Pol. J. Environ. Stud. Vol. 33, No. 4 (2024), 4153-4159
DOI: *10.15244/pjoes/182879* ONLINE PUBLICATION DATE: 2024-02-28

Original Research

Effect of Metals on Smoker Immune System and their Correlation with Viral Infections

Tabinda Ijaz[1], Ayoub Rashid Ch[1*], Aqsa Batool[1], Yasmin Akhtar[1], Muhammad Nauman Khan[2, 3*], Yaseen[4], Rashid Iqbal[5], Saeedah Musaed Almutairi[6], Doaa Bahaa Eldin Darwish[7]

[1] Department of Chemistry, Government College University, Lahore 54000, Pakistan.
[2] Department of Botany, Islamia College Peshawar, 25120 Peshawar, Pakistan.
[3] Biology Laboratory, University Public School, University of Peshawar, 25120 Peshawar, Pakistan.
[4] Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan.
[5] Department of Agroecology-Climate and Water, Aarhus University, 8830 Tjele, Denmark.
[6] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia.
[7] Botany Department, Faculty of science, Mansoura University, Mansoura 35511, Egypt; d_darwish@mans.edu.eg.

Received: 27 September 2023
Accepted: 19 January 2024

Abstract:

This study was standardized to investigate the effects of trace metals in smokers' blood samples and find metals' correlation with virulent diseases. An auxiliary finding was used to detect the inflated amount of metal absorption, which was determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The large metal level does not generate immunity in the smoker's body. Human samples display fickle computation of concentrations of these metals. The Mean±SD of As, B, Pb Sr, and Zn in smoker's samples were (6.1±2.9 ppb), (285.9±118.1 ppb), (60.7±28.5 ppb), (28.6±9.8 ppb), and (420.5±100.4 ppb) reciprocally. The As, B, Pb, Sr, and Zn concentrations in all the smokers' blood samples were over the acceptable limit, withal the *P*-value= (≥0.05) all the samples have displayed no correlation in smokers against viral inflammation. This examination can be used as a support and quotation for further investigations. These findings declare that the number of metals in the body for long periods contributes to toxicity and promotes viral infections.

Keywords: Cytokines; Immunology; Metals; Smokers; Viral Infections

Introduction

Immunity is the capacity of multicellular organisms to prevent adverse microorganisms from penetrating them in the body. Immunity is associated with both specific and nonspecific items. Some other incidents of immune activity observe the promote epidemicity combative [1]. Innate immunity, also called native protection, is tolerated by the integrity of pathogens, that is, its genomic fundaments without a supplementary impetus or an introductory ailment. 'Passive immunity' is acquired over the displacement of immunoglobulin or stimulates [2]. Over an array of impressions called immune feedback, the immunity of our body structure kills viruses as well as

* e-mail: ayoubrashid@gcu.edu.pk (A.R.C); nomiflora@uop.edu.pk (M.N.K)

stuff that infects human anatomy and motivates infection [3]. Anatomical and physiological barriers provide protection, which is supplemented by innate immunity. The innate immune system relies on a small number of invariant receptors to identify invasive pathogens, but it makes up for this by focusing on conserved microbial components that are shared by many different pathogen types. The innate immune system is known for its speed: minutes after being exposed to a pathogen, it begins to produce a protective inflammatory response. Further, the ensuing adaptive immune response is mostly activated by innate immunity [4, 5].

The virus has lethal textures of small size and expanse composition that may multiply in the human body, fern, and bacterium [6]. All viral cells contain a genomic structure that may be DNA/RNA or protein. Viruses boost efficiency with other chemical actions from the host cell [7]. The host must detect the invader and its components, such as uncapped viral RNA [8], as well as cellular stress, metabolic changes, and cellular damage brought on by infection, before the immune response to a virus or other microbe may be launched. Germline-encoded pattern recognition receptors (PRRs) [4, 8] are principally responsible for this early response to infection. There are five different kinds of PRRs. These include the nucleotide oligomerization and binding domain-like receptors (NLRs), the Pyrin-HIN domain (PYHIN) receptors [4, 9, 10], the Toll-like receptors (TLRs) localized to the cell surface or within endosomes, the intracellular retinoic acid-inducible gene-I-like receptors (RLRs), and the C-type lectin receptors. Each of these receptor types contains several members, and each member of a receptor-type recognizes a different pathogen-associated product or damage-associated chemical pattern, such as reactive oxygen species, adenosine triphosphate, or apoptotic/necrotic cells [11, 12].

