

Redox and essential metal status in the brain of Wistar rats acutely exposed to a cadmium and lead mixture

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Most Pb and Cd neurotoxicity studies investigate exposure to either of the toxic metals alone, while data on co-exposure are scarce. The aim of our study was to fill that gap by investigating acute combined effects of Pb and Cd on redox and essential metal status in the brain of Wistar rats. Animals were randomised in four groups of six to eight rats, which received 15 or 30 mg/kg of Cd, 150 mg/kg of Pb, or 150 mg/kg of Pb + 15 mg/kg of Cd by gavage. The fifth, control, group received distilled water only. Co-treatment with Pb and Cd induced significant increase in malondialdehyde (MDA) and thiobarbituric acid-reactive substances (TBARS) compared to control and groups receiving either metal alone. This is of special importance, as MDA presence in the brain has been implicated in many neurodegenerative disorders. The groups did not significantly differ in Zn, Cu, Mn, and Fe brain levels. Our findings highlight the importance of metal mixture studies. Neurotoxicity assessments of single chemicals do not provide a real insight into exposure to mixtures in real life. Further research should look into interactions between these metals to reveal complex molecular mechanisms of their neurotoxicity.

KEY WORDS: Cd; Cu; Fe; MDA; Mn; neurotoxicity; oxidative stress; Pb; TBARS; Zn

Lead has been found to produce a wide range of toxic effects in humans and animals, most often targeting the haematopoietic and nervous systems. Its neurotoxicity is often associated with behavioural abnormalities, learning impairment, anxiety, depression, and headache (1–3). Low-level Pb exposure has been shown to impair cognitive function and lead to maladaptive behaviour (4). Children are particularly sensitive as they develop, absorb more Pb through the digestive tract, and are more exposed to it through dust and soil (5). Exposure at early age can later lead to intellectual deficits, hyperactivity, and functional impairment (6–8).

There is also a growing evidence of the toxic effects of Cd on the nervous system and of an association between Cd exposure and neurodegenerative diseases (9, 10). Its neurotoxicity is manifested through symptoms such as vertigo, headache, learning disabilities, Parkinsonism-like symptoms, and impaired vasomotor function (10, 11).

Investigations aiming to explain the mechanisms of their toxicity are numerous. Both metals affect essential metals, such as zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), and magnesium (Mg) status in various tissues. This

can be attributed to their similarities and competition for the same biological molecules (12–19). Homeostasis of essential metals is crucial for normal physiological processes in all systems and organs, including the central nervous system. In addition, essential metals are components of antioxidant enzymes that protect the body from free radicals and are therefore closely associated with oxidant/antioxidant processes (20).

One of the suggested mechanisms of their toxicity, oxidative stress, has been recognised as pivotal in the pathogenesis of many nervous system diseases (3, 21). It is known that exposure to Pb and/or Cd could induce oxidative stress in cells increasing production of prooxidants and/or decreasing antioxidant safety mechanisms in organism, which results in lipid peroxidation, DNA damage, and depletion of sulphhydryl (SH) groups (22–25). Studies have shown that Cd-induced production of reactive oxygen species (ROS) functions as an upstream signal to mediate mitophagy in mice brain, contributing to Cd neurotoxicity (26). The results of a study on a mouse model (27) evidenced a correlation between 5-aminolevulinic acid and oxidative stress in mice brain, which implies that oxidative stress might be the main mechanism involved in Pb neurotoxicity.

The fact that humans are exposed to metal combinations rather than a single metal (28) could be of vast importance

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for understanding their toxic effects. The last few decades have seen growing evidence that toxic effects of combined exposure – metal plus metal (28–30) or metal plus chemical (such as persistent organic pollutants) (31–33) – do not correspond to those of a single metal. Similar target organs and mechanisms of toxicity raise concerns about synergistic interactions between Pb and Cd (34, 35). However, animal studies examining the effects of Pb and Cd co-exposure on the central nervous system (CNS) are rather scarce. An acute oral toxicity study (34) showed that co-exposure to Pb and Cd had an additive effect. Other research indicates that Cd could antagonise Pb-induced effects on movement and vertical activity in rats (12), but most of it is still focused on single metals.

The aim of our preliminary study was to shed more light on neurotoxic effects of combined exposure to Pb and Cd by measuring oxidative and essential metal parameters in the brain of Wistar rats. We hoped to get a glimpse of the mechanism(s) of their combined action and interactions at doses that are able to induce toxic effects.

MATERIALS AND METHODS

Chemicals

Lead acetate ($\text{Pb}(\text{CH}_3\text{COOH})_2 \cdot 3\text{H}_2\text{O}$, Centrohem, Stara Pazova, Serbia) and cadmium chloride ($\text{CdCl}_2 \cdot x\text{H}_2\text{O}$, Merck, Darmstadt, Germany) solutions were used for rat treatment. Standard solutions of Cd, Pb, Zn, Cu, Mn, and Fe used for calibration were purchased from Merck. Nitric acid (65 % HNO_3 , Merck) and hydrogen peroxide (30 % H_2O_2 , Sigma-Aldrich, Steinheim, Germany) were used for wet digestion of the tissues. All chemicals and reagents for biochemical assays were purchased from Sigma-Aldrich.

