Article

Photosynthetic chlorophyll fluorescence response of *Tradescantia pallida* exposed to different heavy metals alone and in combination

Neelima Meravi[1], **Chandralekha Piparia**[1], **Anjali Verma**[1], **Prashant Kumar Singh**[2], **Santosh Kumar Prajapati**[1]

[1]Department of Botany, School of Life Sciences, Guru GhasidasVishwavidyalaya,Koni,Bilaspur, 495009, Chhattisgarh, India

[2]Department of Botany, Govt. VBSD Girls College, Jashpur, 496331, Chhattisgarh, India

E-mail: sntshprjpt@rediffmail.com

Received 5 April 2023; Accepted 10 May 2023; Published online 25 May 2023; *Published 1 December 2023*

Abstract

Presence of metals in the environment may have adverse impacts on the essential components of ecosystem particularly the phototrophs, In order to assess impacts of heavy metals on *Tradescantia pallida*, plants were grown in pots (5 L capacity) under control and the metal treatment of $MnCl_2$ (10 mM L^{-1}), $CuCl_2$ (10 mM L^{-1}), $CoCl_2$ (10 mM L^{-1}), $FeCl_3$ (10 mM L^{-1}), $NiCl_2$ (10 mM L^{-1}), $ZnCl_2$ (10 mM L^{-1}) and 10 mM combinations of $MnCl_2$ + $CoCl_2$, $CuCl_2$ + $FeCl_3$ and $NiCl_2$ + $ZnCl_2$. Results showed that the Fv/Fm (indication of the maximum and effective photochemical quantum yield of PS II) was ≥ 0.70 for the control (0.773) and Mn (0.705), Fe (0.700), and Cu + Fe (0.709) exhibiting small impact of these metals or combination on the photosynthetic machinery. However, the Fv/Fm value <.70 for Cu (0.645), Co (0.116), Ni (0.440), Zn (0.553), Co + Mn (0.363), and Ni + Zn (0.617) treatments clearly showed adverse impact of these metals and combination on the photosynthetic processes. The greatest negative impact observed by Co treatment alone or with the combination of Mn (Co + Mn). The study highlighted reduced negative impact of Cu metals when used in combination with Fe. However, it is clear from the present study that *Tradescantia pallida* plants are under stress in the presence of heavy metals. It also confirms that presence of heavy metals in ecosystem can cause reduction in the overall primary productivity of the *Tradescantia pallida*.

Keywords heavy metals; phototrophs; Fv/Fm; *Tradescantia pallida.*

Proceedings of the International Academy of Ecology and Environmental Sciences
ISSN 2220-8860
URL: http://www.iaees.org/publications/journals/piaees/online-version.asp
RSS: http://www.iaees.org/publications/journals/piaees/rss.xml
E-mail: piaees@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Presence of excess heavy metals in the environment due to natural calamities, industries, mining, agricultural and other human activities is deleterious. Heavy metals influence not only plant growth and reproduction but also human health (Alengebawy et al., 2021; Felix et al., 2010; Su et al., 2014). Over accumulation of Reactive Oxygen Species (ROS) affecting various regulatory processes such as programmed cell death, cellular transport, enzyme associated processes etc., have been observed due to chromium toxicity (Wakeel et al., 2020). The effect of heavy metals on plant growth and metabolism has been studied extensively but the effect of plant exposed to a single heavy metal has received much attention than the combined effects of multiple metals at the same time. Although contaminated terrestrial and aquatic environments usually contain a mixture of substances. Metals such as copper (Cu), zinc (Zn), cobalt (Co), and iron (Fe) are essential for various metabolic activities in plants (trace amount). But excess of these metals adversely affect plant metabolism (Hall, 2002) and it does create the oxidative stress in plants (Šiukšta et al., 2019) metals exert their toxic effects on plants mostly by damaging chloroplasts and disturbing photosynthesis. Reduction in photosynthesis is the consequence of interference of metal ions with photosynthetic enzymes and chloroplast membranes (Aggarwal et al., 2012). Chlorophyll fluorescence is the best suited and most reliable method for photosynthetic productivity measurement (Ptushenko et al., 2013; Kumar et al., 2014). Accumulation of metals in higher plants leaf influences the functioning of the stomata and hence affects photosynthesis and transpiration rates and thus photosynthesis is indirectly reduced by heavy metals.

Zinc (Zn) is an essential plant nutrient which is required for the catalytic function of many enzymes, structural stability of cell membranes, proteins as well as protection of bio-membranes against oxidative damages (Aravind and Prasad 2003; Broadley et al., 2007). Higher concentration of Zn can also give rise to manganese (Mn) and copper (Cu) deficiencies in plant shoots. Toxicity symptoms of zinc include slow and stunted growth, chlorosis induced by iron deficiency followed by decreased chlorophyll synthesis as well as interference with manganese uptake (Broadley et al., 2007). Copper (Cu) is an essential nutrient which is involved in a wide range of biochemical and physiological processes. It participates in electron-transfer system of photosynthesis in the form of plastocyanin, as a co-factor of Cu-Zn superoxide dismutase, polyphenoloxidase (Maksymiec, 1997). Redox cycling between Cu^{2+} and Cu^{+} leads to ROS production in plant cell. Excess of Cu in soil plays a phytotoxic role and induces stress and causes injury to plants and leads to retardation of growth and leaf chlorosis (Lewis et al., 2001).A study reveals that sample having high 40% of copper plus lead together cause extreme damage to the photosynthetic machinery and generate stress in *Citrus aurantium* L. (Giannakoula et al., 2021)