Smoking is an activity that is observed where a substance is burned and the ensuing smoke breathed in to be enjoyed and absorbed into the blood [13]. Even a minute amount of tar cigarettes and without smoking tobacco was shown to extend the danger of heart disease as compared to non-smokers [14]. *In vivo*, chronic smokers are related to the enlarged levels of many inflammatory markers together with serum globulin, IL-6, and alpha TNF in each male and female smoker [15]. Containing 4000 known chemical components, the roll of tobacco is incredibly toxic and hepatotoxic for an individual's health [16]. Around 2 billion people use tobacco products globally, primarily in the form of cigarettes, and at least 4 million people die from tobacco-related diseases each year [17]. The sharp increase in diseases linked to tobacco use or cigarette smoke, such as cardiovascular disease, chronic obstructive pulmonary disease (COPD), Crohn's disease, and various cancers [18], suggests that smoking may have a negative influence on the development of human diseases [19]. The World Health Organization (WHO) presently estimates that there are 1.1 billion smokers in the world [20]. It is calculable that the ubiquity of smoking is 36% in men's and 9% in women's, and around 1200 kids begin smoking every day [21].

The ordinary components of the planet also consist of non-essential metals such as Pb, As, Cd, and Zn, etc. A few of the metals are fundamental aspects; however, when they endure in a huge amount, they become lethal [22]. The toxicity of heavy metals develops when the concentration level of the metal is very low or very high. Due to the aggregation of heavy metals, many fractious health problems developed [23].

A few metals are called essential metals, but they are also toxic if their amount is increased. Heavy metals are important for human beings on a controlled level. Heavy metals, for example lead, cadmium, and arsenic, are extensively present in the environment [24]. A significant public health issue is heavy metal contamination [25, 26]. Toxic heavy metals include metals including lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg), which can seriously impair human health even at low doses [27]. Even though some metals, such as nickel (Ni) and manganese (Mn), are regarded as essential or trace elements to perform significant physiological functions [28, 29], they can harm a person's body if their concentration crosses a predetermined threshold [30, 31]. Metals that attach with the MHC/peptide complicated inside the TCR receptors can produce neoantigens that are identified as foreign, for that reason blurring the distinction between hypersensitivity and autoimmunity [32]. Stimulation of T cells is essential for hypersensitivity induced by metals. The revival of T cells' desire to engage with TCRs with MHC complexes asserted on the surface of dendritic cells. Metal ions and other tiny fragments attach with MHC proteins in a variety of ways to grant this signal. Some metals act as haptens with the aid of interacting with residues of amino acids on major histo-compatible peptides. T cells recognize metals through this mechanism [33]. Due to its intricate structure, the immune system is vulnerable to a wide range of assaults, including physical and chemical agents from the environment, medications, and biologics. Any type of dysregulation may cause immunological derangements with harmful implications for health since the immune system is a highly regulated network of cells and soluble mediators [34]. Because pollutants build up in the body and may have an impact on the immune system, environmental pollution poses a severe threat to human health [35]. Early (Immuno) toxicological investigations and experimental rodent studies initially identified the immunomodulatory potential of environmental chemicals, often known as xenobiotics, as a result of immune-mediated lung illnesses connected to occupational exposure. Certain heavy metals, well-documented for their impact on the immune system, are associated with immune-mediated illnesses as environmental contaminants. Among these, cadmium (Cd) stands out as one of the most hazardous [36-38]. Analyzing small quantities of heavy metals in a sample pattern can be accomplished for regulatory approval and quality management purposes. We identify the heavy metalloids in actual samples, and we can additionally decide to examine the contaminants in smokers. These metals can be measured to components per billion levels by using our laboratory equipment. In chemical laboratories, metals can be explored through a number of strategies. Most regularly, examining techniques of metal identification rely on different techniques, such as Atomic Absorption Spectrophotometer (AAS), Inductively Coupled Plasma- Optical Emission Spectrophotometer (ICP-OES), and Inductively Coupled Plasma- Mass Spectrophotometer determination of such low quantity demands highly touchy methods [39]. Few investigative strategies have been used for trace analysis of metals. We used the ICP-OES method to detect metals found in our samples [40].

