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# Microelement imbalance in the rat uterus after short-term heavy metals exposure

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*Heavy metals (HMs) are distinguishable among various exogenous elements with a comprehensive impact and high adverse environmental consequences. The danger of their spread is accompanied by risks of entry into living organisms, leading to organ accumulation, homeostasis disruption, and the development of various inflammatory and oncological clinical manifestations. Interestingly, the uterus shows an unpredictable reaction to the influence of these chemical elements, with almost all previous studies providing differing results. Therefore, this study investigated the features of chemical element content in the rat uterus after 30 days of HMs exposure and assessed the benefits of vitamin E against HMs impacts. Spectral analysis of uterine tissue of rats (daily HMs exposure) and animals (HMs exposure with vitamin E treatment) showed a significantly higher accumulation of Zn, Cu, Fe, Mn, Pb, and Cr after 30 days of the experiment. Although the uterus samples of rats treated with vitamin E showed a significant accumulation of HMs, it should be noted, that the tendency for their accumulation was less pronounced than in untreated rats. This study showed the accumulation features of some chemical elements in the rat uterus after 30 days of HMs exposure. Therefore, widespread environmental HMs exposure can leads to high absorption levels in uterine tissues. However, increases in HMs levels were significantly attenuated by vitamin E supplementation. Unfortunately, while a positive trend was found for each chemical element, the complete protection and purification effect of vitamin E use against HMs exposure was not observed.*
*Key words: uterus; heavy metals; accumulation; rats; heavy metals salts.*

## INTRODUCTION

The main technogenic burden on the environment is the different pollutants circulating at all ecosystem levels (solid, liquid, and gaseous states) caused by the emissions from technological processes [1]. Heavy metals (HMs) are distinguishable among various exogenous elements with a comprehensive impact and high adverse environmental risk [1-4]. In principle, the danger of their spread is accompanied by risks of entry into living organisms, leading to organ accumulation, homeostasis disruption, and development of various inflammatory and oncological clinical manifestations. However, the toxic effects of HMs may be specific and may differ depending on their type, combination, and concentration [2-6].

Fine chemical particles in the ambient environment have been reported to be associated with many health problems, including urinary, cardiovascular, hematogenic, neuro, reproductive, and respiratory systems. Due to their large pollution area, strong adsorption capacity, and toxicity mechanisms, HMs can be adsorbed by living organisms and accumulate within them, causing inflammatory or cancerogenic local damage and widespread harm to homeostasis [2, 4-7].

The uterus is one of the largest structurally and hormonally balanced organs of the reproductive system [8] and is subject to the influence of HMs [2, 4-7]. Interestingly, the uterus shows an unpredictable reaction to the influence of chemical elements, with almost all previous studies providing differing results. This variability might reflect differences in pollutants (including HMs), their concentrations, and their impact on organisms across their study areas and objectives [2, 9-12].

Due to numerous reports of pollutants' danger and risks, many developed countries worldwide are pursuing solutions to pollution issues and investing considerably in the development of methods (e.g., degassing and phytoremediation) and drugs (e.g., natural or artificial compounds) for environmental and health protection [13]. Vitamin E can be one of these protective agents since it is one of the most important essential nutrients, with potent antioxidant, anti-inflammatory, and detox activities, known to humanity [2, 4, 14]. Many studies have described vitamin E's effectiveness and protective effects against the adverse impacts of various HMs. Moreover, the potential benefit of vitamin E supplementation for female gynecological health and reproductive-related diseases has also been reported [14, 15].

Therefore, this study investigated the features of chemical element content in the rat uterus after 30 days of HMs exposure and assessed the benefits of vitamin E against HMs impacts.

## METHODS

**Experimental model.** Following the aim of this study, the experimental mixture was presented with six of the most common and potentially dangerous HMs. The experimental model and the list of HMs were approved by the Bioethics Committee of the Medical Institute of Sumy State University (No. 2/10 from 10.10.2019). The list of HMs salts were estimated in accordance with preliminary reports in the following concentrations: zinc ($ZnSO_4 \times 7H_2O$) – 5 mg/l, copper ($CuSO_4 \times 5H_2O$) – 1 mg/l, iron ($FeSO_4$) – 10 mg/l, manganese ($MnSO_4 \times 5H_2O$) – 0.1 mg/l, lead ($Pb(NO_3)_2$) – 0.1 mg/l, and chromium ($K_2Cr_2O_7$) – 0.1 mg/l [4, 5]. The HMs were dissolved in tap water and supplied in a drinking bottle in ad libitum access for oral exposure.