Cobalt (Co) naturally occurs in the earth’s crust as cobaltite, erythrite and smaltite. Plants can accumulate small quantity of Co from the soil. The accumulation of cobalt from soil and its distribution in plants body parts is species dependent and controlled by diverse mechanisms (Kukier et al., 2004; Li and Metherel, 2004). Meagre information is available regarding the phytotoxic effect of Co. Phytotoxicity studies of cobalt in barley (*Hordeum vulgare* L.), oilseed rape (*Brassica napus* L.) and tomato (*Lycopersicon esculentum* L.) have shown the undesirable effect on shoot growth and biomass (Li HF et al., 2009). Excess Co also restricted the concentration of Fe, chlorophyll, protein and catalases activity in leaves and also affected the translocation of P, S, Mn, Zn and Cu from roots to tips in cauliflower. Nickel (Ni) is present in natural soils at very low concentrations. However, Ni^{2+} concentration is increasing in soil by human activities such as mining works, emissions from smelters, burning of coal (Gimeno-Garcia et al., 1996). In humans nickel toxicity leads to skin inflammation, cardiovascular disorders and are prone to cause cancer (Genchi et al., 2020). Excess of Ni^{2+} in soil causes many physiological changes and various toxicity symptoms such as chlorosis and necrosis in different plant species (Pandey and Sharma, 2002; Rahman et al., 2005). Plants growing in soil having high Ni^{2+} showed loss of nutrient balance and a decline in water content of dicot and monocot plant species. The decrease in water uptake is as an indicator of the progression of Ni^{2+} toxicity in plants (Pandey and Sharma, 2002; Gajewska et al., 2006).

Manganese (Mn) is an essential element for plant growth but presence of excess manganese in leaves causes a reduction of photosynthetic rate (Kitao et al., 1997). It has been reported that excess Mn reduce synthesis of chlorophyll by blocking a Fe-concerning process (Clarimont et al., 1986). Mn is easily transported from root to shoot through the xylem, but not easily remobilized through phloem to other organs once it reaches the plant leaves (Loneragan, 1988). Brown necrotic areas on leaves, petioles and stems are common symptom of Mn toxicity which starts on the lower leaves and progresses with passage of time towards the upper leaves (Horiguchi, 1988). Chlorosis in younger leaves due to Mn toxicity may be mediated through Mn-induced Fe deficiency studied in the shoots of sugarcane (Huang et al., 2016). Iron is a very essential metal for all plants as it has plays important role in biological functions such as photosynthesis, chloroplast development and chlorophyll biosynthesis. Iron is a major component of the cell redox systems such as heme proteins including cytochromes, catalase, peroxidase and legheamoglobin and iron sulfur proteins (Marschner, 1995). Almost mineral soils are rich in iron, but iron toxicity symptoms in leaf tissues occurs only under flooded conditions (Becker and Asch, 2005). Excess Fe^{2+} causes free radical production that disturbs cellular structure irreversibly and damages membranes, proteins and DNA (Arora et al., 2002; De-dorlodot et al., 2005). Another group of researchers have shown that iron toxicity is accompanied with reduction of plant photosynthesis and yield and the increase in oxidative stress and ascorbateperoxidise activity (Sinha et al., 1997). Crop productivity and health has been deteriorated due to heavy metal hindrance and to overcome those hindrances various biotechnological interventions are employed (Elango et al., 2022).

Determination of chlorophyll a fluorescence tells us about changes in the efficiency of photochemistry and heat scattering (Baker and Oxborough, 2004; Stirbet et al., 1998). Since such a measurement is handy, non-invasive, highly responsive, rapid as well as reliable and gives quantitative look into oxygenic photosynthesis and therefore suitable tool for determination of plant physiological status (Murchie and Lawson, 2013; Singh et al., 2022). So, the present experiment was conducted to study the adverse effects caused by metals on the photosynthetic efficiency of *Tradescantia pallida* plants using non-destructiveand easy access parameters with the help of JUNIOR PAM fluorometer.

2 Materials and Methods

Photosynthetic responses of *Tradescantia pallida* exposed to different heavy metals alone and in combination were studied by growing 10 healthy *Tradescantia pallida* plants in plastic pots (5 L capacity). The metal treatment was performed once all plant got stabilized with various metal salts $MnCl_2$ (10 mM L^{-1}), $CuCl_2$ (10 mM L^{-1}), $CoCl_2$ (10 mM L^{-1}), $FeCl_3$ (10 mM L^{-1}), $NiCl_2$ (10 mM L^{-1}), $ZnCl_2$ (10 mM L^{-1}) and combination of $MnCl_2 + CoCl_2$, $CuCl_2 + FeCl_3$ and $NiCl_2 + ZnCl_2$.

Various photosynthetic parameters viz. Fv/Fm (maximum photochemical quantum yield of photosystem II), Y II (photochemical quantum yield of photosystem II), qL (coefficient of photochemical fluorescence quenching), qP (coefficient of photochemical fluorescence quenching),NPQ (non-photochemical fluorescence), Y(NPQ) (quantum yield of non-photochemical fluorescence quenching) and Y(NO) (quantum yield of non-photochemical fluorescence quenching)were recorded with the help of JUNIOR - PAM, Chlorophyll Fluorometer, Heinz Walz GmbH, Germany. All experiment start with a leaf which has been acclimated to dark or dim light conditions for 30 minutes. With the dark acclimated leaf kept in the magnetic leaf clip, the Fo fluorescence level should not exceed 600 counts to avoid signal saturation during Fo, Fm determination.