Animals and treatment

The experiment was performed on thirty-three 8–12-week-old male albino Wistar rats weighing 250–350 g [body weight (bw)]. They were purchased from the Military Medical Academy, Belgrade, Serbia. After acclimatisation to the new surroundings, the animals were randomised into five groups: one control and four experimental. Each group counted between 6 and 8 animals. The control group received distilled water. Other groups received aqueous solutions of Pb and/or Cd at acute doses of 15 or 30 mg/kg bw of Cd alone (Cd15 and Cd30 group, respectively) or 150 mg/kg bw of Pb alone (Pb150 group), or their combination (150 mg/kg bw of Pb plus 15 mg/kg bw of Cd; Cd15+Pb150 group) by oral gavage. We chose doses higher than those environmental, as we wanted to obtain observable toxic effects, based on previous reports (18, 30, 36).

All experimental groups had free access to standard pellet food (Veterinary Institute Subotica, Subotica, Serbia) and drinking water. All animals were sacrificed with ketamine administered intraperitoneally 24 h after

treatment. The brain was removed, washed with ice-cold saline (0.9 % NaCl), and frozen to -80°C until metal and oxidative stress analysis. All experimental procedures were approved by the Ethics Committee on Animal Experimentation of the University of Belgrade Faculty of Pharmacy (No. 323-07-11822/2018-05). The study was carried out in accordance with the Animal Welfare Act of the Republic of Serbia (37).

Metal analysis

Brain samples for metal analysis were digested with CHNO_3 (7 mL) and H_2O_2 (1 mL) in a microwave digestion system (START D, SK-10T, Milestone Srl, Sorisole, Italy) according to manufacturer's recommendations. Then we added 25 mL of deionised water to the digested samples. For Pb and Cd measurement we used graphite furnace atomic absorption spectroscopy (GFAAS, with graphite tube atomizer GTA 120 and 200 Series AA; Agilent Technologies, Santa Clara, CA, USA) with 0.5 % $\text{NH}_4\text{H}_2\text{PO}_4$ as matrix modifier, while for Zn, Cu, Mn, and Fe we used flame atomic absorption spectrometry (FAAS, 240FS AA, Agilent Technologies). The accuracy of AAS analyses was validated with standard reference material (SRM 1577c – bovine liver, National Institute of Standards and Technology, Gaithersburg, MD, USA).

Redox status analysis

Brain samples for redox status analysis were homogenised with 0.1 mol/L phosphate buffer (pH 7.4) in the 1:9 weight-to-volume ratio using a T10 basic Ultra-Turrax homogeniser (IKA, Germany). One part of the homogenate was kept at -80°C to measure thiobarbituric acid-reactive substances (TBARS), and the rest was first centrifuged in a cooling centrifuge at $800\times g$ for 10 min and then at $9500\times g$ for 20 min to obtain a postmitochondrial supernatant. Postmitochondrial supernatant was also stored at -80°C until biochemical analysis.

Superoxide anion ($\text{O}_2^{\cdot-}$) was measured as described by Auclair and Voisin (38) by reducing yellow-stained nitroblue tetrazolium (NBT) to blue formazan. The rate of superoxide anion generation was measured as the rate of formation of reduced NBT and expressed as $\mu\text{mol}/\text{min}/\text{g}$ of protein.

Total oxidative status (TOS) was measured with a spectrophotometric method optimised by Erel (39) and based on oxidant capacity to oxidise ferrous ion–o-dianisidine complex to ferric ion, which forms a coloured complex with xylenol-orange in an acidic medium. Absorbance was measured at 560 nm and reflects the total content of oxidants in the sample, such as H_2O_2 and lipid hydroperoxides. Calibration was performed with hydrogen peroxide, and results were expressed in μmol of H_2O_2 equivalent per g of protein.

The spectrophotometric method we used for measuring total antioxidative status (TAS) has also been described by

Erel (40). The method is based on oxidation of 2,2'-azinobis(3-ethylbenzo-thiazoline-6-sulfonate) (ABTS) to colour ABTS⁺ radical cation. Discoloration caused by sample antioxidants was measured at 660 nm. The results were expressed in μmol of Trolox equivalent per g protein.

Superoxide-dismutase (SOD) activity was performed according to the modified method of Misra and Fridovich (41), based on the ability of the enzyme to inhibit auto-oxidation of epinephrine in alkaline medium (pH 10.2). The activity of this enzyme is expressed in relative units, which are obtained by measuring the absorbance of the resulting red oxidation product at 480 nm and 25 °C. SOD activity in the sample is calculated as the percentage of inhibition of epinephrine auto-oxidation (U/g).

Levels of total SH-groups were measured as described by Ellman (42), using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) as reagent in alkaline medium (pH 9.0). Absorbance of yellow reaction product (dianion of 5-thio-2-nitrobenzoic acid) was measured at 412 nm.

MDA was determined as TBARS, according to the method described by Girotti (43). It is based on the absorption maximum of MDA and other TBARS with thiobarbituric acid at 535 nm. Absorbance was measured after heating samples and reagents at 100 °C for 5 min, then ice-cooling them, and then centrifuging them at 10,000 \times g and + 4 °C for 10 min. Calibration was performed with MDA solutions.

