

Effects of Some Heavy Metals on *in vitro* Pollen Germination and Tube Growth of Apricot (*Armenica vulgaris* Lam.) And Cherry (*Cerasus avium* L.)

Nazmi Gür and Aykut Topdemir

Department of Biology, Faculty of Science and Art, Firat University, 23169 Elazig, Turkey

Abstract: The aim of the study was to determine the influence of heavy metals (Cd, Cu, Hg and Pb) on the pollen germination and tube growth of Apricot and Cherry. This study demonstrated heavy metals led to a significant decrease pollen germination and tube growth of apricot and cherry. There was a reduction in pollen germination and tube elongation as metal concentrations increased. Cu had the highest toxic effect on pollen of apricot while Pb had the least effect. Cherry pollen germination and tube growth was mostly inhibited by Hg and Cd, but only weakly by Pb.

Key words: Pollen germination • Apricot • Cherry • Heavy metals

INTRODUCTION

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter our bodies via food, drinking water and air. As trace elements, some heavy metals are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning for human, animal, plant and microorganisms [1-6]. Over the last decades, environmental contamination with heavy metals has increased drastically

Heavy metals have recently received the attention of researchers all over the world, mainly due to their harmful effects on plant. The toxic effects of metals have also been intensively studied at the level of biochemical-physiological process such as photosynthesis [7], transpiration [8], enzyme activity [9] or metal accumulation in tissue [10].

Pollen germination and tube growth are used to detect biological activity of various environmental pollutants such as anthropogenic compounds of the atmosphere, heavy metals, pesticides, acid rain [11-17]. In this paper we report effects of the four heavy metals (Cd, Cu, Hg and Pb) on *in vitro* germination and tube growth of pollen in apricot and cherry plant, which has an economic importance.

MATERIALS AND METHODS

Pollens of apricot (*Armenica vulgaris* Lam) and cherry (*Cerasus avium* L.) flowers were obtained from

Bavunusagi village of Elazig which is situated east part of Turkey. Flowers were placed in polyethylene containers and the experiments were done without any delay in laboratory. Flowers from the same tree were used in every sequence of the experiment. Standard solution of each metal (30, 60, 90, 120 and 240 μ M) were prepared with distilled water from CuCl₂, PbCl₂, HgCl₂ and CdCl₂.

Pollens were germinated in Brewbaker and Kwack culture solution (culture medium). Heavy metal solutions and culture medium at the same volumes were used. Sterile 3 micro slides were prepared for each heavy metal solutions (2 for experiment group, 1 for control group). A 50 μ l culture solution was dripped to 2 various areas on each slide. 50 μ l heavy metal solutions for experiment groups and 50 μ l deionised water for control group (CG) were added onto slides. Pollens on anther were homogeneously cultivated by a sterile syringe into the culture medium under stereomicroscope. Petri dishes (15 cm diameter) with a moist filter paper lining the lower plate served as an improvised humidity chamber. Two glass rods were placed parallel at about 4 cm apart on the moist filter paper to facilitate the handling of the pollen cultures. Then, the petri dishes were settled in incubator at 22 $\pm$ 2°C. Each germination medium was fixed with 10 % ethyl alcohol after 3 hours and then lamella were closed. Germination percentages and tube lengths of pollens were determined under light microscope by method of Shivanna and Rangaswamy [18].

Mean and standard deviation were calculated for pollen germination and tube length in each treatment and control of each species. Multiple range tests (Duncan)

were used to determine significant differences among means ($P<0.05$) in either pollen germination or pollen tube length.

RESULTS

Tables 1 and 2 summarize the results for the effect of heavy metals (Cd, Cu, Hg and Pb) on pollen germination and tube growth of pollen in apricot and cherry plants.

Table 1: The effect of Cd, Pb, Hg and Cu on pollen germination and tube length in apricot (*Armenica vulgaris* Lam.)