Measuring light: Blue LED (wavelength of maximum emission: 450 nm). Two modulation frequencies were used (5 and 100 Hz). Actinic light: Blue LED (wavelength of maximum emission: 450 nm). Photon flux densities at 1 mm distance from the tip of the 400 mm JUNIOR PAM light guide: 25 to 1500 µmol photons/(m^2*s), adjustable at 12 different levels. Far red light: LED with 730 nm maximum emission wavelength) for selective excitation of photosystem I. Saturating pulses: Blue LED (wavelength of maximum emission: 450 nm). Maximum photon flux density 10000 μmol photons/(m^2*s) at 1 mm distance from the tip of the 400 mm JUNIOR-PAM light guide.

Software: WinControl-3 software for PAM fluorometers.

3 Results and Discussion

Tradescantia pallida plants exposed to the metals and their combinations (10 mM L^{-1}) for seven days caused a decrease of maximum quantum yield (Fv/Fm) of PSII (Fig. 1) for all the treatments except for $MnCl_2$. The most prominent decrease in Fv/Fm value (0.102) was noticed in plant treated with $CoCl_2$ followed by $NiCl_2$, $ZnCl_2$, $CuCl_2$ and $FeCl_3$. The treatment of plant with $MnCl_2$ showed increase in the Fv/Fm value (0.742) as compared to observed value (0.739) for control plant. The reduction in Fv/Fm value was also observed for combined metals treatments; however the reduction was less as compared to single metal treatment. The observed value (0.726) of Fv/Fm for combined treatment of $CuCl_2$ + $FeCl_3$ was higher as compared to other combined treatments. The observed value of Fv/Fm also shows that plants are under stress for all the treatment except $MnCl_2$ and $CuCl_2$ + $FeCl_3$ because all plants showed less Fv/Fm value than control plant.

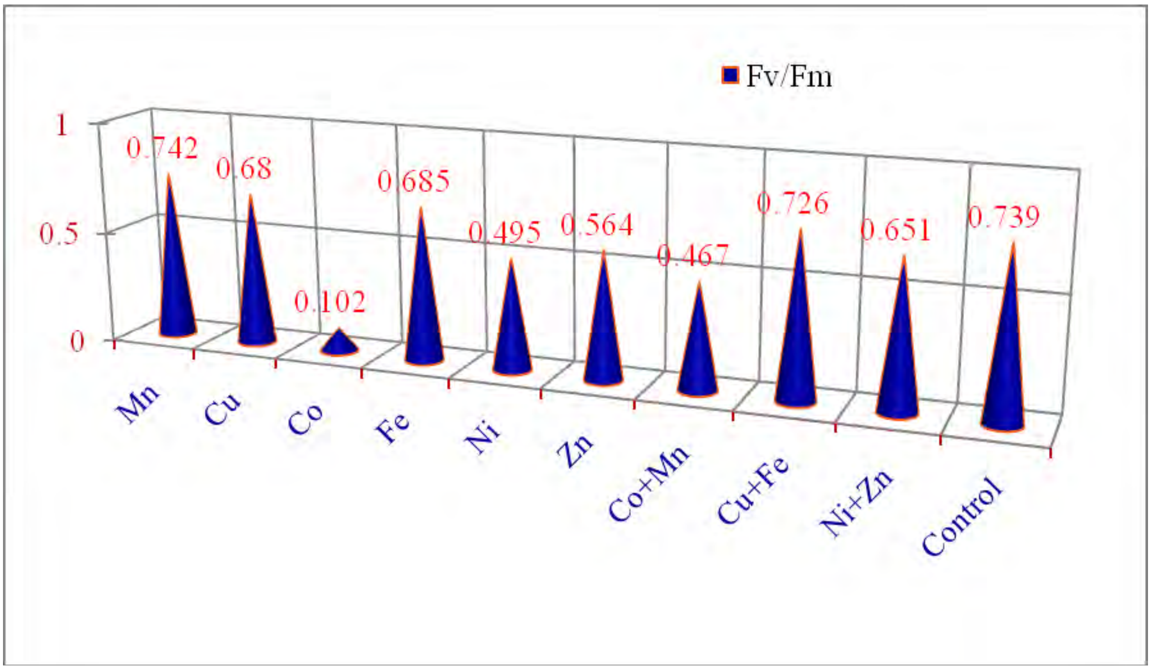

Fig. 1 Effects of different metals/metal combinations on Fv/Fm (Fm-Fo/Fm; maximum photochemical quantum yield of photosystem II).

Tradescantia pallida plants were exposed to the metals and their combinations (10 mM L^{-1}) for seven days to study the effects on Y (II) (photochemical quantum yield of photosystem II) as shown in Fig. 2. The observed value of Y (II) shows that treatments caused a decrease in photochemical quantum yield of photosystem II for all the treatments as compared to Y (II) of control plant (0.582). The maximum reduction was observed for Co (0.039) followed by Ni, Cu, Fe and Mn. For combined treatment the value was lowest for Co + Mn (0.306) and maximum for Fe + Cu (0.565). Therefore, it can be said that treatment with metals and their combinations (10 mM L^{-1}) can effectively reduce the photochemical quantum yield of photosystem II in *Tradescantia pallida* plants.