Protein concentration in brain tissue was determined with the method described by Bradford (44), in which bovine serum albumin is used as the standard. This is a spectrophotometric method based on the binding of Coomassie Brilliant Blue G-250 to a protein molecule in a sample, which causes the absorption maximum to shift from 465 to 595 nm.

Advanced oxidation protein products (AOPP) were determined with a spectrophotometric method described by Witko et al. (45), using glacial acetic acid, 1.16 mol/L potassium-iodide solution, and phosphate buffer (pH 7.4). The absorbance was measured at 340 nm. The reaction was calibrated with chloramine-T, and results expressed as μmol of chloramine-T equivalent per g of protein.

Oxidative stress index (OSI) as an indicator of the degree of oxidative stress was calculated as a ratio between TOS and TAS and presented in arbitrary units (40). All spectrophotometric methods run on ILAB 300 Plus analyser (Instrumentation Laboratory, Milan, Italy), with the exception of TBARS determination, run on a Cary60 UV-VIS spectrophotometer (Agilent Technologies).

Statistical analysis

For all statistical analyses we used the IBM SPSS Statistics version 18 for Windows (IBM, Armonk, NY, USA). After normality and homogeneity of variance check, differences between the groups were analysed with one-way ANOVA or Kruskal-Wallis test, followed by LSD or the

Mann-Whitney *U* test, respectively, with $P < 0.05$ set as the level of significance. All values are expressed as mean \pm standard deviation or median and range. Graphs were made using the GraphPad Prism 5 software (GraphPad software, Inc., La Jolla, CA, USA).

RESULTS

Pb and Cd levels in brain

The levels of Pb (Figure 1) and Cd (Figure 2) in rat brains are expressed as ng of metal per g of wet tissue. Lead brain levels were significantly higher in the Pb150 and Cd15+Pb150 groups than controls ($P < 0.001$), but the two groups did not differ significantly. Cd levels were significantly higher in the Cd15 and Cd30 groups compared to control ($P < 0.01$) and in the Cd30 group compared to Cd15. The Cd15+Pb150 group, however, had significantly lower Cd brain levels than the Cd15 group.

Essential metals

There were no significant differences in Zn, Cu, Mn, and Fe brain levels between the groups (Table 1).

Oxidative stress parameters

Table 2 shows the effects of Pb and/or Cd treatment on oxidative stress parameters in rat brain. MDA and TBARS levels were significantly higher in the Cd30 group compared to control ($P < 0.01$) and the Cd15 group ($P < 0.05$). Co-treatment significantly increased MDA and TBARS compared to control ($P < 0.001$) and either metal administered alone (Cd15 $P < 0.001$, Pb150 $P < 0.05$). However, we

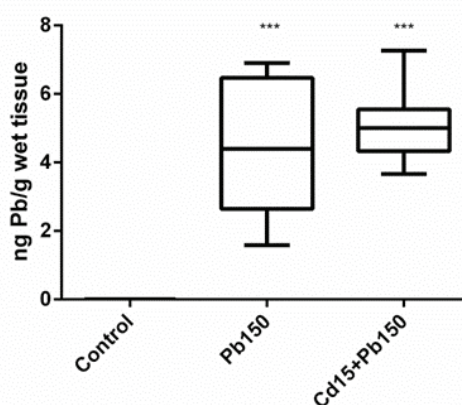


Figure 1 Brain Pb levels in Wistar rats after acute exposure to Pb alone and in combination with Cd ($N=6-8$ per group). *** $P < 0.001$ compared to control (one-way ANOVA followed by LSD). Pb150 – the group treated with Pb in the dose of 150 mg/kg bw; Cd15+Pb150 – the group treated with a combination of 150 mg/kg Pb and 15 mg/kg of Cd. Boxes represent interquartile ranges (25–75th percentile), the line within the box median values, and the ends of the whiskers minimum and maximum values within the group

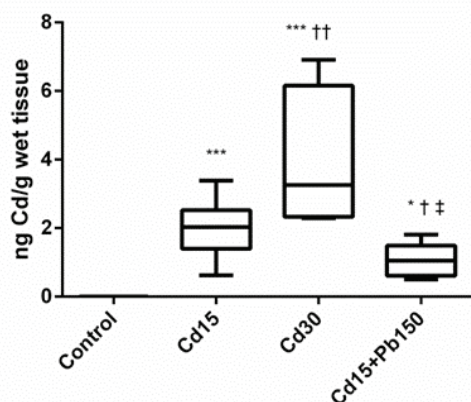


Figure 2 Brain Cd levels in Wistar rats after acute exposure to Cd alone and in combination with Pb ($N=6-8$ per group). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to control; † $P<0.05$, †† $P<0.01$ compared to Cd15; ‡ $p<0.05$, compared to Cd30 (one-way ANOVA followed by LSD). Cd15 – the group treated with Cd in the dose of 15 mg/kg bw; Cd30 – the group treated with Cd in the dose of 30 mg/kg bw; Cd15+Pb150 – the group treated with a combination of 150 mg/kg Pb and 15 mg/kg Cd. Boxes represent interquartile ranges (25–75th percentile), the line within the box median value, and the ends of the whiskers minimum and maximum values within the group

observed no significant changes in AOPP, O_2^- , SOD, and SH. Exposure to toxic metals resulted in significant increase in TAS in all treated groups compared to control, but TOS did not change significantly. Consequently, OSI was significantly lower in the Cd15 ($P<0.05$), Cd30 ($P<0.01$), and Cd15+Pb150 group ($P<0.05$) compared to control.

