Metallothionein gene expression in *Drosophila subobscura* indicates their different function in response to heavy metal exposures

Mina Rakić[1, 2], Mihailo Jelić[1], Marina Stamenković-Radak[1, 2], Aleksandra Patenković[2],
Marija Tanasković[2], Katarina Erić[2], Slobodan Davidović[2], Pavle Erić[2], Marija Savić Veselinović[1]

[1] University of Belgrade, Faculty of Biology, Studentski trg 16, Serbia
[2] University of Belgrade, Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia, Bulevar despota Stefana 142, Serbia

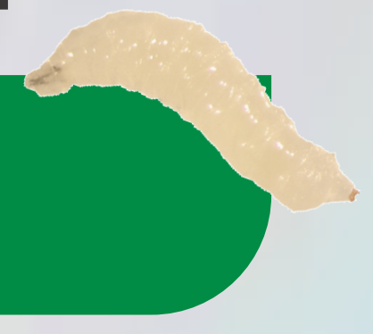
INTRODUCTION AND AIMS OF THE STUDY

Heavy metals are one of the most persistent pollutants that are introduced into the environment through industrialization, exploitation and utilization of heavy metal deposits. Due to their toxicity, heavy metals have severely negative effects on organisms, mainly through oxidative stress.

Metallothioneins (Mtn) are recognized as the first line of defense against heavy metal toxicity in the cell. Due to their ability to bind metal ions, these proteins play the main role in heavy metal detoxification and are present in all living species. Metallothioneins have a wide range of functions in the cell, including homeostasis of essential heavy metals, detoxification of non-essential heavy metals and scavenging of free radical species. There are six currently described metallothionein genes in *Drosophila*: ***MtnA***, ***MtnB***, ***MtnC***, ***MtnD***, ***MtnE*** and ***MtnF***.

The aim of the study was to investigate **the influence of heavy metals on the expression of six metallothionein genes** and to answer the following questions:

1. To which extent does the concentration of metals influence the *Mtn* gene expression?
2. How does the duration of exposure affect the expression?
3. Is there any relation of certain metallothioneins to specific heavy metals or a combination of them?



MATERIALS AND METHODS

The experiment consisted of **short term (24 hours)** and **long term (10 days)** exposure of *Drosophila subobscura* 3rd Instar larvae to **higher and lower concentrations of four heavy metals (zinc, copper, cadmium and lead) and their combination (cadmium and lead).** Larvae grown on standard *Drosophila* medium were included as a control group.

Treatment	Lower concentration (1)	Higher concentration (2)
Zinc (Zn)	2.5mM	5mM
Copper (Cu)	0.5mM	1mM
Cadmium (Cd)	0.05mM	0.1mM
Lead (Pb)	0.25mM	2.5mM
Cadmium and lead (Cd+Pb)	0.05mM and 0.25mM	0.1mM and 2.5mM
Control group	/	/

RNA isolation was performed with TRIzol®, after which it was converted to cDNA. Expression of *Mtn* genes in all treatment groups compared to the control group was quantified with RT-qPCR using SYBR® Green (QuantStudio™ 3 System).