Material and Methods

A cross-sectional record was determined, in which 20 subjects were included in this finding. In this discussion, all symptomatic checks were carried out in the Chemistry department of Government College University Lahore. A survey pattern was set for subjects alongside their age. After obtaining permission, a complete 3ml of blood was drawn from selected subjects. The sample used to be aseptically accumulated from a medial cubital vein in a red-capped vial. After centrifugation, serum was separated, and in serum, nitric acid dropped for storage of metals.

Chemicals

The chemicals used had complete control over pureness and quality. Analytical grade chemicals are used in this study. The chemicals' concentration HNO_3, H_2SO_4, H_2O_2, and double distilled water were received from the chemical store of the Chemistry department of Government College University Lahore.

Conventional Wet Acid

The Digestion Procedure Wet acid digestion method was used in this research work. With the help of an acid (or mixture of acids) a sample is dissolved. Therefore, it is called acid digestion. Open vessels are used to conduct such dissolving procedures with the help of a hot plate. The successive method was followed for sample qualification. In 100 mL of beaker total, 2 g of sample was taken. Samples were digested by adding a 10 mL conc. of Nitric acid (HNO_3) to each sample separately, and heating on a hotplate at 70 ºC for 30 min until orange fumes disappeared. Then 10 mL of conc. sulfuric acid (H_2SO_4) was added to each sample and the temperature increased to 150 ºC (the sample was heated for 1 h). The temperature was increased to 300 ºC after 1 h, and at this temperature, the dissolution of samples was accomplished for about 30 minutes until white fumes disappeared. Then 2 mL of hydrogen peroxide (H_2O_2) was added to each sample and heated at 180º C for 30 min, until the remaining volume of the sample was 20 mL. Then we powered off the hot plate and cooled the samples at room temperature. 20 mL samples were then filtered at the end by using filter paper.

Preparation of Standard Solution

The stock solution of arsenic was prepared with a concentration of 1000 mg/L. From the stock solution, the working solutions of 2, 4, 6, 8, and 10 ppm were made by using the following eq. 1. After that, a calibration curve showing the linear response of the ICP-OES signal versus the concentration of standards was drawn.

$$C_1V_1 = C_2V_2 \quad (1)$$

C_1 = Concentration of stock standard (1000 mg/L), V_1 = Volume of stock standard to be used (unknown), C_2 = Desired concentration of working standard (mg/L), V_2 = Total volume of working standard (mL)

Plan of Work for the Absorption of Selected Samples

The factor was capable in succeeding phases: Determination of mode for pattern arrangements, Detection of samples by way of using ICP, Estimate the number of metals, and Statistical finding out about accumulated data.

ICP-OES

This method has high efficiency, many component examinations, and high potency. The origination is lead into argon plasma. Plasma is the 4th element of the universe, next to the solid, aqueous, and gaseous states. In the ICP-OES the plasma is provoked at the end of a quart's torch by a frosty introducing coil, through which an immense density changes current flow (Figure 1).

Statistical Analysis

The statistical finding, mean ± standard deviation, and correlation test were done using SPSS software 21.