DISCUSSION

Our findings confirm that both metals pass the blood-brain barrier (BBB) and accumulate in the brain. As we expected, Cd levels were almost two times higher in the brains of the Cd30 group than the Cd15 group.

However, combined administration with 15 mg/kg bw of Cd led to lower Cd brain levels than administration of Cd alone in the same dose. At the same time, Pb in the

combination group was higher, but not significantly, than when administered alone in the same dose. These findings point to toxicokinetic interactions between Pb and Cd – most likely to a competition for metal transporters in the BBB (46, 47).

Increased brain Pb levels were also observed in a chronic interaction study (48), but these were nearly five times higher than control. The main pathway of Pb crossing the BBB is that it mimics and substitutes Ca ions. Another is divalent metal ion transporter (DMT1) (47). One study showed that combined exposure to Pb and Cd synergistically increased the expression of this transporter in the CNS of developing rats treated for 45 days (49). Other *in vivo* studies have also shown higher Pb levels after co-exposure with other metals. In a recent eight-day study (50) this was attributed to high Pb affinity for specific proteins in the brain, which implies that Pb more efficiently binds to protein target sites than Cd.

Even though exposure to Pb and Cd has been shown to interfere with essential metals in many tissues (15, 30, 51–53), our findings fail to confirm this in the rat brain. No visible interference in the brain was also reported by other similar studies (54, 55). This suggests that changes in brain essential metals are not the major mechanism of Pb and Cd neurotoxicity after acute exposure. However, this hypothesis needs to be investigated further.

The most important finding of our study is that lipid peroxidation in the rat brain observed after acute co-administration of Pb and Cd was significantly higher than the one induced by treatment with either metal alone. This points to additive or even synergistic neurotoxicity of these metals. However, the limitation of our study design is that it cannot inform about the type of toxicodynamic interactions observed in the rat brains.

We were surprised to find that Pb or the lower Cd dose (15 mg/kg bw) did not change MDA and TBARS brain levels when given alone, as many other studies reported this increase with Pb or Cd (9, 23, 54, 56–60). MDA and TBARS significantly increased only at the Cd dose of 30 mg/kg bw, which suggests that the dose can have an important role in redox disturbances in the brain. The

Table 1 Brain Zn, Cu, Mn, and Fe levels in Wistar rats after acute exposure to Pb and/or Cd ($N=6-8$ per group)

Essential metals	Groups				
	Control ($\mu\text{g/g}$)	Cd15 ($\mu\text{g/g}$)	Cd30 ($\mu\text{g/g}$)	Pb150 ($\mu\text{g/g}$)	Cd15+Pb150 ($\mu\text{g/g}$)
Zn	11.12 10.69–15.10	12.35 11.39–13.71	12.68 12.12–15.66	12.74 10.87–14.89	14.84 11.16–15.40
Cu	1.99±0.41	2.24±0.27	2.17±0.23	2.26±0.26	1.88±0.22
Mn	1.93±0.44	1.38±0.24	1.72±0.34	1.65±0.40	1.90±0.51
Fe	22.42 15.52–26.35	18.12 16.08–30.81	19.31 15.85–33.61	21.19 19.12–22.86	16.87 13.44–24.93

Values are represented as means \pm standard deviations or medians and ranges. Cd15 – the group treated with Cd in the dose of 15 mg/kg bw; Cd30 – the group treated with Cd in the dose of 30 mg/kg bw; Pb150 – the group treated with Pb in the dose of 150 mg/kg bw; Cd15+Pb150 – the group treated with a combination of 150 mg/kg Pb and 15 mg/kg Cd

Table 2 Effects of acute Pb and/or Cd treatment on oxidative stress parameters in the brain of Wistar rats (N=6–8 per group)

Parameter	Groups				
	Control	Cd15	Cd30	Pb150	Cd15+Pb150
TBARS ($\mu\text{mol}/\text{mg}$ protein)	161.36 \pm 47.74	175.25 \pm 43.23	309.64\pm106.78 ^{***†}	230.95 \pm 47.82	390.57\pm95.15 ^{***††§}
AOPP ($\mu\text{mol}/\text{min}/\text{g}$ protein)	27.57 \pm 6.28	24.76 \pm 4.90	27.68 \pm 6.43	25.4 \pm 6.18	24.83 \pm 8.83
O₂⁻ ($\mu\text{mol}/\text{min}/\text{g}$ protein)	91.74 \pm 6.65	99.78 \pm 17.30	91.38 \pm 18.94	95.13 \pm 11.82	90.24 \pm 7.92
SOD (U/g)	57.35 \pm 10.63	47.52 \pm 5.03	57.73 \pm 18.82	58.29 \pm 5.87	49.32 \pm 10.92
SH (mmol/g protein)	0.14 \pm 0.03	0.15 \pm 0.02	0.13 \pm 0.02	0.15 \pm 0.02	0.12 \pm 0.02
TAS (μmol Trolox Equiv. /g protein)	130.00 87.70–188.28	268.66* 232.28–368.51	300.07 ^{***} 274.71–346.72	222.61 ^{**‡} 108.48–336.72	264.91 ^{**} 221.20–274.67
TOS (μmol H ₂ O ₂ Equiv. /g protein)	2.36 \pm 0.68	2.46 \pm 0.53	2.05 \pm 0.54	2.77 \pm 0.65	2.89 \pm 1.89
OSI	0.0162 \pm 0.0078	0.0106\pm0.0049*	0.0068\pm0.0016**	0.0149\pm0.0087*	0.0099\pm0.0037*