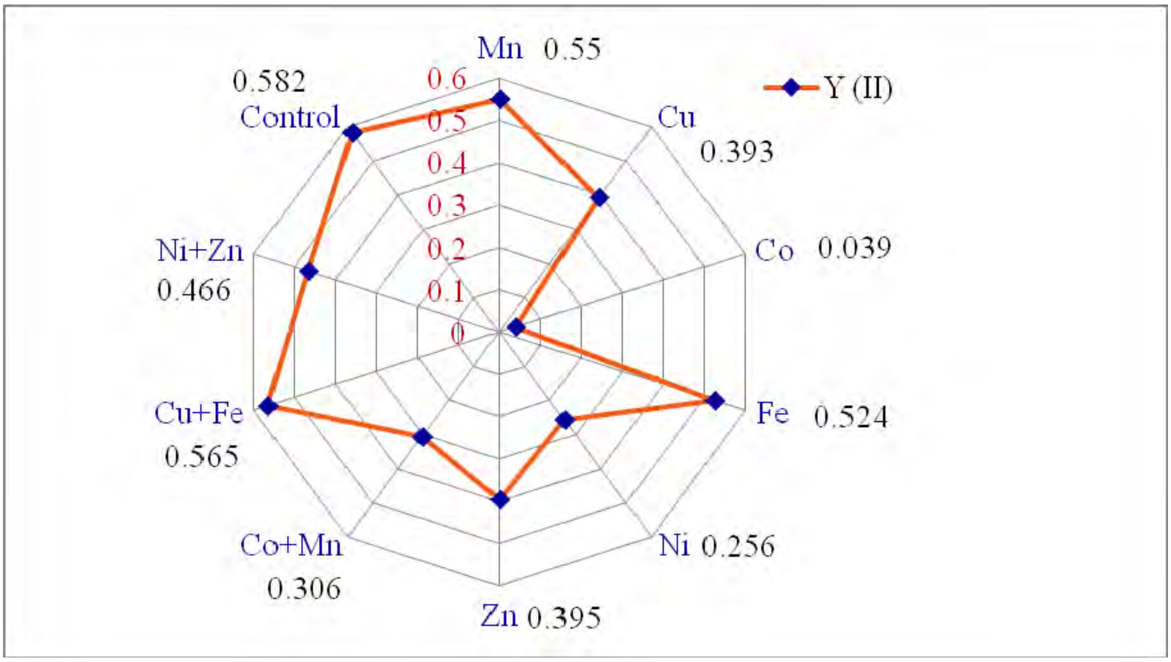

Fig. 2 Effects of different metals/metal combinations on Y(II) (photochemical quantum yield of photosystem II).

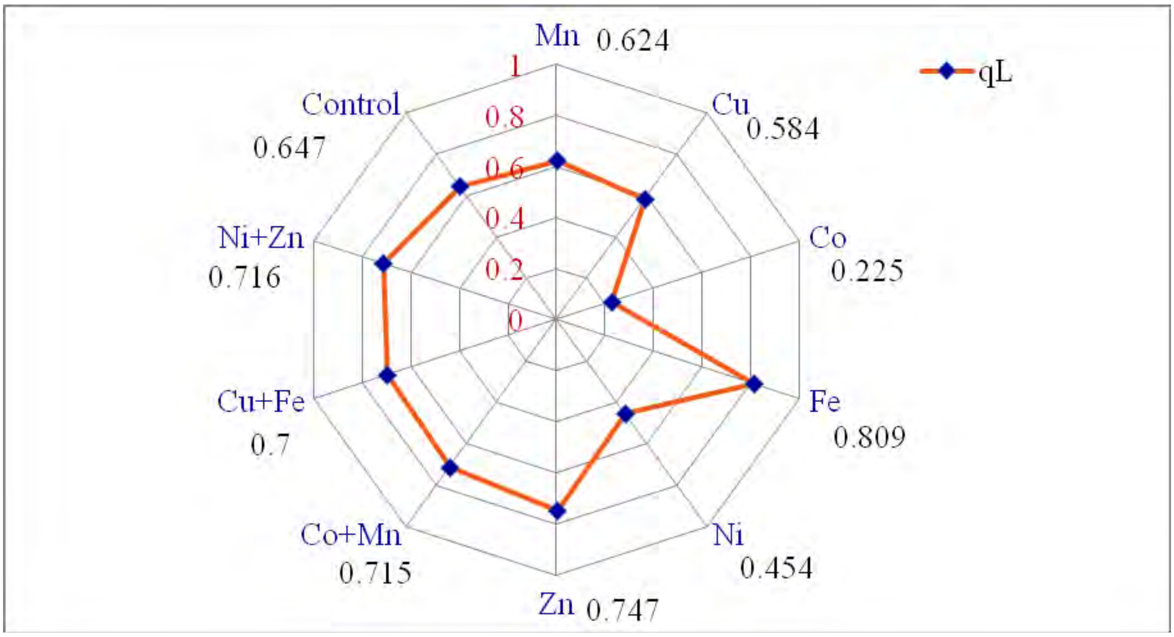

Fig. 3 Effects of different metals/metal combinations on qL (coefficient of photochemical fluorescence quenching assuming that the many photosystem complexes form a combined light-harvesting antenna so that an absorbed photon becomes available for many reaction centers (Lake Model).

The parameter qL (coefficient of photochemical fluorescence quenching) is considered to be more precise indicator of PS II redox state and also estimates the fraction of open PS II centres. It is clear from the Fig. 3 that in case of single metal treatment qL value for all plants was lower than control plant (0.645) except for Fe (0.809) and Zn (0.747). When metals were used in combinations they increased the fraction of open PS II centres for all treatments compared to control. The minimum value of qL was observed for Co (0.225) and followed by Ni, Cu and Mn and maximum value of qL was recorded for Fe (0.809) and followed by Zn, Ni + Zn, Co + Mn and Cu + Fe.