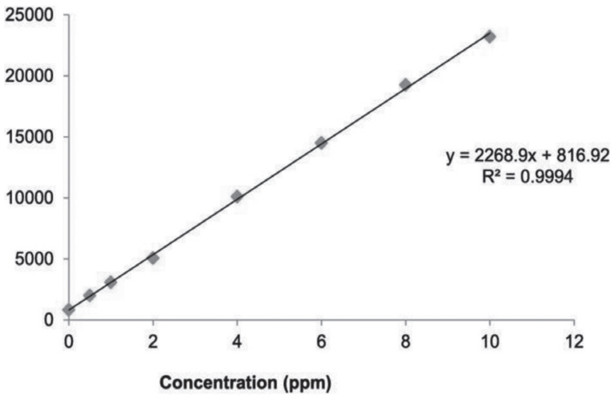

Figure 1. A calibration curve showing the linear response of the ICP-OES signal versus the concentration of standards prepared

Results

Demographic Characteristics

The total subjects was 20, which are included in this examination. 5 samples were excluded due to age issues. 15 smokers' samples were also included in this study.

Determination of Elements by ICP-OES and Wavelength Used for Metal Detection

The estimation and evaluation of heavy metals in smoker samples were carried out using an analytical technique ICP-OES, and different wavelengths were used for the analysis of different metals (Table 1).

Table 1: Wavelength used for the analysis of metals in Samples

Element	Wavelength	Unit	Type
As	189.042	ppb	Analyte
B	249.773	ppb	Analyte
Pb	220.353	ppb	Analyte
Sr	407.771	ppb	Analyte
Zn	213.856	ppb	Analyte

Results of Smokers

We used ICP-OES to estimate the heavy metals in the blood of smokers and coded them as S 1-S 15, respectively. The research shows the estimated results for the level of concentrations of metals in all samples. These are discussed below in Table 2.

Table 2: Concentration (ppb) of (As, B, Pb, Sr, and Zn) in blood samples of smokers

Sample Code	As	B	Pb	Sr	Zn
S1	6.33	340.4	78.33	25.20	434.20
S2	2.7	312.7	45.7	12.80	321.70
S3	8.8	220.6	98.23	34.7	5438
S4	9.12	432.54	25	35.78	487.34
S5	3.7	123.7	56.23	34.81	316
S6	3.32	154.21	34.30	14.26	316.40
S7	5.7	230.5	44	34.8	424
S8	5	325.8	35	27	234.8
S9	3.21	431.42	65.12	21.8	366
S10	7.7	276.9	32.43	18.8	432.43
S11	3.9	345.9	24	23.7	287.34
S12	4.8	322.8	23.8	12.8	345.12
S13	2.8	234.6	35	31.34	298.45
S14	7.84	145.9	46	26.12	345.81
S15	4.76±1	456.73	76.56	42.65	234.34
Mean±SE	5.3±0.55	290±27.2	25±6.0	26.4±2.4	7.0±34

Correlation of Metals with Immunity

To detect the effects of metals (As, B, Pb, Sr, and Zn) on the ability of the host cells to increase viral infections, the viral masses in metals define toxic effects after different kinds of viral contamination were determined. The induction of an immune response to a viral microbe was examined upon metal exposure to assess the effects of metals on immunity.

Comparison of Metals with Immunity Against Viral Infection in Smokers

On a subset of samples (n=15) a correlation analysis was done. Results indicate there is no significant association between metals (As, B, Pb, Sr, and Zn) and immunity in smokers (P value= ≥0.05) as given in Table 3.

Table 3: Comparison of metals with immunity against viral infection in smokers

Parameters	P-value	Mean±SD
As ppb	0.228	5.3±2.2
B ppb	0.893	290.3±11.3
Pb ppb	0.120	47.4±22.5
Sr ppb	0.228	26.4±9.2
Zn ppb	0.698	36.2±89.09

Determination of Elements by ICP-OES and Wavelength Used for Metal Detection

The estimation and evaluation of heavy metals in smokers and RT-PCR patients' negative samples was carried out using an analytical technique ICP-OES. Different wavelengths were used for the analysis of different metals.

Discussion

This study aimed to find out the affiliation between alloys and immunity towards viral samples in smokers. This is findings-categorical, that the outcomes of metals viewed as unprotected and not essential aspects of the innate immune response and host increase the chances of viral infection and toxicity. Various analyses were used to detect the correlation between immunity with viral infection, due to the presence of metals in the body. Metals, such as cadmium, lead, arsenic, and nickel, are found in cigarette smoke. These metals can accumulate in the body over time, primarily affecting the respiratory system due to inhalation. They can trigger inflammation and oxidative stress, as well as disrupt immune function.