Values are represented as means \pm standard deviations or medians and ranges. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to control; † $P < 0.05$, †† $P < 0.01$ compared to Cd15; ‡ $P < 0.05$, ‡‡ $P < 0.01$ compared to Cd30; § $P < 0.05$ compared to Pb150 (one-way ANOVA or Kruskal-Wallis test followed by LSD or the Mann-Whitney U test). Cd15 – the group treated with Cd in the dose of 15 mg/kg bw; Cd30 – the group treated with Cd in the dose of 30 mg/kg bw; Pb150 – the group treated with Pb in the dose of 150 mg/kg bw; Cd15+Pb150 – the group treated with a combination of 150 mg/kg Pb and 15 mg/kg Cd; TBARS – thiobarbituric acid-reactive substances; O₂⁻ – superoxide anion; AOPP – advanced oxidation protein products level; SH – total thiol groups; SOD – superoxide dismutase; TAS – total antioxidant status; TOS – total oxidative status; OSI – oxidative stress index (TOS/TAS)

question remains, however, to what extent does Cd contribute to MDA and TBARS production when combined with Pb, considering that the Cd brain levels in the Cd15+Pb150 group were lower than in the Cd15 or Cd30 groups.

As for antioxidative defences, acute exposure to Pb and/or Cd did not affect SOD. Significantly higher TAS observed in our study, however, implies that these metals may have induced higher production or redistribution of antioxidants, as reported elsewhere (61), which in turn restored oxidant/antioxidant balance to normal. However, this may not be the case with prolonged exposure to Pb and Cd (as opposed to acute effects we measured), as it seems to deplete antioxidant enzyme defences and tip the balance toward a more oxidative state or stress (23, 24, 54).

We therefore believe that changes in MDA and TBARS levels might be related to direct interaction of Pb and Cd with membrane unsaturated lipids as a mechanism leading to oxidative damage (62, 63).

CONCLUSION

Our findings confirm concerns that single exposure studies can underestimate the toxic effect of Pb and Cd. Further subacute and subchronic experimental studies are warranted to better understand the complex toxicokinetic and toxicodynamic mechanisms of neurotoxic effects and interactions between Pb and Cd in mixtures.

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Conflicts of interest

None to declare.

REFERENCES

- Sanders T, Liu Y, Buchner V, Tchounwou PB. Neurotoxic effects and biomarkers of lead exposure: A review. *Rev Environ Health* 2009;24:15–45. doi: 10.1515/REVEH.2009.24.1.15
- Mason LH, Harp JP, Han DY. Pb neurotoxicity: neuropsychological effects of lead toxicity. *Biomed Res Int* 2014;2014:840547. doi: 10.1155/2014/840547
- Orisakwe OE. The role of lead and cadmium in psychiatry. *N Am J Med Sci* 2014;6:370–6. doi: 10.4103/1947-2714.139283
- Rocha A, Trujillo KA. Neurotoxicity of low-level lead exposure: history, mechanisms of action, and behavioral effects in humans and preclinical models. *Neurotoxicology* 2019;73:58–80. doi: 10.1016/j.neuro.2019.02.021
- Safarik AM, McGregor E, Whitfield Aslund ML, Cheung PH, Pinsent C, Jackson BJ, Hair AT, Lee M, Sigal EA. The influence of lead content in drinking water, household dust, soil, and paint on blood lead levels of children in Flin Flon, Manitoba and Creighton, Saskatchewan. *Sci Total Environ* 2017;593–594:202–10. doi: 10.1016/j.scitotenv.2017.03.141