The parameter qP is more consistent with separated light-harvesting antennae of photosystems as compared to qL and relates PS II maximum efficiency to operating efficiency. It is clear from the Fig. 4 that value of qP was lower for all plants as compared to control plant (0.825) except for Fe (0.869) in the case of single metal treatment. Co caused maximum reduction in qP value (0.231) followed by Ni, Cu, Mn and Zn. The observed value of qP was also lower than control plant when metals were used in combinations except for the combined treatment of Cu + Fe (qP value is 0.83). Co + Mn treatment gave minimum qP value (0.778) and Ni + Zn treatment have maximum value (0.809).

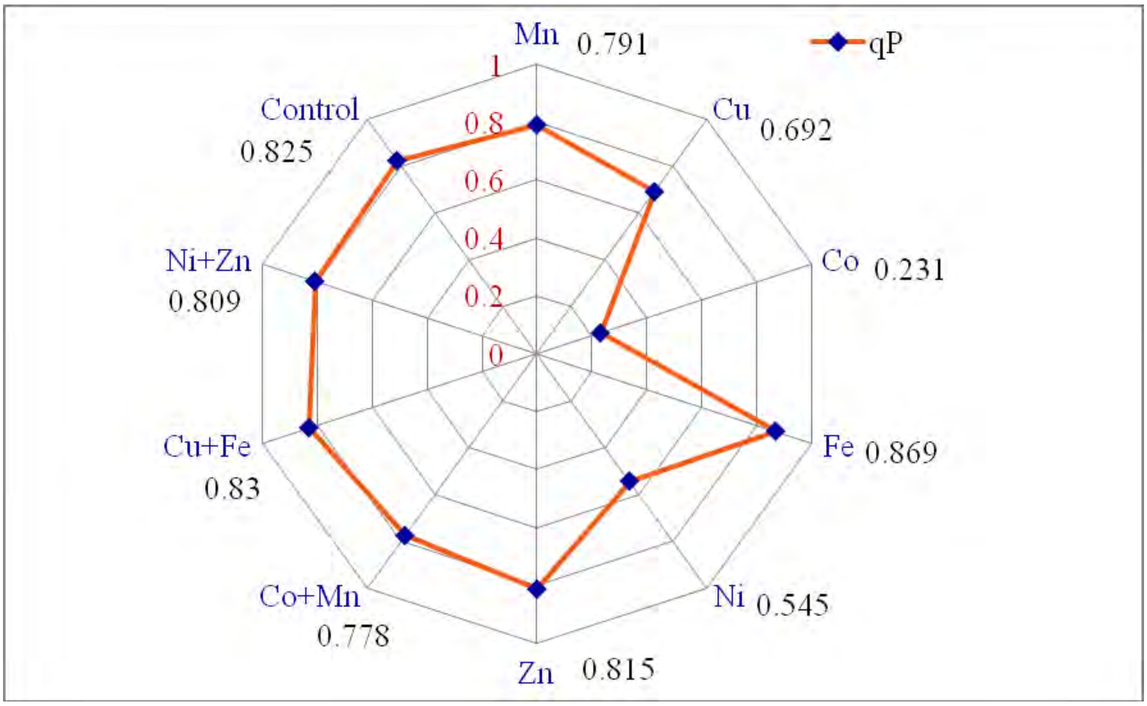

Fig. 4 Effects of different metals/metal combinations on qP (coefficient of photochemical fluorescence quenching). In comparison, the qP is more consistent with separated light-harvesting antennae of photosystems (puddle model).

NPQ (non-photochemical quenching of chlorophyll fluorescence) is an indication of the intensity of non-radiative energy loss in the light harvesting complex II of photosystem II, which is credited to prevent lessening of the electron transfer chain. NPQ is a means employed by plants to protect themselves from the undesirable effects of high light intensity. NPQ serves as an index of stress for the plants. It is clear from the Fig. 5 that NPQ was recorded maximum for Cu (1.189) treatment and minimum for Co (0.019) treatment. It is also clear from the figure that single and combined metal treatments of plants excepting Co have more NPQ values as compared to control plant. In combined treatment NPQ was maximum for Co + Mn and minimum for Cu + Fe treatments. Higher value of NPQ for a given metal treatment means higher non-radiative energy loss in the light harvesting complex II of photosystem II and thus reducing stress. Fig. 5 shows that Cu was able to reduce the maximum stress and Co was able to induce stress.