Acknowledgements

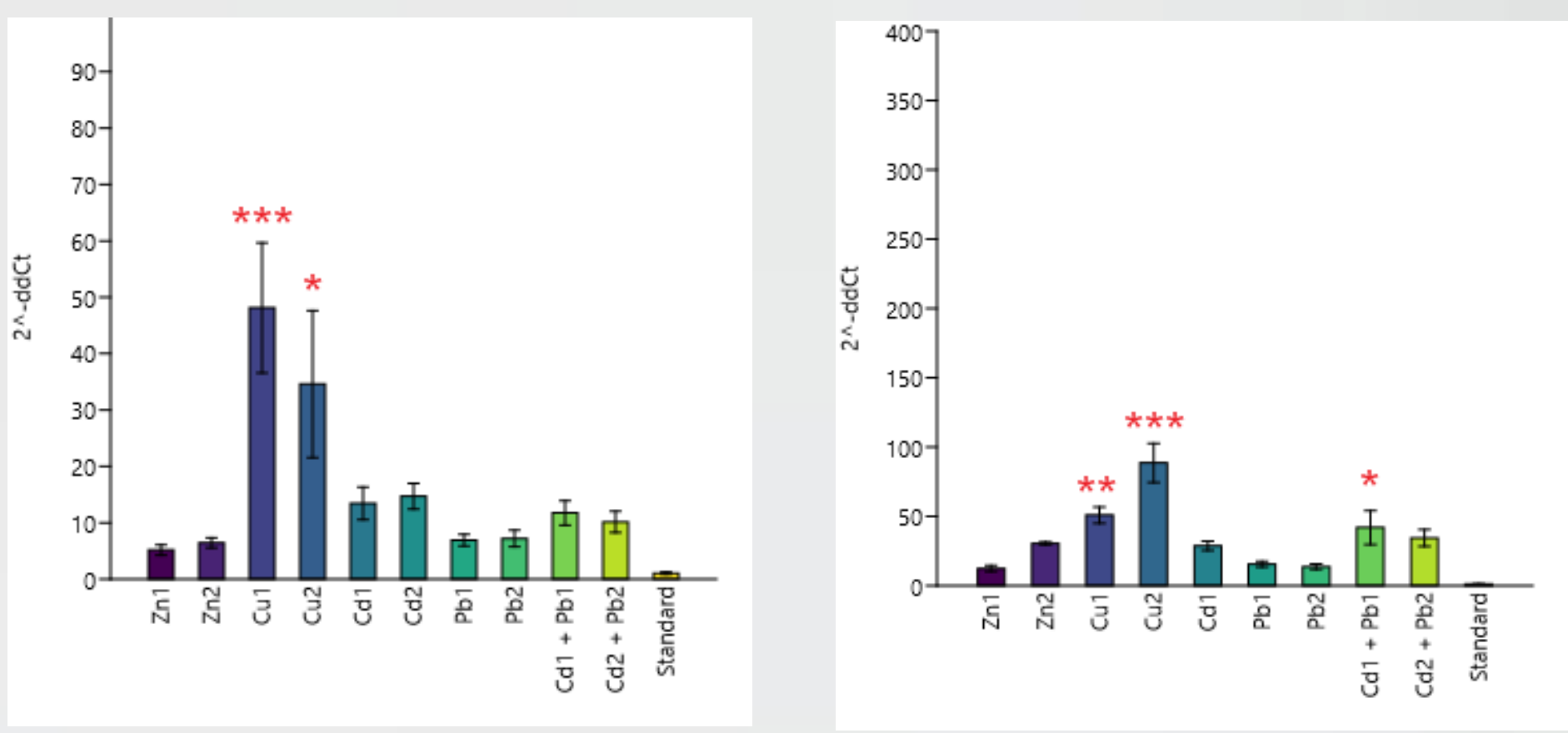
Mina Rakić is a holder of Ministry of Education, Science and Technological Development of Republic of Serbia PhD student scholarship.