CdCl ₂ .H ₂ O			PbCl ₂		
Treatment	Polen germ. (%)	Tube lenght (μ)	Treatment	Polen germ. (%)	Tube lenght (μ)
CG	72.80a	189.05a	CG	72.80a	189.05a
30	71.32b	111.70b	30	56.15b	152.10b
60	66.98c	73.20c	60	53.72c	106.05c
90	52.48d	65.20c	90	45.56d	95.20c
120	41.71e	32.25d	120	41.25e	68.95d
240	19.53f	12.65e	240	23.22f	31.25e
HgCl ₂			CuCl ₂		
Treatment	Polen germ. (%)	Tube lenght (μ)	Treatment	Polen germ. (%)	Tube lenght (μ)
CG	72.80a	189.05a	CG	72.80a	189.05a
30	60.70b	84.90b	30	50.00b	85.85b
60	50.99c	55.60c	60	32.94c	26.35c
90	34.38d	46.95c	90	30.68d	23.90c
120	19.58e	46.45c	120	28.84e	23.25c
240	11.41f	15.15d	240	23.12f	9.70d

Table 2: The effect of Cd, Cu, Hg and Pb on pollen germination and tube length in Cherry (*Cerasus avium* L.)

CdCl ₂ .H ₂ O			PbCl ₂		
Treatment	Polen germ. (%)	Tube lenght (μ)	Treatment	Polen germ. (%)	Tube lenght (μ)
CG	88.37a	181.70a	CG	88.37a	181.70a
30	68.84b	85.50b	30	63.69b	137.75b
60	26.84c	33.95c	60	40.93c	84.85c
90	22.68d	28.85c	90	35.68d	74.65c
120	19.09e	14.15d	120	30.79e	52.00d
240	11.65f	8.20d	240	15.32f	14.95d
HgCl ₂			CuCl ₂		
Treatment	Polen germ. (%)	Tube lenght (μ)	Treatment	Polen germ. (%)	Tube lenght (μ)
CG	88.37a	181.70a	CG	88.37a	181.70a
30	72.61b	54.90b	30	48.58b	104.70b
60	20.61c	35.30c	60	40.26c	47.15c
90	17.32d	30.50c	90	32.56d	42.55c
120	15.97e	19.15d	120	28.04e	24.05d
240	11.31f	5.60e	240	22.05f	7.95e

As can be seen, there was a reduction in pollen germination and tube elongation as metal concentrations increased. Apricot pollen germination was mostly inhibited as 90 μm as by Cu. 120 and 240 μm Hg was shown mostly inhibitor effect on apricot pollen germination. Cd was shown weakly affect on apricot pollen germination. Cherry pollen germination mostly inhibited by Hg, but only least by Pb. Significant differences ($P<0.05$) are observed between all heavy metal concentrations on apricot and cherry pollen germination.

Pollen tube elongation of both plants were inhibited by all heavy metal concentrations. Significant differences ($P<0.05$) on apricot and cherry pollen tube length are not observed between the treatments with all heavy metals at 60 and 90 μm. In addition, no statistically significant differences were found between 60, 90 and 120 μm concentrations of Hg and Cu on pollen tube length of apricot plants. Cu was shown mostly inhibitor effect on apricot pollen tube elongation, but only weakly by Pb. Pollen tube elongation in cherry was mostly inhibited by Hg and Cd.

DISCUSSION

Our study exhibited that Cu had the highest inhibition on apricot pollen germination and tube growth. As known, Cu is an essential role in many physiological pathways in plant, such as photosynthesis, respiration, carbohydrate distribution, protein metabolism etc. But it is a toxic to plant at high concentration. Phytotoxic effect of Cu on plant are higher than most of heavy metal contaminants. It was reported that toxic effect of Cu and other heavy metals on wheat growth was as follow: Cd>Cu>Ni>Zn>Pb>Cr [19]. Another studies demonstrated that Cu was the second most effective metal (within Hg, Cd, Co, Pb and Zn) on the seed germination, root elongation and coleoptile and hypocotyls growth in *Triticum aestivum* and *Cucumis sativus* [20]. Our observations are in accordance with the above reported results. Furthermore, another study has indicated that Cu, Ni and Hg most inhibited elements on pollen germination in tobacco plant [16]. Others have also reported that compounds with Hg among heavy metals prevent DNA replication and protein synthesis, causing mitotic anomalies and that Cu has similar effects, causing chromosome anomalies [21]. Our results lend credence to this report. However, different effect of heavy metal application has been also observed in our other experimental model in which we reported that Cu weakly affected on pollen germination and tube growth in quince

and plum plant [17]. This different observations may be due to different toleration of different plants to heavy metal stress.