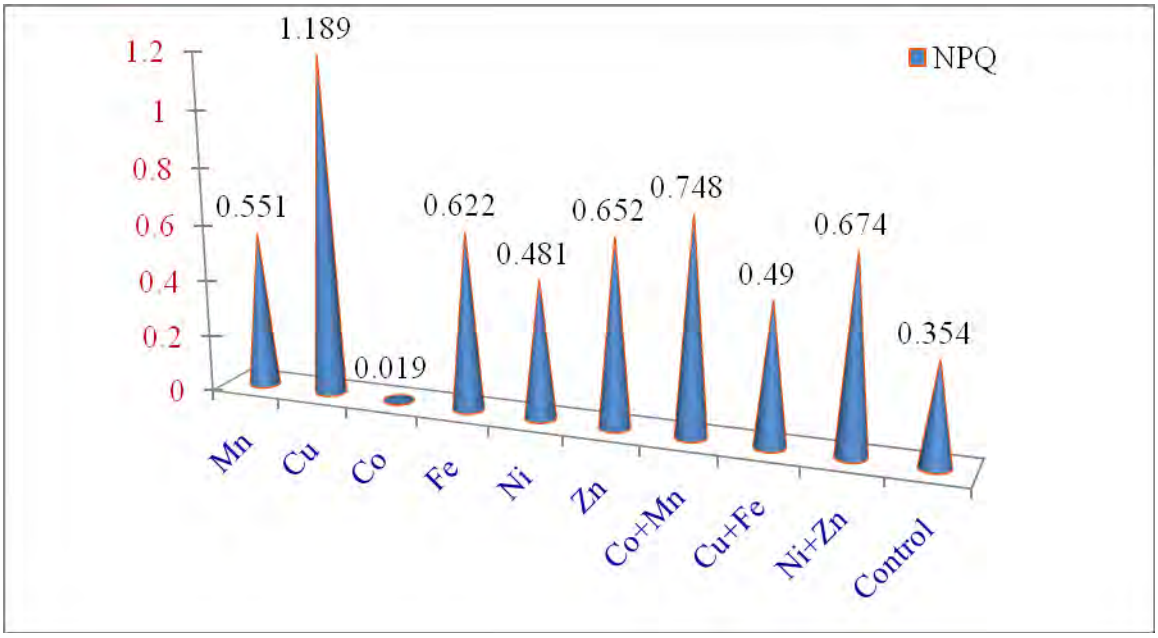

Fig. 5 Effects of different metals/metal combinations on NPQ (non-photochemical fluorescence). The extent of NPQ has been suggested to be associated with the number of quenching centers in the light-harvesting antenna.

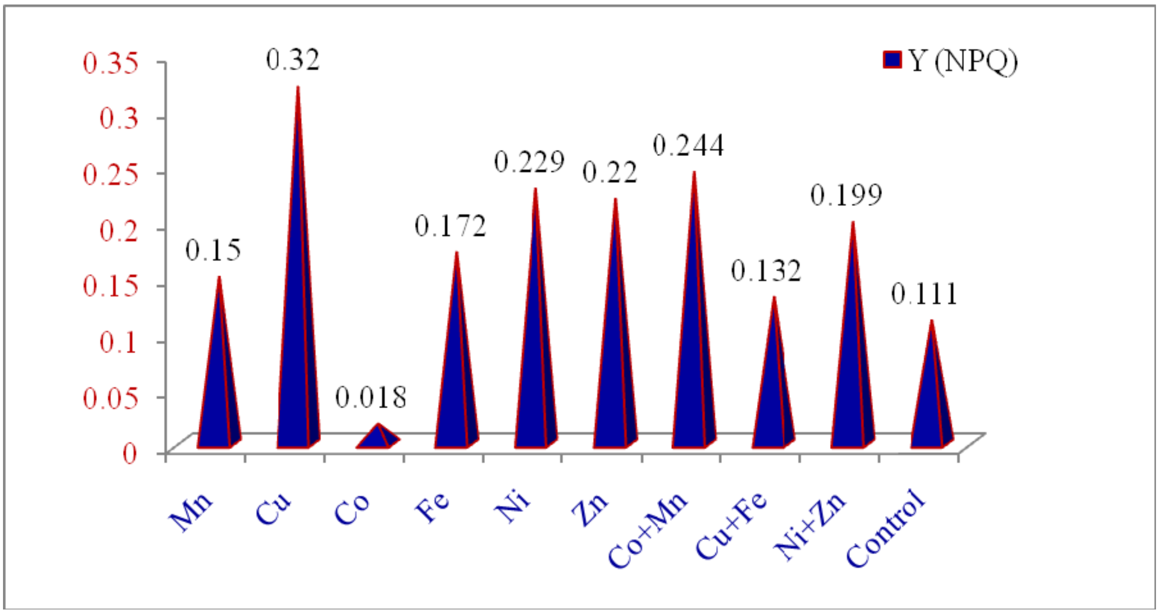

Fig. 6 Effects of different metals/metal combinations on Y(NPQ) (quantum yield of non-photochemical fluorescence quenching due to down regulation of the light-harvesting function).

Y (NPQ) is used to quantify the fraction of excitation energy which is dissipated as heat via photo-protective mechanism. It is clear from Fig. 6 that Y (NPQ) was minimal for Co and maximum for Cu in single metal treatment because the NPQ was more for Cu as compared to Co. Co + Mn treatment gave maximum value while Cu + Fe resulted in minimum for Y (NPQ). Y (NPQ) followed the similar trends as that of NPQ for various treatments. Y (NO) calculates the yield of all other non-photochemical losses except excitation energy which is dissipated as heat via photo-protective mechanism (Fig. 7). The figure clearly indicates that Cu treatment resulted in minimum Y (NO) whereas Co has maximum Y (NO). It can be observed from the figure that Y (NO) values are higher for those metals/metal combination treatments whose Y (NPQ) values were lower and vice-versa.