For screening potential therapy for the treatment against HMs impact, vitamin E was selected for oral exposition (based on the literature recommendations) [4, 13-15]. The rats were administered vitamin E (daily at 10.00 am) using a disposable tip and automatic pipette at an average prophylactic dose (9.1 mg/kg to rats' body weight considering species' characteristics).

The 24 female rats were selected for this study after physical and behavioral examinations. Further, the rats were randomly divided into three groups (8 rats each): Group I - control (received ordinary drinking water); Group II – rats treated by HMs mixture; Group III - rats administered by HMs in combination with vitamin E treatment. The duration of the experiment was 30 days. The rats were housed in polypropylene cages under environmentally controlled laboratory conditions of temperature 22 ± 1°C, relative humidity 55 ± 5%, and 12 h light/dark cycle. The animals had ad libitum access to standard pellets and water during the experiment. After 30 days, female rats were euthanized with the next low abdominal midline incision and uteruses collection. For the following atomic absorption spectrometric analysis, the uterine wall of 1.0 cm in length was excised from one random horn (proximal part - in the direction from the partial caudal fusion to the ovaries). All animal handling and experimental procedures were performed according to international, national, and institutional ethical guidelines.

**Atomic absorption spectrometry**. The atomic absorption spectrometric method was used for the determination of the chemical composition of the uterine tissues. Samples were weighed on analytical balance with an accuracy of 0.001g, dried out, and burned in porcelain crucibles in an oven at 450°C for 48 h to remove the organic matrix. Obtained ash was dissolved in 2.0 ml hydrochloric 10% and 1.0 ml nitric acids. Thereafter, to determine the concentration of microelements, spectrophotometer C-115M1

(Ukraine) with electrothermal atomizer and computer recording of the analytical signal with calculated program “AAS SPEKTR” (Ukraine) was used. Optical density measures were taken into account at a specific wavelength of each element**:** zinc, 213.9 nm; copper, 324.7 nm; iron, 248.3 nm; manganese, 279.4 nm; lead, 283.3 nm; chrome, 357.9 nm.

**Statistical analysis.** Results are expressed as mean ± standard deviation or as a percentage of the difference. Data were analyzed by using a one-way analysis of variance (ANOVA) followed by Bonferroni’s post-hoc test. In all cases, P < 0.05 was considered statistically significant. All data collected from the experiment were analyzed by Graph Pad® 6.0 software.

## RESULTS

Baseline levels of the studied chemical elements in uterine tissue were determined using a control group (group I). Their average zinc content was 38.44 ± 2.04 µg/g, copper was 4.84 ± 0.17 µg/g, iron was 86.07 ± 4.83 µg/g, manganese was 2.43 ± 0.11 µg/g, lead was 0.144 ± 0.005 µg/g, and chromium was 0.908 ± 0.009 µg/g. The total content of these microelements was 132.83 ± 5.69 µg/g. The quantitative HMs content differed significantly among them, in descending order Fe > Zn > Cu > Mn > Cr > Pb.

Spectral analysis of rat uterine tissue from experimental groups II (HMs exposure only) and III (HMs exposure with vitamin E treatment) showed a significantly higher accumulation of chemical elements after 30 days of HMs exposure (Fig. 1). Group II showed a rapid increase in Zn (34.11%; 51.55 ± 3.32 µg/g; P < 0.0001), Cu (44.83%; 7.01 ± 0.39 µg/g; P < 0.0001), Fe (61.95%; 139.39 ± 11.56 µg/g; P < 0.0001), Mn (38.68%; 3.37 ± 0.19 µg/g; P < 0.0001), Pb (53.15%; 0.219 ± 0.008 µg/g; P < 0.0001), and Cr (47.91%;1.34 ± 0.032 µg/g; P < 0.0001) content.