Xiong and Peng [22] revealed that Cd at 2,51 mg/ml or higher inhibited pollen germination of five species and tube growth was inhibited at concentrations of 1,58 or higher while it stimulate more pollen tube growth below 1,58 mg/ml Cd. However, when this study compared with our study regarding the pollen tube growth stimulation under different conditions it was seen that pollen tube growth was not stimulated due to high concentrations of Cd. This result is consistent with our studies without Cd, which has an important role in the pollen tube growth but high Cd may not favor to the pollen tube growth as in our case.

Growth of apricot and cherry with and without Pb was compared under similar condition. Both species exhibited no remarkable differences in growth. Lead is common heavy metal pollutant, released from loaded gasoline and industrial processes and Pb has not be shown to be essential in plant metabolism and demonstrated to stimulate formation of free radicals and reactive oxygen species which can damage plant cells [23, 24]. In plants, it can adversely affect different processes such as germination, growth and photosynthesis etc [25]. In another study on the bioconcentrations of heavy metals in the plant structure, it has also been claimed that Cd, Cu, Hg and Ni are more toxic than Pb and Zn for plants [26]. An [27] indicate that Cu is more toxic than Pb to the plants in their study. Our results are in accordance with the above reported results.

Based on the results, we concluded that heavy metals was responsible for a decrease in the pollen germination and tube growth of the apricot and cherry plant. Lowered heavy metals concentration in the soil may be important to obtain more agricultural products.

REFERENCES

1. Nodelkoska, T.V. and P.M. Doran, 2000. Interactive effects of temperature and metal stress on the growth and some biochemical compounds in wheat seedlings. *Environ. Pollut.* 107, 315-320.
2. Donderski, W. and M.S. Brzezinska, 2005. The influence of heavy metals on activity of chitinases produced by planktonic, benthic and epiphytic bacteria. *Polish Journal of Environmental Studies*, 14: 851-859.
3. Tkaczuk, C., 2005. The effect of selected heavy metal ions on the growth and conidial germination of the arhid pathogenic fungus *Pandora neoaphidis* (Remaudiere et Hennbert) Humber. *Polish Journal of Environmental Studies*, 14: 897-902.
4. Topolska, K., K. Sawicka-kapusta and E. Cieslik, 2004. The effect of contamination of the Krakow region on heavy metals content in the organs of Bank Voles (*Clethrionomys glareolus*, Schreber, 1780). *Polish Journal of Environmental Studies*, 13: 103-109.
5. Nasiadek, M., T. Krawczyk and A. Sapota, 2005. Tissue levels of cadmium and trace elements in patients with myoma and uterine cancer. *Human Exp. Toxicol.*, 12: 623-630.
6. Rydzewska, A., 2001. A comprasion of concentrations of selected heavy metals in neoplastic and peri-neoplastic lung tissues in inhabitants from Wielkopolska and Upper Silesian industrial district in Poland. *Polish Journal of Environmental Studies*, 4: 289-292.
7. Kupper, H., I. Setlik, M. Spiller, F.C. Kupper and O. PRASIL, 2002. Heavy metal-induced inhibition of photosynthesis: targets of *in vivo* heavy metal chlorophyll formation. *Journal of Phycology*, 38: 429-441.
8. Pandey, N. and C.P. Sharma, 2002. Effects of heavy metals Cu^{+2} , Ni^{+2} and Cd^{+2} on growth and metabolism of cabbage. *Plant Science*, 163: 753-758.
9. Astolfi, T., S. Zuchi and C. Passera, 2005. Effect of cadmium on H^+ATPase activity of plasma membrane vesicles isolated from roots of different s-supplied maize (*Zea mays* L.) plant. *Plant Sciences*, 169: 361-368.
10. Palmieri, R.M., L.L. Pera, G.D. Bella and G. Dugo, 2005. Simultaneous determination of Cd(II), Cu(II), Pb(II) and Zn(II) by derivative stripping chronopotentiometry in *Pittosporum tobira* leaves: a measurement of local atmospheric pollution in Messina (Sicily, Italy). *Chemosphere*, 8: 1161-1168.
11. Wolters, J.H.B. and M.J.M. Matens, 1987. Effects of Air Pollutants on Pollen. *The Botanical Review*, 53: 372-414.
12. Kappler, R. and U. Kristen, 1987. Photometric quantification of *in vitro* pollen tube growth: a new method suited to determine the cytotoxicity of various environmental substances. *Environmental and Experimental Botany*, 27: 305-309.