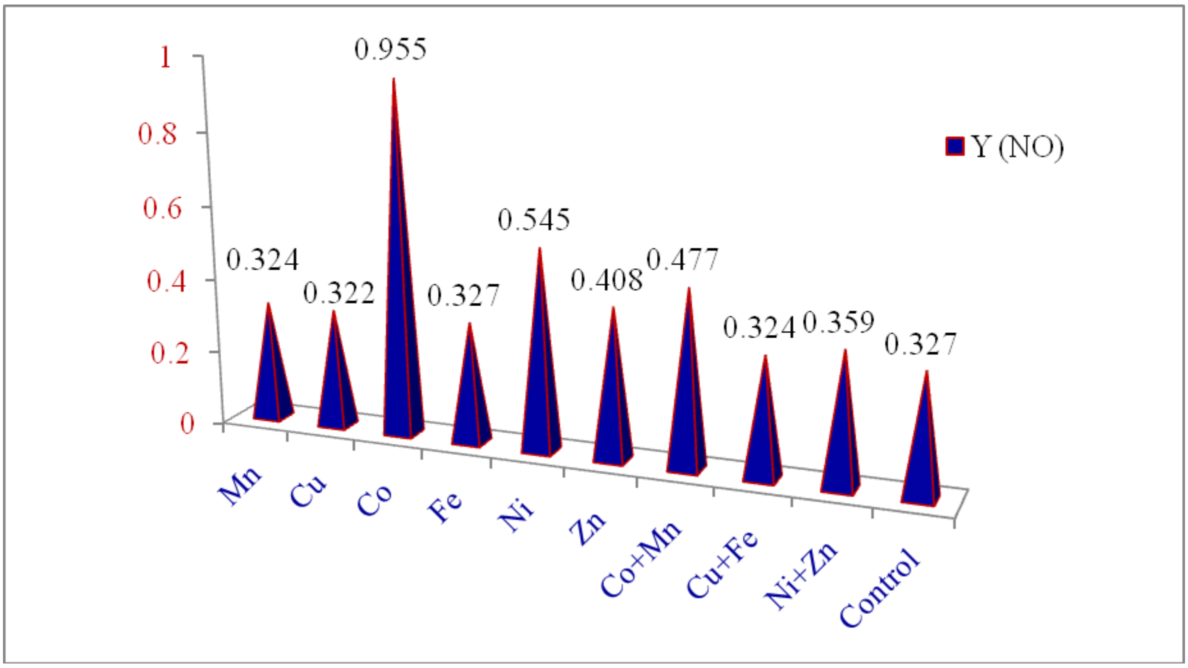

Fig. 7 Effects of different metals/metal combinations on Y(NO) (quantum yield of non-photochemical fluorescence quenching other than that caused by down regulation of the light-harvesting function).

The effect of metals and their combinations on different chlorophyll afluorescence parameters were significant as confirmed by one-way ANOVA. The calculated F-value (6.6078) was greater than the F-critical value (2.2656) for the alpha level 0.05 and the *p* value was very less as compared to alpha level 0.05.

Huang et al. (2017) have studied toxic effects of cadmium on *Festuca arundinacea* and different responses of the photosynthetic activities in the photosystem electron donor and acceptor where they have shown that chlorophyll a fluorescence parameters are adversely affected. Chlorophyll a fluorescence was studied in some perennial grasses where soil was contaminated by heavy metals and it was reported that presence of heavy metals were reducing photosynthetic efficiency of these grasses (Zurek et al., 2014). Other workers has shown the effects of cadmium and zinc on chlorophyll fluorescence in durum wheat and established that heavy metals are detrimental for photochemistry (Paunov et al., 2018).

Acknowledgement
The authors are thankful to Department of Botany, Guru Ghasidas Vishwavidyalaya for providing necessary facilities.

References

Alengebawy A, Abdelkhalek ST, Qureshi SR, et al. 2021. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological Risks and Human Health Implications. Toxics, 9(3): 42

Aravind P, Prasad MNV. 2003. Zinc alleviates cadmium-induced oxidative stress in *Ceratophyllum demersum* L: a free floating freshwater macrophyte. Plant Physiology and Biochemistry, 41: 391-397

Arora A, Sairam RK, Srivastava GC. 2002. Oxidative stress and antioxidative system in plants. Current Science, 82: 1227-1238

Baker NR, Oxborough K. 2004. Chlorophyll fluorescence as a probe of photosynthetic productivity. Chlorophyll a fluorescence: A Signature of Photosynthesis, 19: 66-79

Becker M, Asch F. 2005. Iron toxicity in rice-conditions and management concepts. Journal of Plant Nutrition and Soil Science, 168: 558-573

Broadley MR, White PJ, Hammond JP, et al. 2007. Zinc in plant. New Phytologist, 173: 677

Clairmont K B, Hagar WG, Davis EA. 1986. Manganese toxicity to chlorophyll synthesis in tobacco callus. Plant Physiology, 80: 291-293

De Dorlodot S, Lutts S, Bertin P. 2005. Effects of ferrous iron toxicity on the growth and mineral composition of an interspecific rice. Journal of Plant Nutrition, 28: 1-20

Elango D, Devi KD, Jeyabalakrishnan HK, et al. 2022. Agronomic, breeding, and biotechnological interventions to mitigate heavy metal toxicity problems in agriculture. Journal of Agriculture and Food Research, 10: 100374

Felix-Henningsen P, Urushadze T, Steffens D, et al. 2010. Uptake of heavy metals by food crops from highly-polluted Chernozem-like soils in an irrigation district south of Tbilisi, Eastern Georgia. Agronomy Research, 8: 781 - 795

Gajewska E, Skłodowska M, Słaba M, et al. 2006. Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. BiologiaPlantarum, 50: 653-659

Genchi G, Carocci A, Lauria G, Sinicropi MS, et al. 2020. Nickel: Human health and environmental toxicology. International Journal of Environmental Research and Public Health, 17: 679