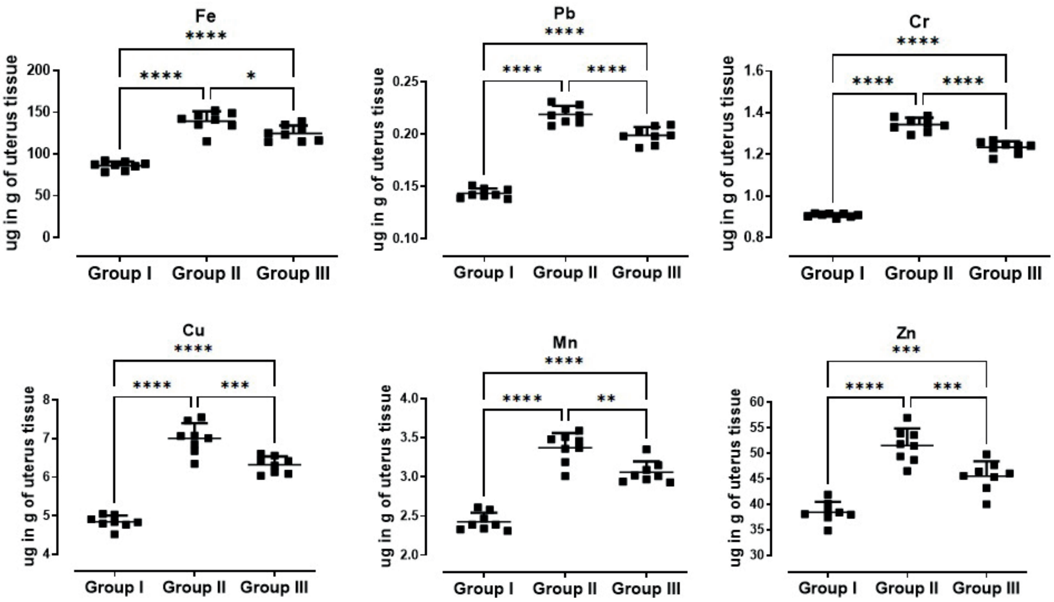
Uterus samples from group III rats showed a similar tendency, an increasing exogenous HMs content in the uterine wall, even against the treatment background. Their HMs content exceeded the control group values by 44.85% (124.67 ± 9.29 µg/g; P < 0.0001) for Fe, 39.16% (0.199 ± 0.008 µg/g; P < 0.0001) for Pb, 35.9% (1.234 ± 0.029 µg/g; P < 0.0001) for Cr, 30.58% (6.32 ± 0.22 µg/g; P < 0.0001) for Cu, 25.93% (3.06 ± 0.14 µg/g; P < 0.0001) for Mn, 18.44% (45.53 ± 2.93 µg/g; P < 0.0001) for Zn, accordingly.

Notably, the tendency for HMs to accumulate in the uterine wall of group III rats was less pronounced than in group II rats. Zn content was lower by 11.67% (P < 0.0001), Fe - 10.56% (P = 0.014), Cu - 9.84% (P < 0.0001), Mn - 9.2% (P = 0.002), Pb - 9.13% (P < 0.0001), Cr - 8.12% (P < 0.0001) in vitamin E-treated (group III) rats than in untreated (group II) rats.

In addition, the relative HMs increases in rat uterine tissue were the same for both groups II and III, in decreasing order Fe > Pb > Cr > Cu > Mn > Zn (Figure). Moreover, the increases in each chemical element’s content above baseline led to rises in their total content of 52.74% (202.88 ± 13.26 µg/g; P < 0.0001) for group II and 36.27% (181.01 ± 7.82 µg/g; P < 0.0001) for group III. Therefore, total HMs content in group III was 16.47% (21.87 µg/g; P = 0.001) lower than in group II.

## DISCUSSIONS

We have shown that consuming water enriched with a mixture of environmentally common and potentially dangerous HMs for 30 days leads to abnormal increases (52.74%; P < 0.0001) in their content in rat uterine tissue. However, a protective treatment attenuated this increase by 16.47% (P = 0.001), possibly reflecting their enhanced removal. Nevertheless, the content of all HMs was consistently reduced in vitamin E-treated rats. Unfortunately, they did not return to the baseline values of the control group. We hypothesize that this indicates the promising use of vitamin E for controlling the intake of pollutants (both alone and in combination




The imbalance of Fe, Pb, Cr, Cu, Mn and Zn levels in uterine tissue in rats after 30 days HMs exposition. Data are expressed as Mean ± SD (Scatter dots; error bars – T style with above direction). One-way ANOVA followed by Bonferroni's post-hoc test (n = 24): Group I (Control) vs. Group II (HMs) vs. Group III (HMs + vitamin E). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$

with other drugs), with protocols aiming to maximize protection against HM accumulation and facilitate their rapid removal.