For instance, cadmium has been shown to impair immune responses by affecting the function of immune cells and reducing antibody production. Smoking is a well-known risk factor for various diseases, including respiratory infections. The chemicals in tobacco smoke can impair the immune system, making smokers more susceptible to infections and hindering the body's ability to fight off pathogens. Smoking can reduce the effectiveness of immune cells, compromise the respiratory tract's defenses, and impair the clearance of mucus and foreign particles. Metals in cigarette smoke can synergistically interact with the detrimental effects of smoking on the immune system. These metals can further contribute to oxidative stress, inflammation, and immune dysfunction, compounding the negative impact of smoking on the immune response. The presence of metals may intensify the damage caused by smoking, making the immune system even more compromised.

Many studies show that there are negative effects to smoking, both on the immune system and many body organs [41-43]. Our results also showed there is no correlation between the low amounts of arsenic exposure in the smoker in different ways. The most important findings have been that boron consumption is inversely related to lung cancer, although low boron consumption combined with no HRT use is associated with increased odds for this disease in women, and our results show low-level boron produces immunity in smokers P value=0.228 [44]. Another study explains the immunological outcomes of low-level occupational exposure to lead, investigates the phenotypic guidelines and practical purity of peripheral blood lymphocytes in a group of firearms advisors, and contrasts the records to those bought from some unexposed controls. Their results indicate that among humans with delicately extended blood lead levels (>25 μg/dL), hazardous results on the host's immune features [45, 46], and our results also indicate a small amount of lead and strontium in the body for a long time can produce toxicity in smokers and enhance the chances of diseases. *P*-value= (0.120) and (0.228). Zn is a cofactor in greater than 300 enzymes, altering several organ functions and having a secondary effect on the immune system. A weakened immune system resulting from smoking and metal exposure can increase susceptibility to viral infections. Viruses, including respiratory viruses like influenza and respiratory syncytial virus (RSV), can cause more severe illnesses in smokers due to their impaired immune responses. Viral infections may also exacerbate the negative effects of metals and smoking on the immune system, creating a vicious cycle of immune dysfunction and susceptibility to infections. Some experiments propose that anti-oxidative enzyme activities alternate relying on their cofactor concentrations in tobacco people who smoke and indicate that zinc inflects the presumed effect of cadmium through its improvement of T-helper 2 cytokines interpretations and down-regulation of T-helper 1 cytokines. Our results have shown a high amount of zinc can cause toxicity *P*-value = (0.698) [47].

Conclusions

In summary, metals in cigarette smoke can worsen the adverse effects of smoking on the immune system, making individuals more susceptible to viral infections. Efforts to reduce smoking and minimize exposure to metals are essential to enhance immune function and protect individuals from the detrimental consequences of both smoking and viral infections. We confirm the accumulation of trace metals (As, B, Pb, Sr, Zn) in distinctive human samples that have been collected from Government College University Lahore. The amount of arsenic, boron, lead, strontium, and zinc in all blood samples of smokers is high, following WHO (World Health Organization) set limits, and all of these alloys have proven no correlation with immunity against virulent diseases. So, it can be concluded that trace metals accumulate in the body for a long duration, causing toxicity and making the immune system weak.

Acknowledgements

The authors extend their appreciation to the Researchers supporting project number (RSP2024R470), King Saud University, Riyadh, Saudi Arabia.

Funding

Not Applicable

Conflict of Interest

The authors declare no conflict of interest.