6. Sanders T, Liu Y, Buchner V, Tchounwou PB. Neurotoxic effects and biomarkers of lead exposure: A review. *Rev Environ Health* 2009;24:15–45. doi: 10.1515/REVEH.2009.24.1.15
7. Chin-Chan M, Navarro-Yepes J, Quintanilla-Vega B. Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. *Front Cell Neurosci* 2015;9:1–22. doi: 10.3389/fncel.2015.00124
8. Dominguez S, Flores-Montoya MG, Sobin C. Early chronic exposure to low-level lead alters total hippocampal microglia in pre-adolescent mice. *Toxicol Lett* 2019;302:75–82. doi: 10.1016/j.toxlet.2018.10.016
9. Agnihotri SK, Agrawal U, Ghosh I. Brain most susceptible to cadmium induced oxidative stress in mice. *J Trace Elem Med Biol* 2015;30:184–93. doi: 10.1016/j.jtemb.2014.12.008
10. Wang B, Du Y. Cadmium and its neurotoxic effects. *Oxid Med Cell Longev* 2013;2013:898034. doi: 10.1155/2013/898034
11. Branca JJV, Morucci G, Pacini A. Cadmium-induced neurotoxicity: still much ado. *Neural Regen Res* 2018;13:1879–82. doi: 10.4103/1673-5374.239434
12. Pohl HR, Roney N, Abadin HG. Metal ions affecting the neurological system. *Met Ions Life Sci* 2011;8:247–62. doi: 10.1515/9783110436624-015
13. Goyer RA. Toxic and essential metal interactions. *Annu Rev Nutr* 1997;17:37–50. doi: 10.1146/annurev.nutr.17.1.37
14. Bulat ZP, Djukić-Ćosić D, Maličević Ž, Bulat P, Matović V. Zinc or magnesium supplementation modulates Cd intoxication in blood, kidney, spleen, and bone of rabbits. *Biol Trace Elem Res* 2008;124:110–7. doi: 10.1007/s12011-008-8128-5
15. Bulat Z, Djukić-Ćosić D, Antonijević B, Buha A, Bulat P, Pavlović Z, Matović V. Can zinc supplementation ameliorate cadmium-induced alterations in the bioelement content in rabbits? *Arh Hig Rada Toksikol* 2017;68:38–45. doi: 10.1515/aiht-2017-68-2919
16. Bulat Z, Djukić-Ćosić D, Antonijević B, Bulat P, Vujanović D, Buha A, Matović V. Effect of magnesium supplementation on the distribution patterns of zinc, copper, and magnesium in rabbits exposed to prolonged cadmium intoxication. *Sci World J* 2012;2012:1–9. doi: 10.1100/2012/572514.
17. Djukić-Ćosić D, Ninković M, Maličević Ž, Plamenac-Bulat Z, Matović V. Effect of supplemental magnesium on the kidney levels of cadmium, zinc, and copper of mice exposed to toxic levels of cadmium. *Biol Trace Elem Res* 2006;114:281–91. doi:10.1385/BTER:114:1:281.
18. Buha A, Bulat Z, Djukić-Ćosić D, Matović V. Effects of oral and intraperitoneal magnesium treatment against cadmium induced oxidative stress in plasma of rats. *Arh Hig Rada Toksikol* 2012;63:247–54. doi:10.2478/10004-1254-63-2012-2217.
19. Buha A, Jugdaohsingh R, Matovic V, Bulat Z, Antonijevic B, Kerns JG, Goodship A, Hart A, Powell JJ. Bone mineral health is sensitively related to environmental cadmium exposure- experimental and human data. *Environ Res* 2019;176:108539. doi:10.1016/j.envres.2019.108539.
20. Chui D, Yang H, Wang H, Ji T, Yu J, Zhang S, Chen Z, Xiao W. The dishomeostasis of metal ions plays an important role for the cognitive impairment. *Mol Neurodegener* 2013;8:S17. doi:10.1186/1750-1326-8-s1-p13.
21. Salim S. Oxidative stress and the central nervous system. *J Pharmacol Exp Ther* 2017;360:201–5. doi:10.1124/jpet.116.237503.
22. Matović V, Buha A, Djukić-Ćosić D, Bulat Z. Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys. *Food Chem Toxicol* 2015;78:130–40. doi:10.1016/j.fct.2015.02.011.
23. Patra RC, Rautray AK, Swarup D. Oxidative stress in lead and cadmium toxicity and its amelioration. *Vet Med Int* 2011;2011. doi:10.4061/2011/457327.
24. Pandya C, Pillai P, Nampoothiri LP, Bhatt N, Gupta S, Gupta S. Effect of lead and cadmium co-exposure on testicular steroid metabolism and antioxidant system of adult male rats. *Andrologia* 2012;44:813–22. doi:10.1111/j.1439-0272.2010.01137.x.
25. Nampoothiri LP, Agarwal A, Gupta S. Effect of co-exposure to lead and cadmium on antioxidant status in rat ovarian granulosa cells. *Arch Toxicol* 2007;81:145–50. doi:10.1007/s00204-006-0133-x.
26. Wei X, Qi Y, Zhang X, Gu X, Cai H, Yang J, Zhang Y. ROS act as an upstream signal to mediate cadmium-induced mitophagy in mouse brain. *Neurotoxicology* 2015;46:19–24. doi:10.1016/j.neuro.2014.11.007.
27. Wang J, Wu J, Zhang Z. Oxidative stress in mouse brain exposed to lead. *Ann Occup Hyg* 2006;50:405–9. doi:10.1093/annhyg/mei079.
28. Caito S, Aschner M. Neurotoxicity of metals. *Handb. Clin. Neurol.*, vol. 131, 2015, p. 169–89. doi:10.1016/B978-0-444-62627-1.00011-1.
29. Jadhav SH, Sarkar SN, Patil RD, Tripathi HC. Effects of subchronic exposure via drinking water to a mixture of eight water-contaminating metals: A biochemical and histopathological study in male rats. *Arch Environ Contam Toxicol* 2007;53:667–77. doi:10.1007/s00244-007-0031-0.
30. Andjelkovic M, Djordjevic AB, Antonijevic E, Antonijevic B, Stanic M, Kotur-Stevuljovic J, Spasojevic-Kalimanovska V, Jovanovic M, Boricic N, Wallace D, Bulat Z. Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney. *Int J Environ Res Public Health* 2019;16. doi:10.3390/ijerph16020274.
31. Buha A, Antonijević B, Bulat Z, Jačević V, Milovanović V, Matović V. The impact of prolonged cadmium exposure and co-exposure with polychlorinated biphenyls on thyroid function in rats. *Toxicol Lett* 2013;221:83–90. doi:10.1016/j.toxlet.2013.06.216.
32. Curcic M, Buha A, Stankovic S, Milovanovic V, Bulat Z, Đukić-Ćosić D, Antonijević E, Vučinić S, Matović V, Antonijevic B. Interactions between cadmium and decabrominated diphenyl ether on blood cells count in rats – Multiple factorial regression analysis. *Toxicology* 2017;376:120–5. doi:10.1016/j.tox.2016.05.011.
33. Čurčić M, Janković S, Jačević V, Stanković S, Vučinić S, Durgo K, Bulat Z, Antonijević B. Combined effects of cadmium and decabrominated diphenyl ether on thyroid hormones in rats. *Arh Hig Rada Toksikol* 2012;63:255–62. doi:10.2478/10004-1254-63-2012-2179.
34. Yuan G, Dai S, Yin Z, Lu H, Jia R, Xu J, Song X, Li L, Shu Y, Zhao X. Toxicological assessment of combined lead and cadmium: Acute and sub-chronic toxicity study in rats. *Food Chem Toxicol* 2014;65:260–8. doi:10.1016/j.fct.2013.12.041.
35. Wu X, Cobbina SJ, Mao G, Xu H, Zhang Z, Yang L. A review of toxicity and mechanisms of individual and mixtures of

