in plants: A review. Plants 9(562): 2-22. https://doi.org/10.3390/plants9050562

Baran U & Ekmekçi Y (2022). Physiological, photochemical, and antioxidant responses of wild and cultivated *Carthamus* species exposed to nickel toxicity and evaluation of their usage potential in phytoremediation. Environmental Science and Pollution Research 29: 4446-4460. https://doi.org/10.1007/s11356-021-15493-y

Beauchamp C & Fridovich I (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44: 276-287. https://doi.org/10.1016/0003-2697(71)90370-8

Beyer W F & Fridovich I (1987). Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. Analytical Biochemistry 161(2): 559-566. https://doi.org/10.1016/0003-2697(87)90489-1

Bradford M M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72(1-2): 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Calvo B O, Parapugna T L & Lagorio M G (2017). Variability in chlorophyll fluorescence spectra of eggplant fruit grown under different light environments: A case study. Photochemical & Photobiological Sciences16: 711-720. https://doi.org/10.1039/c6pp00475j

Chaney R L (1993). Zinc Phytotoxicity. In: Robson A D (ed) Zinc in Soils and Plants. Developments in Plant and Soil Sciences, 55. Springer, Dordrecht, pp 135-150. https://doi.org/10.1007/978-94-011-0878-2-10

Chen Q, Zhang X, Liu Y, Wei J, Shen W, Shen Z & Cui J (2017). Hemin-mediated alleviation of zinc, lead and chromium toxicity is associated with elevated photosynthesis, antioxidative capacity; suppressed metal uptake and oxidative stress in rice seedlings. Plant Growth Regulation 81: 253-264. https://doi.org/10.1007/s10725-016-0202-y

Çiçek N & Çakirlar H (2008). Effects of salt stress on some physiological and photosynthetic parameters at three different temperatures in six soya bean (*Glycine max* L. Merr.) cultivars. *Journal of Agronomy & Crop Science* 194: 34-46. https://doi.org/10.1111/j.1439-037X.2007.00288.x

Cordon G, Iriel A, Cirelli A F & Lagorio M G (2018). Arsenic effects on some photophysical parameters of *Cichorium intybus* under different radiation and water irrigation regimes. Chemosphere 204: 398-404. https://doi.org/10.1016/j.chemosphere.2018.04.048

DalCorso G, Manara A, Piasentin S & Furini A (2014). Nutrient metal elements in plants. Metallomics 6: 1770-1788. https://doi.org/10.1039/c4mt00173g

Díaz-Pontones D M, Corona-Carrillo J I, Herrera-Miranda C & González S (2021). Excess zinc alters cell wall class III peroxidase activity and flavonoid content in the maize scutellum. Plants 10: 197. https://doi.org/10.3390/plants10020197

Dobrikova A, Apostolova E, Adamakis I S & Han A (2022). Combined impact of excess zinc and cadmium on elemental uptake, leaf anatomy and pigments, antioxidant capacity, and function of photosynthetic apparatus in clary sage (*Salvia sclarea* L.). Plants 11: 2407. https://doi.org/10.3390/plants11182407

Dobrikova A, Apostolova E, Hanć A, Yotsova E, Borisova P, Sperdouli I, Adamakis I-D S & Moustakas M (2021). Tolerance mechanisms of the aromatic and medicinal plant *Salvia sclarea* L. to excess zinc. Plants 10: 194. https://doi.org/10.3390/plants10020194

Ekmekçi Y, Tanyolaç D & Ayhan B (2008). Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars. Journal of Plant Physiology 165: 600-611.

Fatemi H, Zaghdoud C, Nortes P A, Carvajal M & Martínez-Ballesta M C (2020). Differential aquaporin response to distinct effects of two Zn concentrations after foliar application in pak choi (*Brassica rapa* L.) plants. Agronomy 10: 450. https://doi.org/10.3390/agronomy10030450

Genty B, Briantais J M & Baker N R (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta (BBA) - General Subjects 990(1): 87-92. https://doi.org/10.1016/S0304-4165(89)80016-9

Glińska S, Gapińska M, Michlewska S, Skiba E & Kubicki J (2016). Analysis of *Triticum aestivum* seedling response to the excess of zinc. Protoplasma 253: 367-377.https://doi.org/10.1007/s00709-015-0816-3