RESULTS

Short term — Long term

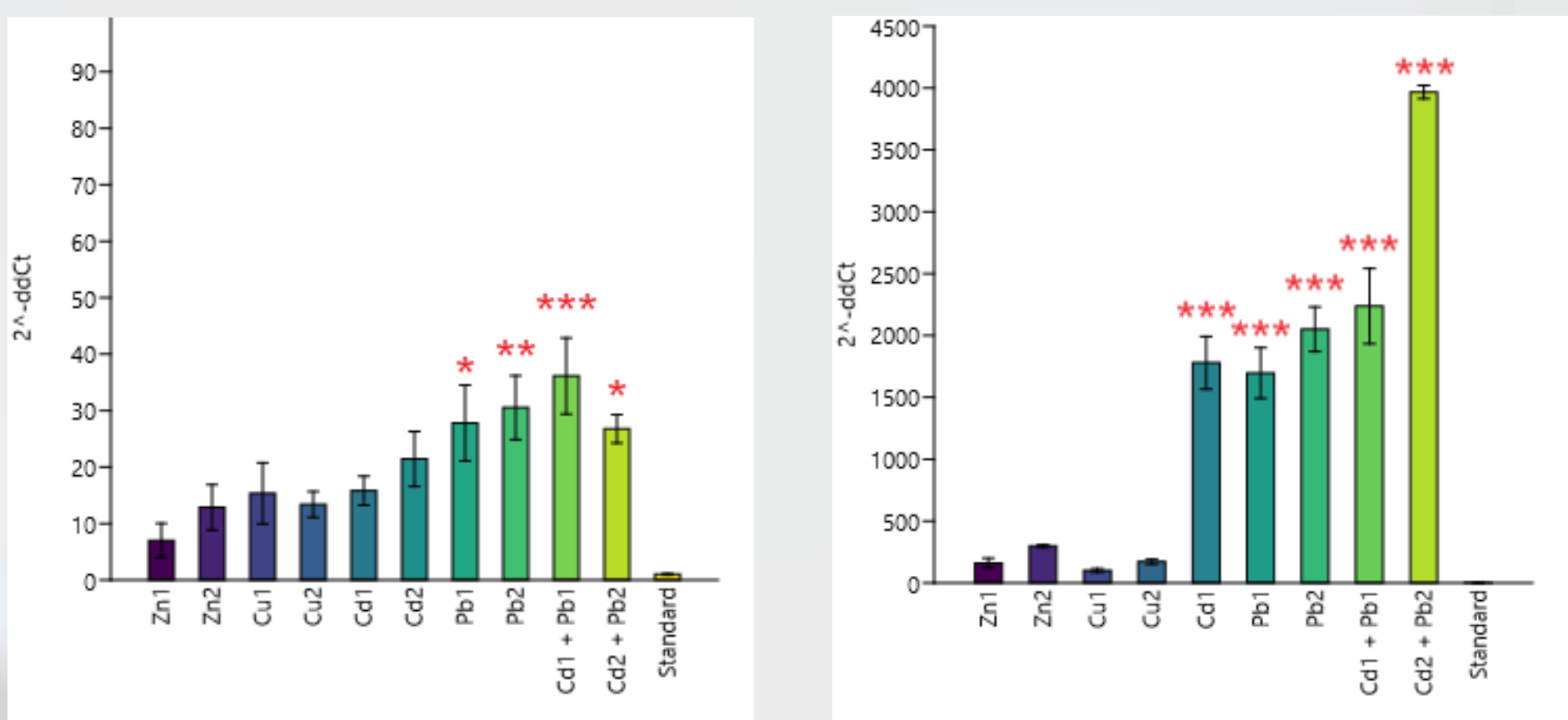
MtnA

Exposure	Source	SS	df	MS	F	p
Short term	Sample	6016.653	10	601.665	6.68458	0.000105
	Error	1980.176	22	90.008		
Long term	Sample	16882.62	9	1875.85	14.1544	0.000001
	Error	2650.56	20	132.53		