Interestingly, Fe, an essential trace element, showed the greatest accumulation. Toxic elements, such as Pb and Cr, accumulated to a lesser extent, although with a great tendency than Zn, which accumulated the least. Differences in metal accumulation may be due to the synergism and antagonism of certain metals [2, 7], where some metals enhance the accumulation of others or displace them. Many studies have shown that essential metals are necessary and absorbed easily by the body. However, it is difficult to control their circulation with excessive intake. Even when the signaling pathway mechanisms of toxic metals or other dangerous chemical pollutants are largely predictable, the effect of extremely high doses of necessary trace elements remains unpredictable, highlighting the negative impact of environmental pollution [2-7, 9, 10, 16].

HM accumulation in the uterus likely perturbs female sex hormone expression and the estrous cycle. It also likely causes blood circulation disorders and dystrophies; inflammation; enzymatic activity inhibition; gene mutations and pathological methylation; perturbed cell integrity, hypoxia, division, and apoptosis; hyper- or hypo-atrophic changes; antioxidant mechanism impairment; increased free radicals production, oxidative stress, lipid peroxidation, and DNA damage [2, 5, 9,10, 17-22]. Unfortunately, the risk of exposure of plants, microorganisms, fish, animals, and humans to HMs and other organic and inorganic elements is significant. It is important to note that short- and long-term (acute, sub-chronic or chronic) influx of some experimental substances (including HM) can have both similar and opposite effects [2, 4, 5, 9, 10, 15, 23-28]. Moreover, changes caused by pollutants can manifest even in subsequent generations [18, 23].

Increasingly, pharmaceuticals and environmental agents testing in animal models, followed by interpolation of the results, have been improved because of good correlation with in-human results, which can boost awareness and predict drug effects in humans. Understanding the screening diagnostic criteria and the search for effective drugs can serve as a basis for optimizing and personalizing therapeutic protocols and predicting the course of pathology [4, 5, 13-15, 17, 24, 26, 28]. Much additional work is required to understand this phenomenon completely. Therefore, these findings are essential for understanding the role of metals in uterine homeostasis and pathology.

## CONCLUSIONS

This study showed the accumulation features of some chemical elements (i.e., Zn, Cu, Fe, Mn, Cr, and Pb) in the rat uterus after 30 days of HMs exposure. Therefore, widespread environmental HM exposure can leads to high absorption levels in uterine tissues. However, increases in HMs levels were significantly attenuated by vitamin E supplementation. Unfortunately, while a positive trend was found for each chemical element, the complete protection and purification effect of vitamin E use against HM exposure was not observed. These findings indicate that the accumulation potential of essential and nonessential metals in the rat uterus might have adverse effects.

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## ДИСБАЛАНС МІКРОЕЛЕМЕНТІВ У МАТЦІ ЩУРІВ ПІСЛЯ КОРОТКОТРИВАЛОГО ВПЛИВУ ВАЖКИХ МЕТАЛІВ

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Поширення важких металів (ВМ) супроводжується ризиками проникнення в живі організми, що призводить до їх накопичення в органах, порушення гомеостазу, розвитку різноманітних запальних та онкологічних клінічних проявів. Цікаво, що матка виявляє непередбачувану реакцію на вплив цих хімічних елементів, що відображається на різниці результатів проведених досліджень. У цьому дослідженні ми вивчали особливості вмісту хімічних елементів у матці щурів після 30 днів впливу суміші ВМ та оцінювали переваги застосування вітаміну Е на тлі дії полютантів. Спектральний аналіз тканини матки щурів, яких піддавали щоденному впливу ВМ, і тварин, яким комбінували експозицію ВМ з лікуванням вітаміном Е, показав достовірне зростання вмісту Zn, Cu, Fe, Mn, Pb та Cr. Хоча у зразках матки щурів, лікованих вітаміном Е, спостерігалася тенденція до акумуляції ВМ, яка була менше вираженою порівняно з тваринами без лікування. Таким чином, широке поширення останніх у навколишньому середовищі може призводити до високих рівнів їх накопичення в тканині матки. Водночас застосування вітаміну Е супроводжувалося певним пригніченням зростання вмісту ВМ. Хоча позитивна тенденція накопичення була виявлена для кожного хімічного елемента на тлі застосування вітаміну Е, повного захисту від впливу ВМ не спостерігалося.

Ключові слова: матка; важкі метали; накопичення; щури; солі важких металів.

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