Guadagno C R, Virzo De Santo A & D'Ambrosio N (2010). A revised energy partitioning approach to assess the yields of non-photochemical quenching components. Biochimica et Biophysica Acta 1797: 525-530. https://doi.org/10.1016/j.bbabio.2010.01.016

Iriel A, Cordon G, Fernández Cirelli A & Lagorio M G (2019). Non-destructive methodologies applied to track the occurrence of natural micropollutants in watering: *Glycine max* as a biomonitor. Ecotoxicology and Environmental Safety 182: 109368. https://doi.org/10.1016/j.ecoenv.2019.109368

Janeeshma E, Kalaji H M & Puthur J T (2021). Differential responses in the photosynthetic efficiency of *Oryza sativa* and *Zea mays* on exposure to Cd and Zn toxicity. Acta Physiologiae Plantarum 43: 12. https://doi.org/10.1007/s11738-020-03178-x

Jayasri M A & Suthindhiran K (2017). Effect of zinc and lead on the physiological and biochemical properties of aquatic plant *Lemna minor*: its potential role in phytoremediation. Applied Water Science 7: 1247-1253. https://doi.org/10.1007/s13201-015-0376-x

Karahan F, Ozyigit I I, Saracoglu I A, Yalcin I E, Ozyigit A H & Ilcim A (2020). Heavy metal levels and mineral nutrient status in different parts of various medicinal plants collected from eastern mediterranean region of Turkey. Biological Trace Element Research 197: 316-329. https://doi.org/10.1007/s12011-019-01974-2

Kaur H & Garg N (2021). Zinc toxicity in plants: a review. Planta 253: 129.https://doi.org/10.1007/s00425-021-03642-z

Küpper H & Andresen E (2016). Mechanisms of metal toxicity in plants. Metallomics 8: 269-285. https://doi.org/10.1039/c5mt00244c

Laemmli U K (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685. https://doi.org/10.1038/227680a0

Lichtenthaler H K, Buschmann C & Knapp M (2005). How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio $R_{Fd}$ of leaves with the PAM fluorometer. Photosynthetica 43(3): 379-393. https://doi.org/10.1007/s11099-005-0062-6

Lichtenthaler H K (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods in Enzymology 148: 350-382. https://doi.org/10.1016/0076-6879(87)48036-1

M'Rah S, Marichali A, M'Rabet Y, Chatti S, Saber C, Casabianca H & Hosni K (2023). Morphology, physiology, and biochemistry of zinc-stressed caraway plants. Protoplasma 260: 853-868. https://doi.org/10.1007/s00709-022-01818-2

Mancinelli A L, Yang C P H, Lindquist P, Anderson O R & Rabino I (1975). Photocontrol of anthocyanin synthesis: III. The action of streptomycin on the synthesis of chlorophyll and anthocyanin. Plant Physiology 55(2): 251-257. https://doi.org/10.1104/pp.55.2.251

Marschner H (1995). Mineral nutrition of higher plants. London: Academic Press.

Maxwell K & Johnson G H (2000). Chlorophyll fluorescence-a practical guide. *Journal of Experimental Botany* 51(345): 659-668. https://doi.org/10.1093/jxb/51.345.659

Miller G, Shulaev V & Mittler R (2008). Reactive oxygen signaling and abiotic stress. Physiologia Plantarum 133: 481-489. https://doi.org/10.1111/j.1399-3054.2008.01090.x

Mittler R & Zilinskas B A (1993). Detection of ascorbate peroxidase activity in native gels by inhibition of the ascorbate-dependent reduction of nitroblue tetrazolium. Analitical Biochemistry 212: 540-546. https://doi.org/10.1006/abio.1993.1366

Moustaka J, Panteris E, Adamakis I-D S, Tanou G, Giannakoula A, Eleftheriou E P & Moustakas M (2018). High anthocyanin accumulation in poinsettia leaves is accompanied by thylakoid membrane unstacking, acting as a photoprotective mechanism, to prevent ROS formation. Environmental and Experimental Botany 154: 44-55. https://doi.org/10.1016/j.envexpbot.2018.01.006

Mukhopadhyay M, Das A, Subba P, Bantawa P, Sarkar B, Ghosh P & Mondal T D (2013). Structural, physiological, and biochemical profiling of tea plants under zinc stress. Biologia Plantarum 57(3): 474-480. https://doi.org/10.1007/s10535-012-0300-2