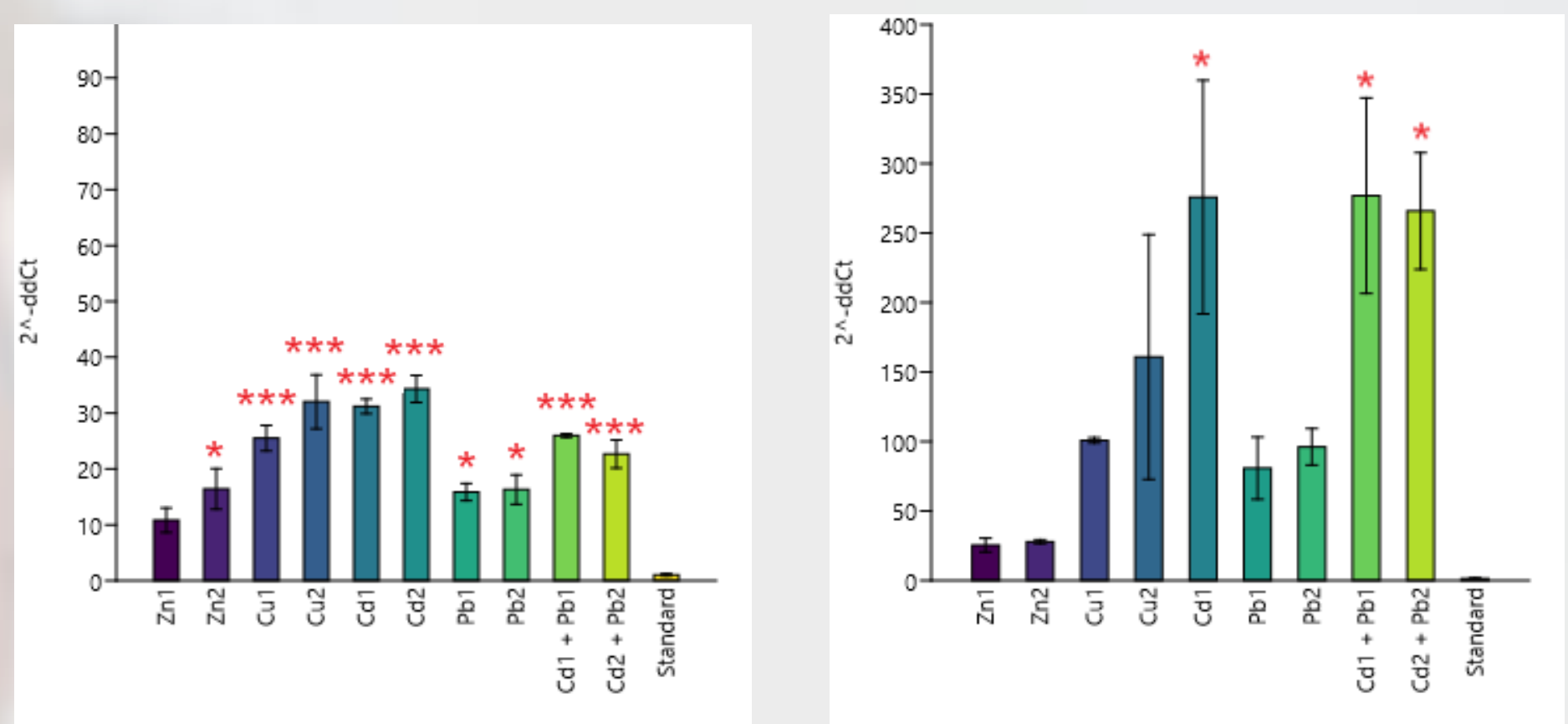
MtnB

Exposure	Source	SS	df	MS	F	p
Short term	Sample	3390.31	10	339.03	5.6670	0.000343
	Error	1316.15	22	59.83		
Long term	Sample	46854855	9	5206095	79.8214	0.000000
	Error	1304436	20	65222		