Giannakoula A, Therios I, Chatzissavvidis C. 2021. Effect of lead and copper on photosynthetic apparatus in citrus (*Citrus aurantium* L.) plants.The role of antioxidants in oxidative damage as a response to heavy metal stress. Plants, 10: 155

Gimeno-García E, Andreu V, Boluda R. 1996.Heavy metals incidence in the application of inorganic fertilizers and pesticides to rice farming soils. Environmental Pollution, 92: 19-25

Hall JÁ. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany, 53: 1-11

Horiguchi T. 1988. Mechanism of manganese toxicity and tolerance of plants: IV. Effects of silicon on alleviation of manganese toxicity of rice plants. Soil Science and Plant Nutrition, 34: 65-73

Huang M, Zhu H, Zhang J, et al. 2017. Toxic effects of cadmium on tall fescue and different responses of the photosynthetic activities in the photosystem electron donor and acceptor sides. Scientific Reports, 7:1-10

Huang YL, Yang S, Long GX, et al. 2016. Manganese toxicity in sugarcane plantlets grown on acidic soils of southern China. PLoS One, 11: e0148956

Kitao M, Lei TT, Koike T. 1999. Effects of manganese in solution culture on the growth of five deciduous broad-leaved tree species with different successional characters from northern Japan. Photosynthetica, 36: 31

Kukier U, Peters CA, Chaney RL, et al. 2004. The effect of pH on metal accumulation in two Alyssum species. Journal of Environmental Quality, 33: 2090-2102

Kumar KS, Dahms HU, Lee JS, et al. 2014. Algal photosynthetic responses to toxic metals and herbicides assessed by chlorophyll a fluorescence. Ecotoxicology and Environmental Safety, 104: 51-71

Lewis S, Donkin ME, Depledge MH. 2001. Hsp70 expression in *Enteromorpha intestinalis* (Chlorophyta) exposed to environmental stressors. Aquatic Toxicology, 51: 277-291

Li HF, Gray C, Mico C, Zhao FJ, et al. 2009. Phytotoxicity and bioavailability of cobalt to plants in a range of soils. Chemosphere, 75: 979-986

Li Z, McLaren RG, Metherell AK. 2004. The availability of native and applied soil cobalt to ryegrass in relation to soil cobalt and manganese status and other soil properties. New Zealand Journal of Agricultural Research, 47: 33-43

Loneragan J F. 1988. Distribution and movement of manganese in plants. In: Manganese in Soils and Plants: Proceedings of the International Symposium on ‘Manganese in Soils and Plants’, Waite Agricultural Research Institute, The University of Adelaide, Glen Osmond, South Australia, Springer, Netherlands

Maksymiec W.1998. Effect of copper on cellular processes in higher plants.Photosynthetica, 34: 321-342

Marschner H. 1995. Mineral Nutrition of Higher Plants (2^{nd} ed). Institute of Plant Nutrition University of Hohenheim, Germany

Murchie EH, Lawson T. 2013. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. Journal of Experimental Botany, 64: 3983-3998

Pandey N, Sharma CP. 2002. Effect of heavy metals Co^{2+}, Ni^{2+} and Cd^{2+} on growth and metabolism of cabbage. Plant Science, 163: 753-758

Paunov M, Koleva L, Vassilev A, et al. 2018. Effects of different metals on photosynthesis: cadmium and zinc affect chlorophyll fluorescence in durum wheat. International Journal of Molecular Sciences, 19: 787

Ptushenko VV, Ptushenko EA, Samoilova OP, et al. 2013. Chlorophyll fluorescence in the leaves of Tradescantia species of different ecological groups: induction events at different intensities of actinic light. Biosystems, 114: 85-97

Rahman H, Sabreen S, Alam S, et al. 2005. Effects of nickel on growth and composition of metal micronutrients in barley plants grown in nutrient solution. Journal of Plant Nutrition, 28: 393-404

Singh H, Kumar D, Soni V. 2022. Performance of chlorophyll a fluorescence parameters in Lemna minor under heavy metal stress induced by various concentration of copper. Scientific Reports, 12: 10620

Sinha S, Gupta M, Chandra P. 1997. Oxidative Stress Induced by Iron in *Hydrilla verticillata* (lf) Royle: Response of Antioxidants. Ecotoxicology and Environmental Safety, 38: 286-291

Šiukšta R, Bondzinskaitė S, Kleizaitė V, et al. 2019. Response of Tradescantia plants to oxidative stress induced by heavy metal pollution of soils from industrial areas. Environmental Science and Pollution Research, 26: 44 - 61

Stirbet A, Strasser BJ, Strasser RJ. 1998. Chlorophyll a fluorescence induction in higher plants: modelling and numerical simulation. Journal of Theoretical Biology, 193: 131-151

Su C, Jiang LQ, Zhang WJ. 2014. A review on heavy metal contamination in the soil worldwide: Situation, impact and remediation techniques. Environmental Skeptics and Critics, 3(2): 24-38

Wakeel A, Xu M, and Gan Y. 2020. Chromium-induced reactive oxygen species accumulation by altering the enzymatic antioxidant system and associated cytotoxic genotoxicultrastructural and photosynthetic changes in plants. International Journal of Molecular Sciences, 21(3): 728

Żurek G, Rybka K, Pogrzeba M, et al. 2014. Chlorophyll a fluorescence in evaluation of the effect of heavy metal soil contamination on perennial grasses. Plos One, 9: e91475