Natasha N, Shahid M, Bibi I, Iqbal J, Khalid S, Murtaza B, Bakhat H F, Farooq A B U, Amjad M, Hammadd H M, Niazi N K & Arshad M, (2022). Zinc in soil-plant-human system: A data-analysis review. Science of the Total Environment 808: 152024. https://doi.org/10.1016/j.scitotenv.2021.152024

Pan L, Li J, Yin H, Fan Z & Li X (2020). Integrated physiological and transcriptomic analyses reveal a regulatory network of anthocyanin metabolism contributing to the ornamental value in a novel hybrid cultivar of *Camellia japonica*. Plants 9: 1724. https://doi.org/10.3390/plants9121724

Paunov M, Koleva L, Vassilev A, Vangronsveld J & Goltsev V (2018). Effects of different metals on photosynthesis: Cadmium and zinc affect chlorophyll fluorescence in durum wheat. *International Journal of Molecular Science* 19: 787. https://doi.org/10.3390/ijms19030787

Petrovic D & Krivokapic S (2020). The effect of Cu, Zn, Cd, and Pb accumulation on biochemical parameters (proline, chlorophyll) in the water caltrop (*Trapa natans* L.), Lake Skadar, Montenegro. Plants 9: 1287. https://doi.org/10.3390/plants9101287

Pütter J (1974). Peroxidases. In: Bergmeyer HU (ed) In Methods of Enzymatic Analysis, Academic P. Academic Press, NY, USA, pp. 685-690

Rai P K, Lee S S, Zhang M, Tsang Y F & Kim K-H (2019). Heavy metals in food crops: Health risks, fate, mechanisms, and management. Environment International 125: 365-385. https://doi.org/10.1016/j.envint.2019.01.067

Ramakrishna B & Rao S S R (2015). Foliar application of brassinosteroids alleviates adverse effects of zinc toxicity in radish (*Raphanus sativus* L.) plants. Protoplasma 252: 665-677. https://doi.org/10.1007/s00709-014-0714-0

Rao M V, Hale B A & Ormrod D P (1995). Amelioration of ozone-induced oxidative damage in wheat plants grown under high carbon dioxide: Role of antioxidant enzymes. Plant Physiology 109(2): 421-432. https://doi.org/10.1104/pp.109.2.421

Roccotiello E, Manfredi A, Drava G, Minganti V, Mariotti M G, Berta G & Cornara L (2010). Zinc tolerance and accumulation in the ferns *Polypodium cambricum* L. and *Pteris vittata* L. Ecotoxicology and Environmental Safety 73: 1264-1271. https://doi.org/10.1016/j.ecoenv.2010.07.019

Saboor A, Ali M A, Hussain S, Enshasy H A E, Hussain S, Ahmed N, Gafur A, Sayyed R Z, Fahad S, Danish S & Datta R (2021). Zinc nutrition and arbuscular mycorrhizal symbiosis effects on maize (*Zea mays* L.) growth and productivity. *Saudi Journal of Biological Sciences* 28: 6339-6351. https://doi.org/10.1016/j.sjbs.2021.06.096

Sapeta H, Yokono M, Takabayashi A, Ueno Y, Cordeiro A M, Hara T, Tanaka A, Akimoto S, Oliveira M. M. & Tanaka R (2023). Reversible down-regulation of photosystems I and II leads to fast photosynthesis recovery after long-term drought in *Jatropha curcas*. *Journal of Experimental Botany* 74(1): 336-351. https://doi.org/10.1093/jxb/erac423

Seregin I V, Ivanova T V, Voronkov A S, Kozhevnikova A D & Schat H (2023). Zinc- and nickel-induced changes in fatty acid profiles in the zinc hyperaccumulator *Arabidopsis halleri* and non-accumulator *Arabidopsis lyrata*. Plant Physiology and Biochemistry 197: 107640. https://doi.org/10.1016/j.plaphy.2023.107640

Sgherri C L M, Loggini B, Puliga S & Navari-Izzo F (1994). Antioxidant system *in Sporobolus stapfianus*: Changes in response to desiccation and rehydration. Phytochemistry 35(3): 561-565. https://doi.org/10.1016/S0031-9422(00)90561-2