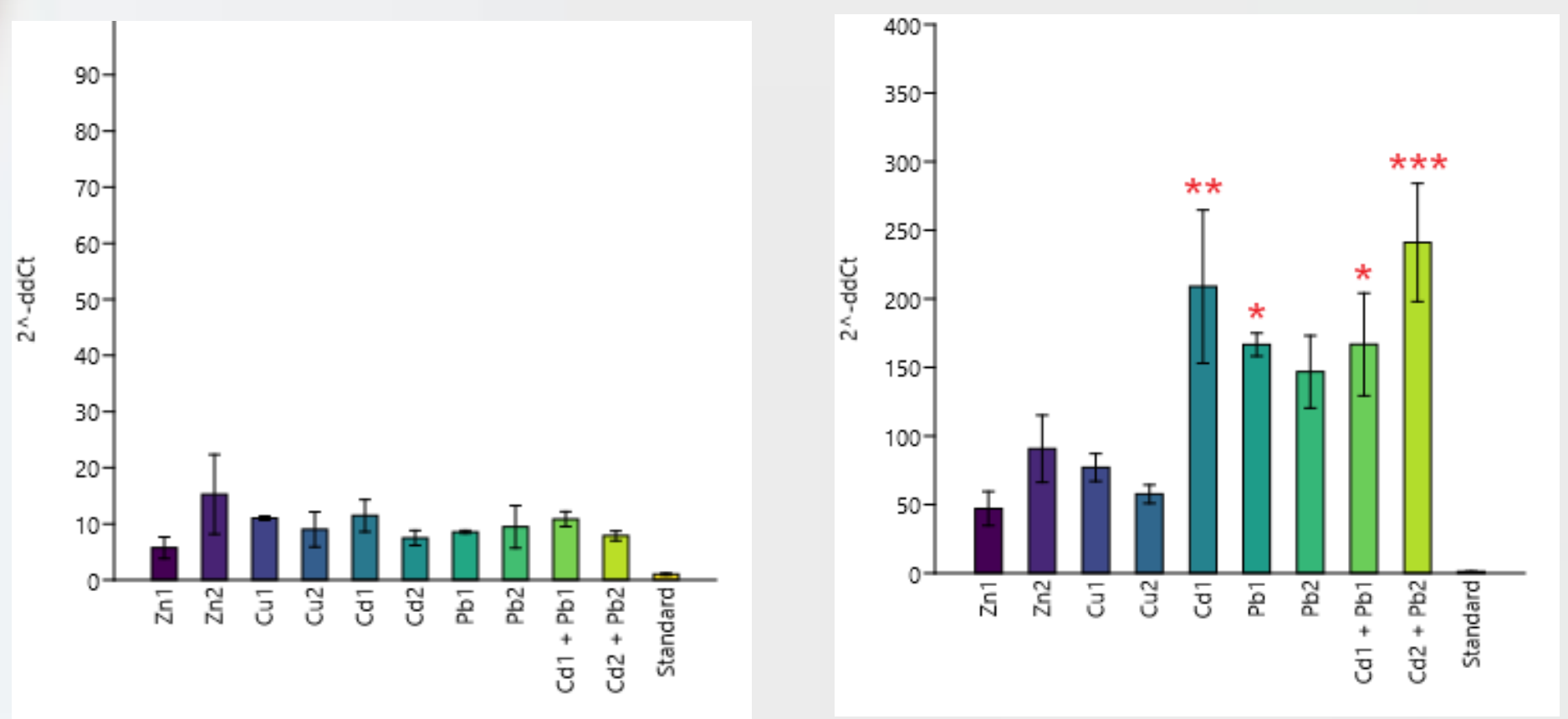
MtnC

Exposure	Source	SS	df	MS	F	p
Short term	Sample	3067.46	10	306.75	16.0513	0.000000
	Error	420.43	22	19.11		
Long term	Sample	313709.7	9	34856.6	5.22908	0.001012
	Error	133318.5	20	6665.9		