References and Notes

1. SUBRAMANIAN N., TORABI-PARIZI P., GOTTSCHALK R. A., GERMAIN R. N., DUTTA B. Network representations of immune system complexity. Wiley Interdisciplinary Reviews: Systems Biology and Medicine, **7** (1), 13, **2015**.
2. GUO Y., WANG S.Z., ZHANG X., JIA H.R., ZHU Y.X., ZHANG X., WU F.G. In situ generation of micrometer-sized tumor cell-derived vesicles as autologous cancer vaccines for boosting systemic immune responses. Nature Communications, **13** (1), 6534, **2022**.
3. DEWITT W.S., EMERSON R.O., LINDAU P., VIGNALI M., SNYDER T.M., DESMARAIS C., SANDERS C., UTSUGI H., WARREN E.H., MCELRAHT J., MAKAR K.W., WALD A., ROBINS, H. S. Dynamics of the cytotoxic T cell response to a model of acute viral infection. Journal of Virology, **89** (8), 4517, **2015.**
4. SATOH T., AKIRA S. Toll-like receptor signaling and its inducible proteins. Microbiology Spectrum, **4** (6), **2016.**
5. KAUR B. P., SECORD E. Innate immunity. Pediatric Clinics, **66** (5), 905, **2019**.

6. WANG H., COHEN A.A., GALIMIDI R.P., GRISTICK H.B., JENSEN G. J., BJORKMAN P.J. Cryo-EM structure of a CD4-bound open HIV-1 envelope trimer reveals structural rearrangements of the gp120 V1V2 loop. Proceedings of the National Academy of Sciences, **113** (46), E7151-E7158, **2016**.
7. REITSMA M.B., FULLMAN N., NG M., SALAMA J.S., ABAJOBIR A., ABATE K. H., ABBAFATI C., ABERA S.F., ABRAHAM B., ABYU G. Y. Smoking prevalence and attributable disease burden in 195 countries and territories, 1990-2015: a systematic analysis from the Global Burden of Disease Study. The Lancet, **389** (10082), 1885, **2017**.
8. KELL A.M., GALE J.R.M. RIG-I in RNA virus recognition. Virology, **479** (110), **2015**.
9. DÍAZ-ALVAREZ L., ORTEGA, E. The many roles of Galectin-3, a multifaceted molecule, in innate immune responses against pathogens. Mediators of Inflammation, **9247574**, 1-10, **2017**.
10. OKAMOTO M., TSUKAMOTO H., KOUWAKI T., SEYA T., OSHIUMI H. Recognition of viral RNA by pattern recognition receptors in the induction of innate immunity and excessive inflammation during respiratory viral infections. Viral Immunology, **30** (6), 408, **2017**.
11. NOMURA J., SO A., TAMURA M., BUSSO N. Intracellular ATP decrease mediates NLRP3 inflammasome activation upon nigericin and crystal stimulation. The Journal of Immunology, **195** (12), 5718, **2015**.
12. GALLUZZI L., VITALE I., WARREN S., ADJEMIAN S., AGOSTINIS P., MARTINEZ A.B., MARINCOLA F.M. Consensus guidelines for the definition, detection and interpretation of immunogenic cell death. Journal for Immunotherapy of Cancer, **8** (1), **2020**.
13. LEE P.N. Tar level of cigarettes smoked and risk of smoking-related diseases. Inhalation Toxicology, **30** (1), 5, **2018**.
14. SANDHYA M., SATYANARAYANA U., JOYARANI D. Study of ECG changes and left ventricular diastolic dysfunction as hemodynamic markers of myocardial stress in chronic smokers. International Journal of Research in Medical Sciences, **3** (3), 588, **2015**.
15. ZITO G., BUSCETTA M., CIMINO M., DINO P., BUCCHIERI F., CIPOLLINA C. Cellular models and assays to study NLRP3 inflammasome biology. International Journal of Molecular Sciences, **21** (12), 4294, **2020**.
16. GNEITING U. From global agenda-setting to domestic implementation: successes and challenges of the global health network on tobacco control. Health Policy and Planning, **31** (S_1), i74-i86, **2016**.
17. SABAH J.T., ALHACHAMI, F.R. Potential Anti-Cancer Properties of Frankincese (*Boswellia sarca*) Chewing Gum and its Role in Reduction of Tobacco Smoking Genotoxicity. Biomedical and Pharmacology Journal, **16** (1), 213, **2023**.
18. DURAZZO T.C., MATTSSON N., WEINER M. W., INITIATIVE A. S. D. N. Smoking and increased Alzheimer's disease risk: a review of potential mechanisms. Alzheimer's & Dementia, **10**, S122, **2014**.
19. HUANG C., SHI G. Smoking and microbiome in oral, airway, gut and some systemic diseases. Journal of Translational Medicine, **17** (1), 1, **2019**.