Sharma J K, Kumar N, Singh N P& Santal A R (2023). Phytoremediation technologies and their mechanism for removal of heavy metal from contaminated soil: An approach for a sustainable environment. Frontier in Plant Science 14: 1076876. https://doi.org/10.3389/fpls.2023.1076876

Sofo A, Moreira I, Gattullo C E, Martins L L & Mou M (2018). Antioxidant responses of edible and model plant species subjected to subtoxic zinc concentrations. Journal of Trace Elements in Medicine and Biology 49: 261-268. https://doi.org/10.1016/j.jtemb.2018.02.010

Sofo A, Vitti A, Nuzzaci M, Tataranni G, Scopa A, Vangronsveld J, Remans T, Falasca G, Altamura M M, Degola F & di Toppi L S (2013). Correlation between hormonal homeostasis and morphogenic responses in *Arabidopsis thaliana* seedlings growing in a Cd/Cu/Zn multi-pollution context. Physiologia Plantarum 149: 487-498. https://doi.org/10.1111/ppl.12050

Sperdouli I, Adamakis I D S, Dobrikova A, Apostolova E, Hanć A & Moustakas M (2022). Excess zinc supply reduces cadmium uptake and mitigates cadmium toxicity effects on chloroplast structure, oxidative stress, and photosystem II photochemical efficiency in *Salvia sclarea* plants. Toxics 10: 36. https://doi.org/10.3390/toxics10010036

Sperdouli I, Mellidou I & Moustakas M (2021). Harnessing chlorophyll fluorescence for phenotyping analysis of wild and cultivated tomato for high photochemical efficiency under water deficit for climate change resilience. Climate 9:154. https://doi.org/10.3390/cli9110154

Stanton C, Sanders D, Krämer U & Podar D (2022). Zinc in plants: Integrating homeostasis and biofortification. Molecular Plant 15: 65-85. https://doi.org/10.1016/j.molp.2021.12.008

Suganya A, Saravanan A & Manivannan N (2020). Role of zinc nutrition for increasing zinc availability, uptake, yield, and quality of maize (*Zea Mays* L.) grains: an overview. Communications in Soil Science and Plant Analysis 51(15): 2001-2021. https://doi.org/10.1080/00103624.2020.1820030

Szopiński M, Sitko K, Gieroń Ż, Rusinowski S, Corso M, Hermans C, Verbruggen N & Małkowski E (2019). Toxic effects of Cd and Zn on the photosynthetic apparatus of the *Arabidopsis halleri* and *Arabidopsis arenosa* pseudo-metallophytes. Frontier in Plant Science 10: 748. https://doi.org/10.3389/fpls.2019.00748

Tiecher T L, Tiecher T, Ceretta C A, Ferreira P A A, Nicoloso F T, Soriani H H, De Conti L, Kulmann M S S, Schneider R O & Brunetto G (2017). Tolerance and translocation of heavy metals in young grapevine (*Vitis vinifera*) grown in sandy acidic soil with interaction of high doses of copper and zinc. Scientia Horticulturae 222: 203-212. https://doi.org/10.1016/j.scienta.2017.05.026

Ünalan Ş (2006) Response of antioxidant enzyme defence system on the maize cultivars under the heavy metal stress and investigation of maize's usability for removal of heavy metal. MSc thesis, Hacettepe University (in Turkish).

Vaillant N, Monnet F, Hitmi A, Sallanon H & Coudret A (2005). Comparative study of responses in four *Datura* species to a zinc stress. Chemosphere 59: 1005-1013. https://doi.org/10.1016/j.chemosphere.2004.11.030

Wang S Y, Jiao H J & Faust M (1991). Changes in ascorbate, glutathione, and related enzyme activities during thidiazuron-induced bud break of apple. Physiologia Plantarum 82(2): 231-236. https://doi.org/10.1111/j.1399-3054.1991.tb00086.x

Wieczorek J, Baran A, Bubak A (2023). Mobility, bioaccumulation in plants, and risk assessment of metals in soils. Science of The Total Environment 882: 163574. https://doi.org/10.1016/j.scitotenv.2023.163574

Yin J, Gentine P, Zhou S, Sullivan S C, Wang R, Zhang Y, & Guo S (2018). Large increase in global storm runoff extremes driven by climate and anthropogenic changes. Nature communications 9(1): 4389. https://doi.org/10.1038/s41467-018-06765-2