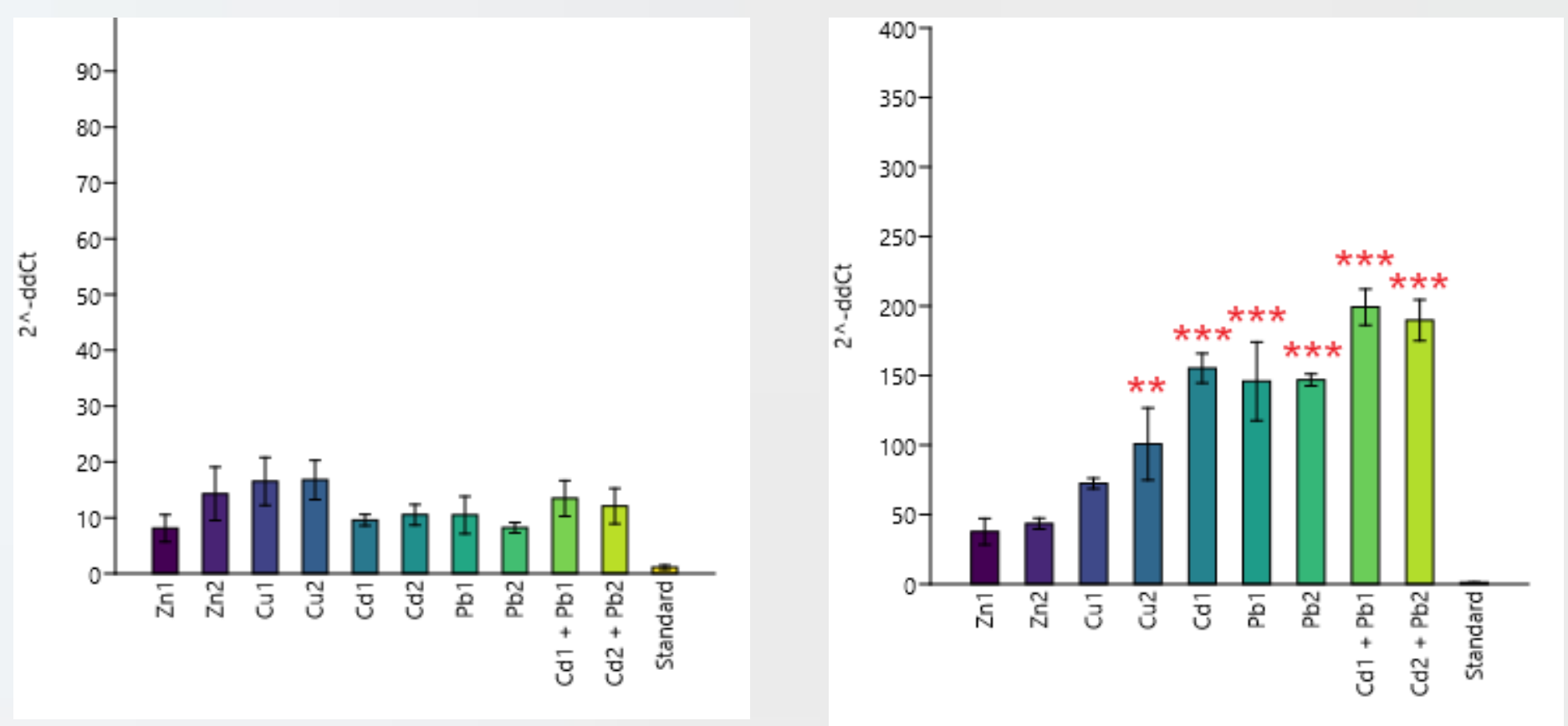
MtnD

Exposure	Source	SS	df	MS	F	p
Short term	Sample	391.671	10	39.167	1.5793	0.178213
	Error	545.614	22	24.801		
Long term	Sample	161005.3	9	17889.5	7.4092	0.000103
	Error	48290.2	20	2414.5		