20. NUTT D.J., PHILLIPS L.D., BALFOUR D., CURRAN H.V., DOCKRELL M., FOULDS J., FAGERSTROM K., LETLAPE K., MILTON A., POLOSA R. Estimating the harms of nicotine-containing products using the MCDA approach. European Addiction Research, **20** (5), 218, **2014**.
21. ENGWA G.A., FERDINAND P.U., NWALO F.N., UNACHUKWU M.N. Mechanism and health effects of heavy metal toxicity in humans. Poisoning in the Modern World-New Tricks for an Old Dog, **10**, 70-90, **2019**.
22. ISMAEL D.S., MOHAMMED M.Y., SADEQ, T.W. Heavy Metal Toxicity and its Effect on Living Organisms: A Review in Toxicology Study. Pharmacy and Applied Health Sciences, **1** (2), 11, **2022**.
23. YANG M.H., WAN W.Q., LUO J.S., ZHENG M.C., HUANG K., YANG L.H., MAI H.R., Li J., CHEN H.Q., SUN X.F., LIU R.Y., CHEN G.Y., FENG X., KE Z.Y., LI B., TANG Y.L., HUANG L.B., LUO, X. Q. Multicenter randomized trial of arsenic trioxide and Realgar-Indigo naturalis formula in pediatric patients with acute promyelocytic leukemia: Interim results of the SCCLG-APL clinical study. American Journal of Hematology, **93** (12), 1467, **2018**.
24. FONTENOT A.P., FALTA M.T., KAPPLER J.W., DAI S., MCKEE A. S. Beryllium-induced hypersensitivity: genetic susceptibility and neoantigen generation. The Journal of Immunology, **196** (1), 22, **2016**.
25. MONCHANIN C., DEVAUD J.M., BARRON A.B., LIHOREAU M. Current permissible levels of metal pollutants harm terrestrial invertebrates. Science of the Total Environment, **779**, 146398, **2021**.
26. ZHENG X., XU X., LU F., WANG Q., ZENG Z., HUO X. High serum IgG subclass concentrations in children with e-waste Pb and Cd exposure. Science of the Total Environment, **764**, 142806, **2021**.
27. CLEMENS S., MA J. F. Toxic heavy metal and metalloid accumulation in crop plants and foods. Annual Review of Plant Biology, **67**, 489, **2016**.
28. GENCHI G., CAROCCI A., LAURIA G., SINICROPI M. S., CATALANO A. Nickel: Human health and environmental toxicology. International Journal of Environmental Research and Public Health, **17** (3), 679, **2020**.
29. CLEMENS S., MA J. F. Toxic heavy metal and metalloid accumulation in crop plants and foods, Annual Review of Plant Biology, **67**, 489, **2016**.
30. HAQ S., BHATTI A.A., DAR Z.A., BHAT S.A. Phytoremediation of heavy metals: an eco-friendly and sustainable approach. Bioremediation and Biotechnology: Sustainable Approaches to Pollution Degradation, 215-231, **2020**.
31. ZHENG K., ZENG Z., TIAN Q., HUANG J., ZHONG Q., HUO X. Epidemiological evidence for the effect of environmental heavy metal exposure on the immune system in children. Science of the Total Environment. **868**, 161691, **2023**.
32. CARTER E.L., GUPTA N., RAGSDALE S.W. High affinity heme binding to a heme regulatory motif on the nuclear receptor rev-erbβ leads to its degradation and indirectly regulates its interaction with nuclear receptor corepressor. Journal of Biological Chemistry, **291** (5), 2196, **2016**.
33. JIA Y., WANG L., LI S., CAO J., YANG, Z. Species-specific bioaccumulation and correlated health risk of arsenic compounds in freshwater fish from a typical mine-impacted river. Science of the Total Environment, **625**, 600, **2018**.
34. GERMOLEC D., LUEBKE R., ROONEY A., SHIPKOWSKI K., VANDEBRIEL R., VAN LOVEREN, H. Immunotoxicology: A brief history, current status and strategies for future immunotoxicity assessment. Current Opinion in Toxicology, **5**, 55, **2017**.
35. SUZUKI T., HIDAKA T., KUMAGAI Y., YAMAMOTO M. Environmental pollutants and the immune response. Nature Immunology, **21** (12), 1486, **2020**.
36. WANG C., ZHANG R., WEI X., LV M., JIANG Z. Metalloimmunology: The metal ion-controlled immunity. Advances in Immunology, **145**, 187, **2020**.
37. GERA R., SINGH V., SHARMA A. K., GHOSH D. Immunomodulation by environmental chemicals: insights into mammalian immune responses to arsenic, cadmium, and lead. Elsevier. **9,** 105, **2015**.