- heavy metals in the environment. *Environ Sci Pollut Res* 2016;23:8244–59. doi:10.1007/s11356-016-6333-x.
35. Matović V, Buha A, Bulat Z, Dukić-Čosić D, Miljković M, Ivanišević J, Kotur-Stevuljević J. Route-dependent effects of cadmium/cadmium and magnesium acute treatment on parameters of oxidative stress in rat liver. *Food Chem Toxicol* 2012;50:552–7. doi:10.1016/j.fct.2011.12.035.
 36. Republic of Serbia. Zakon o dobrobiti životinja [Animal Welfare Act, in Serbian]. Official Gazette of the Republic of Serbia No. 41/2009
 37. Auclair C, Voisin E. Nitroblue tetrazolium reduction. *Handb Methods Oxyg Radic Res* 1985;1:23–32. doi:10.1201/9781351072922.
 38. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 2005;38:1103–11. doi:10.1016/j.clinbiochem.2005.08.008.
 39. Aycicek A, Erel O. Estado oxidante/antioxidante total em recém-nascidos ictericos antes e depois da fototerapia. *J Pediatr (Rio J)* 2007;83:319–22. doi:10.2223/JPED.1645.
 40. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972;247:3170–5.
 41. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;82:70–7. doi:10.1016/0003-9861(59)90090-6.
 42. Girotti MJ, Khan N, McLellan BA. Early measurement of systemic lipid peroxidation products in the plasma of major blunt trauma patients. *J Trauma - Inj Infect Crit Care* 1991;31:32–5. doi:10.1097/00005373-199101000-00007.
 43. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248–54. doi:10.1016/0003-2697(76)90527-3.
 44. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, Jungers P, Descamps-Latscha B. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int* 1996;49:1304–13. doi:10.1038/ki.1996.186.
 45. Karri V, Schuhmacher M, Kumar V. Heavy metals (Pb, Cd, As and MeHg) as risk factors for cognitive dysfunction: A general review of metal mixture mechanism in brain. *Environ Toxicol Pharmacol* 2016;48:203–13. doi:10.1016/j.etap.2016.09.016
 46. Andrade VM, Aschner M, Marreilha dos Santos AP. Neurotoxicity of metal mixtures. *Adv Neurobiol.*, vol. 18, 2017, p. 227–65. doi:10.1007/978-3-319-60189-2_12.
 47. Cobbina SJ, Chen Y, Zhou Z, Wu X, Feng W, Wang W, Mao G, Xu H, Zhang Z, Wu X, Yang L. Low concentration toxic metal mixture interactions: Effects on essential and non-essential metals in brain, liver, and kidneys of mice on sub-chronic exposure. *Chemosphere* 2015;132:79–86. doi:10.1016/j.chemosphere.2015.03.013.
 48. Gu C, Chen S, Xu X, Zheng L, Li Y, Wu K, Liu J, Qi Z, Han D, Chen G, Huo X. Lead and cadmium synergistically enhance the expression of divalent metal transporter 1 protein in central nervous system of developing rats. *Neurochem Res* 2009;34:1150–6. doi:10.1007/s11064-008-9891-6.
 49. Andrade V, Mateus ML, Batoréu MC, Aschner M, dos Santos APM. Urinary delta-ALA: A potential biomarker of exposure and neurotoxic effect in rats co-treated with a mixture of lead, arsenic and manganese. *Neurotoxicology* 2013;38:33–41. doi:10.1016/j.neuro.2013.06.003.
 50. Wang L, Zhou X, Yang D, Wang Z. Effects of lead and/or cadmium on the distribution patterns of some essential trace elements in immature female rats. *Hum Exp Toxicol* 2011;30:1914–23. doi:10.1177/0960327111405865.
 51. Ademuyiwa O, Agarwal R, Chandra R, Raj Behari J. Effects of sub-chronic low-level lead exposure on the homeostasis of copper and zinc in rat tissues. *J Trace Elem Med Biol* 2010;24:207–11. doi:10.1016/j.jtemb.2010.01.002.
 52. Ashby SL, King LJ, Parke DVW. Effect of acute administration of cadmium on the disposition of copper, zinc, and iron in the rat. *Environ Res* 1980;21:177–85. doi:10.1016/0013-9351(80)90019-5.
 53. Cobbina SJ, Chen Y, Zhou Z, Wu X, Zhao T, Zhang Z, Feng W, Wang W, Li Q, Wu X, Yang L. Toxicity assessment due to sub-chronic exposure to individual and mixtures of four toxic heavy metals. *J Hazard Mater* 2015;294:109–20. doi:10.1016/j.jhazmat.2015.03.057.
 54. Seth TD, Agarwal LN, Satija NK, Hasan MZ. The effect of lead and cadmium on liver, kidney, and brain levels of cadmium, copper, lead, manganese, and zinc, and on erythrocyte ALA-D activity in mice. *Bull Environ Contam Toxicol* 1976;16:190–6. doi:10.1007/BF01685226.
 55. Saleh HA, Abd El-Aziz GS, Mustafa HN, El-Fark MO, Tashkandi J, Alzahrani A, Mal A, Aburas M, Salem AH. Beneficial effects of curcumin in maternal and fetal oxidative stress and brain damage induced by gestational lead administration. *Biomed Pharmacol J* 2018;11:871–87. doi:10.13005/bpj/1444.
 56. Villeda-Hernández J, Barroso-Moguel R, Méndez-Armenta M, Nava-Ruiz C, Huerta-Romero R, Ríos C. Enhanced brain regional lipid peroxidation in developing rats exposed to low level lead acetate. *Brain Res Bull* 2001;55:247–51. doi:10.1016/S0361-9230(01)00512-3.
 57. Bennet C, Bettaiya R, Rajanna S, Baker L, Yallapragada PR, Brice J, White S, Bokara KK. Region specific increase in the antioxidant enzymes and lipid peroxidation products in the brain of rats exposed to lead. *Free Radic Res* 2007;41:267–73. doi:10.1080/10715760600889855.
 58. Takeda A, Akiyama T, Sawashita J, Okada S. Brain uptake of trace metals, zinc and manganese, in rats. *Brain Res* 1994;640:341–4. doi:10.1016/0006-8993(94)91891-0.
 59. Braga MM, Dick T, de Oliveira DL, Scopel-Guerra A, Mussulini BHM, de Souza DO, da Rocha JBT. Evaluation of zinc effect on cadmium action in lipid peroxidation and metallothionein levels in the brain. *Toxicol Reports* 2015;2:858–63. doi:10.1016/j.toxrep.2015.05.014.
 60. Shukla A, Girja S, RC Srimal. Cadmium-induced alterations in blood-brain barrier permeability and its possible correlation with decreased microvessel antioxidant potential in rat. *Hum Exp Toxicol* 1996. doi: 10.1177/096032719601500507.
 61. Kerek EM, Prenner EJ. Inorganic cadmium affects the fluidity and size of phospholipid based liposomes. *Biochim Biophys Acta - Biomembr* 2016;1858:3169–81. doi:10.1016/j.bbmem.2016.10.005.
 62. Choe M, Jackson C, Yu BP. Lipid peroxidation contributes to age-related membrane rigidity. *Free Radic Biol Med* 1995;18:977–84. doi:10.1016/0891-5849(94)00217-8.