13. Cox, R.M., 1983. Sensitivity of forest plant reproduction to long range transported air pollutants: in vitro sensitivity of pollen to stimulated acid rain. *New Phytol.*, 109: 193-201.
14. Munzuroglu, O. and N. Gur, 2000. The effects of Heavy Metals on the pollen germination and pollen tube growth of apples (*Malus sylvestris* Miller cv. Golden). *Turk J. Biol.*, 24: 677-684.
15. Abbott, J.D., B.D. Bruton and C.L. Patterson, 1991. Fungicidal inhibition of pollen germination and germ-tube elongation in muskmelon. *Hort. Sci.*, 26: 529-530.
16. Tuna, L., B. Burun, I. Yokas and E. Coban, 2002. The effects of heavy metals on pollen germination and pollen tube length in the tobacco plant. *Turk J. Biol.*, 26: 109-113.
17. Gur, N. and A. Topdemir, 2005. Effects of heavy Metals (Cd^{++} , Cu^{++} , Pb^{++} , Hg^{++}) on Pollen Germination and Tube Growth of Qince (*Cydonia oblonga* M.) and Plum (*Prunus domestica* L.). *Fresenius Environmental Bulletin*, 14: 36-39.
18. Shivanna, K.R. and N.S. Rangaswamy, 1992. *Pollen Biology Laboratory Manual*. Springer Verlag, Berlin, pp: 119.
19. Athar, R. and A. Masood, 2002. Heavy metal toxicity; effect on plant growth and metal uptake by wheat and on free living *Azotobacter*. *Water Air Soil Pollut.* 138: 165-180.
20. Munzuroglu, Ö. and H. Geckil, 2002. Effects of Metals on Seed Germination, Root Elongation and Coleoptile and Hypocotyl Growth in *Triticum aestivum* and *Cucumis sativus*. *Ach. Environ. Contam. Toxicol.*, 43: 203-213.
21. De Flora, S., C. Bennicelli and M. Bagnasco, 1994. Genotoxicity of mercury compounds. A review *Mutat Res.*, 317: 57-79.
22. Xiong, Z.T. and Y.H. Peng, 2001. Response of pollen germination and tube growth to cadmium with special reference to low concentration exposure. *Ecotoxicology and Environmental Safety*, 48: 51-55.
23. Halliwell, B. and M.C. Gutteridge, 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.*, 219: 1-14.
24. Dietz, K.J., M. Baier and U. Kramer, 1999. Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. In: Prasad, M.N.V., Hageneyer, J. (Eds.), *Heavy Metal Stress in Plant from Molecular Ecosystems*. Verlag/Springer, Berlin, Heidelberg and New York, pp: 73-98.
25. Xiong, Z.T., 1988. Lead uptake and effects on seed germination and plant growth in a Pb hyperaccumulator *Brassica pekinensis* Rupr. *Bulletin of Environmental Contamination and Toxicology*, 60: 285-291.
26. Raskin, I., S. Dushenkov and D. Salt, 1994. Bioconcentration of heavy metals by plants. *Current Opinion in Biotechnology*, 5: 285-290.
27. An, Y., 2006. Assessment of comparative toxicities of lead and copper using plant assay. *Chemosphere*, 62: 1359-1365.