38. ALEKSANDROV A.P., MIRKOV I., TUCOVIC D., KULAS J., ZELJKOVIC M., POPOVIC D., NINKOV M., JANKOVIC S., KATARANOVSKI M. Immunomodulation by heavy metals as a contributing factor to inflammatory diseases and autoimmune reactions: Cadmium as an example. Immunology Letters, **240**, 106, **2021**.
39. POITEAU L., SOULIER A., ROSA I., ROUDOT-THORAVAL F., HÉZODE C., PAWLOTSKY J. M., CHEVALIEZ S. Performance of rapid diagnostic tests for the detection of antibodies to hepatitis C virus in whole blood collected on dried blood spots. Journal of Viral Hepatitis, **23** (5), 399, **2016**.
40. MOON K.A., OBEROI S., BARCHOWSKY A., CHEN Y., GUALLAR E., NACHMAN K.E., RAHMAN M., SOHEL N., D'LPPOLITI D., WADE T.J., JAMES K.A., FARZAN S.F., KARAGAS M.R., AHSAN H., NAVAS-ACIEN, A. A dose-response meta-analysis of chronic arsenic exposure and incident cardiovascular disease. International Journal of Epidemiology, **46** (6), 1924, **2017**.
41. SHAH P., TRINH E., QIANG L., XIE L., HU W.Y., PRINS G. S., PI J., HE Y.Y. Arsenic induces p62 expression to form a positive feedback loop with Nrf2 in human epidermal keratinocytes: implications for preventing arsenic-induced skin cancer. Molecules, **22** (2), 194, **2017**.
42. RAHMAN M., SOHEL N., YUNUS F.M., ALAM N., NAHAR Q., STREATFIELD P. K., YUNUS, M. Arsenic exposure and young adult's mortality risk: A 13-year follow-up study in Matlab, Bangladesh. Environment International, **123**, 358, **2019.**
43. SORIANO-URSÚA M.A., FARFÁN-GARCÍA E.D., GENINATTI-CRICH S. Turning fear of boron toxicity into boron-containing drug design. Current Medicinal Chemistry, **26** (26), 5005, **2019**.
44. FENGA C., GANGEMI S., DI SALVATOR V., FALZONE L., LIBRA M. Immunological effects of occupational exposure to lead. Molecular Medicine Reports, **15** (5), 3355, **2017**.
45. RICHARDS D. Prevalence of oral potentially malignant disorders. Evidence-Based Dentistry, **19** (4), 120, **2018**.
46. GODALA M.M., MATEREK-KUŚMIERKIEWICZ I., MOCZULSKI D., RUTKOWSKI M., SZATKO F., GASZYŃSKA E., TOKARSKI S., KOWALSKI J. Lower plasma levels of antioxidant vitamins in patients with metabolic syndrome: a case control study. Advances in Clinical and Experimental Medicine, **25** (4), 689, **2016**.
47. YANG F., LI L., CHEN J.P., LIU X.Q., ZHONG C.L., YANG Y., REN Y.F., YUAN W., LIANG H., MIAO M.H. Couple's infertility in relation to male smoking in a Chinese rural area. Asian Journal of Andrology, **19** (3), 311, **2017**.