Redoks status i razine bioelemenata u mozgu štakora akutno izloženih smjesi kadmija i olova

Dosadašnji podaci o neurotoksičnosti olova (Pb) i kadmija (Cd) uglavnom su opisani nakon izloženosti pojedinačnim metalima, a podaci o neurotoksičnom djelovanju smjese tih dvaju metala prilično su oskudni. Cilj ovoga rada bio je ispitati učinak akutnoga izlaganja smjesi olova i kadmija na parametre oksidacijskoga stresa i status bioelemenata u mozgu štakora. Životinje su bile podijeljene u pet skupina: Cd15 (15 mg/kg), Cd30 (30 mg/kg), Pb150 (150 mg/kg), Pb150+Cd15 i kontrolna, netretirana skupina. Smjesa olova i kadmija dovela je do značajno viših vrijednosti malondialdehida (MDA) i drugih produkata u mozgu štakora u usporedbi s kontrolnom skupinom i obama pojedinačnim metalima. To je iznimno važno, imajući u vidu da je prisutnost MDA-a u mozgu povezana s mnogim neurodegenerativnim poremećajima. Nije dobivena statistički značajna razlika između ispitivanih skupina u razinama cinka (Zn), bakra (Cu), mangana (Mn) i željeza (Fe). Rezultati ovoga istraživanja važan su doprinos budućim istraživanjima smjesa i upućuju na to da istraživanja toksičnosti u kojima se procjenjuje neurotoksičnost pojedinačnih kemikalija ne pružaju stvarni uvid u neurotoksičnost njihovih smjesa kojima smo izloženi u stvarnom životu. Dobiveni rezultati otvaraju daljnja pitanja o interakciji i mehanizmima toksičnosti smjese tih metala.

KLJUČNE RIJEČI: Cd; Cu; Fe; MDA; Mn; neurotoksičnost; oksidacijski stres; Pb; TBARS; Zn