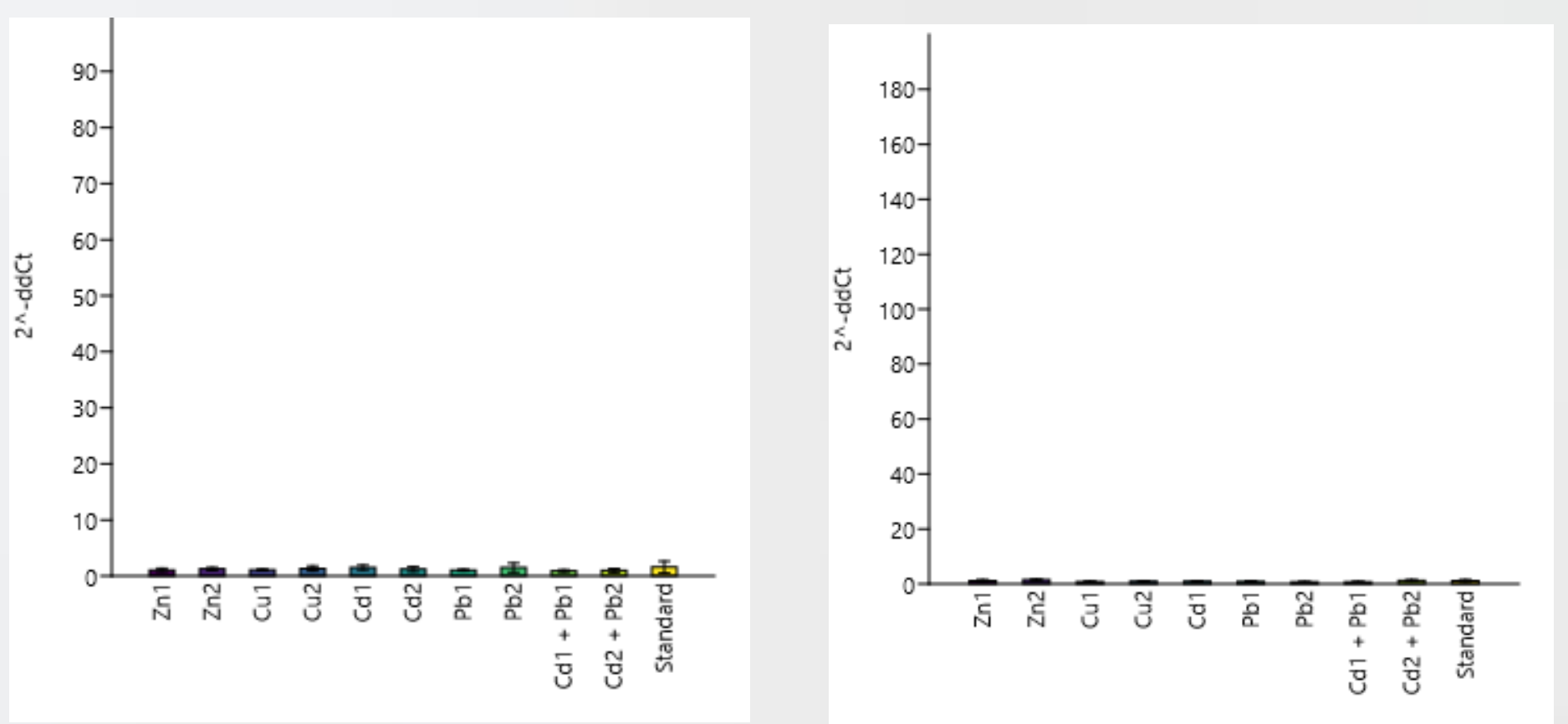
MtnE

Exposure	Source	SS	df	MS	F	p
Short term	Sample	594.202	10	59.420	2.2603	0.053291
	Error	578.362	22	26.289		
Long term	Sample	126019.1	9	14002.1	22.1465	0.000000
	Error	12645.0	20	632.3		



MtnF

Exposure	Source	SS	df	MS	F	p
Short term	Sample	1.59973	10	0.15997	0.22147	0.991222
	Error	15.89138	22	0.72234		
Long term	Sample	1.88623	9	0.20958	1.5006	0.214600
	Error	2.79329	20	0.13966		



Asterisk symbols (*) designate statistical significance of differences in treated samples relative to control group.
*** $p<0.001$, ** $p<0.01$, * $p<0.5$

CONCLUSIONS

- The present study confirmed that the presence of heavy metals in the cell induces the expression of all known *Mtn* genes, except *MtnF*.
- Generally, there was no difference between the lower and higher concentration of each metal, except for *MtnA* and *MtnE* in copper treatments.
- The longer time of the exposure predominantly influenced a significant increase in the expression of almost all *Mtn* genes.
- Expression levels of metallothionein genes in analyzed treatments indicate *MtnA* specificity to copper, and *MtnB* and *MtnD* specificity to cadmium and lead, both separately and conjointly. Other metallothioneins, *MtnC* and *MtnE,* did not show specificity to a certain metal, but their expression was elevated in multiple experimental treatments.
- The newly discovered *MtnF* showed no specificity for any of the heavy metals included in this research. Thus, his function has yet to be determined.