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# Effect of Glyoxal on Cytotoxicity in Human Umbilical Vein Endothelial Cell Culture

**A. Şebnem İlhan[1], Zehra Çiçek[2]**

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**Abstract**

*Objectives:* Glyoxal is used as a biocide and disinfecting agent in pharmacy and dye production is released to the environment, air and water with emissions. Furthermore, glyoxal is produced endogenously in non–enzyme-mediated pathways in intracellular metabolism which can be detected frequently in fermented food and beverages. For this purpose, in this study we investigated the effect of glyoxal on cell viability and proliferation in human umbilical vein endothelial cell (HUVEC) line *in vitro*.

*Method:* In our study, cell culture was performed using HUVEC. Cell proliferation and viability were evaluated by spectrophotometry with tetrazolium salt (MTT) by applying different doses of glyoxal to HUVECs. Data were analyzed by SPSS 21.0 program.

*Results:* Glyoxal in doses of 320, 16, 0.8 $\mu$M significantly decreased cell proliferation when compared with control group ($p < 0.05$). The doses of $4 \times 10^{-2}$, $2 \times 10^{-3}$, $1 \times 10^{-4}$, $5 \times 10^{-5}$, $2 \times 10^{-5}$, $1 \times 10^{-6}$, $6 \times 10^{-7}$, $3 \times 10^{-7}$ $\mu$M, were found to increase cell proliferation significantly ($p < 0.05$).

*Conclusion:* According to our study, when compared to human plasma glyoxal level (0.1 – 1 $\mu$M), doses of glyoxal over 1 $\mu$M showed cytotoxic effect on cells, whereas doses below 1 $\mu$M increased the proliferation of endothelial cells. It is accepted as an important intermediate in the formation of advanced glycation end products (AGEs) by binding to the amino groups, nucleotides and lipids of the glyoxal proteins entering the cell. AGEs modification may activate cell proliferation pathways at low doses by altering protein function and influencing intracellular signaling pathways, while at high doses it may affect repair mechanisms and apoptotic processes, leading to cell damage.

**Keywords:** Cytotoxicity; Endothelium; Glyoxal; Proliferation.

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## Introduction

Diabetes mellitus (DM) develops as a result of absolute deficiency of insulin, impaired synthesis and secretion, and/or insufficient effect at the receptor and postreceptor level and characterized by impaired carbohydrate, lipid and protein metabolism and chronic hyperglycemia as such a metabolic disease.[1] Fasting and postprandial hyperglycemia are responsible for acute, early and late complications of diabetes that affect all body organs and systems. Increased incidance in both

type diabetes (Type 1 and Type 2) has a crucial role in morbidity and mortality all over the world. Hyperglycemia is associated with microvascular diseases such as nephropathy, retinopathy, neuropathy as well as macrovascular disease like coronary heart disease, stroke and peripheral artery disease.[2] Chronic hyperglycemia plays a key role in the onset of vascular complications in diabetes.[3] The mechanism underlying the vascular pathology caused by high glucose levels in diabetes is explained by five different pathways, including the polyol pathway leading to sorbitol and fructose accumulation; the pathway for advanced glycation end products (AGEs) formation; protein kinase C (PKC); the hexosamine pathway; and RAS activation but which way is dominant is still controversial.[4-6] In circulation, hypergycemia during diabetes leads to an increased concentration of α-oxoaldehydes.[7] Alpha-oxoaldehydes are highly reactive carbonyles and cause structural and functional failures by binding proteins, nucleic acids and other proteins that leads to several complications such as vascular diorders, atherosclerosis, hypertension and Alzheimer's disease.[8] In literature, complications associated with diabetes attributed to glycation of glucose with proteins. It was shown that α-oxoaldehydes like glyoxal, methylglyoxal and 3-deoxyglucosone are made up of proteins during glucose-mediated glycation.[9]

AGEs are the molecules those formed by non-enzymatic glycation of proteins, lipids or nucleic acids.[10,11] This process is ascribed to Louis Camille Maillard (1878–1936) and so called as "Maillard reaction". Non-enzymatically formed sugars by covalent binding of proteins to aldehyde and ketone groups, reduced to free amino acids leads to labile Schiff bases.[12] After that, more stable ketoamins called "Amadori products" are formed by irreversible reactions (e.g. HbA1c).[13] Amadori products are transformed into highly stable AGE compounds by further structural changes through oxidation, dehydration and degradation.[14] The reaction leads to AGE formation may take weeks or years and can effect long-lived substrates especially like collogen.[15] Increased substrates, high temperature and increaqsed oxidative stress can reduce AGE formation to several hours.[16] Hormones, enzymes, amino acids or lipids are affected short-lived products.[17–21] Although AGEs are known to be by-products of hyperglycemia, foods cooked at high temperatures[22] and cigarette smoke[23] are important exogeneous source of AGEs. Especially, dietary AGEs are important *in vivo* source for circulation.[24–26] AGEs in circulation is thought to play key role in pathogenesis of micro- and macrovascular diseases arised in hyperglycemia.[27–29] Acculumation of AGEs are proportional to age under physiological conditions[30] and this accumulation can be increased by DM, renal failure, cardiovascular disease, Alzheimer's disease, romatoid arthritis; etc.[25,32–34] Inflammation and oxidative stress,[35] increased glycation of LDL and HDL,[36] activation of proinflammatory iNOS[37] and decreaed NO[38] can be the part of underlying mechanism in detrimental effects of AGEs on the vascular system. Another mechanism involves increased monocyte and macrophage migration as well as increased production of cytokines, such as IGF-1 or PDGF, which modify the proliferation of vascular smooth muscle cells.[39,40] Effects caused by AGEs can be classified by impact areas or receptor dependencies. In vasculature, AGEs cause damage in vascular cells by[41] changing intracellular proteins which regulates gene transcription.[18] AGE precursors leave cells by diffusion and change extracellular matrix proteins firstly, then, by changing signaling between matrix and cells leads to cellular disfunction.[42] AGEs and their precursors can change circulatory proteins leading to functional change. AGEs-modified proteins in circulation binds to AGE receptors and activate them thus changes the production of inflammatory cytokines and growth factors that promote cell and tissue damage.[24,39] In fact, activation of certain receptors for AGEs (e.g. RAGE) promote inflammatory response, mainly by nuclear factor kB (NFkB), apoptosis, prothrombotic activity, expression of adhesion molecules, and activation of oxidative stress[43-45] in addition, AGE-RAGE interaction can activate iNOS. iNOS is mainly found in inflammatory cells, regulated by inflammatory cytokines, and can be produced by toxic concentrations of NO when stimulated by NFkB-induced oxidative stress.[46,47] Subsequent reaction with the oxygen radical generates an excessively reactive metabolite called peroxynitrite. Peroxynitrite interacts with protein and DNA to cause nitrative stress, DNA damage, further NFkB, caspase-3 activation, and vascular cell apoptosis.[48] Animal models and *in vitro* studies support that AGEs affect different cells in atherosclerosis such as endothelial cells,[39,49,50] platelets[51] monocytes/macrophages[52] or vascular smooth muscle cells[53] and thus are associated with vascular disease. Interaction of AGEs with endothelial cells expressing RAGE decreases endothelial barrier function with increased permeability and subendothelial lipid entry. AGE-RAGE interaction triggers the expression of adhesion molecules such as VCAM-1[54] and also promotes the formation of Foam cells by promoting

transendothelial migration of monocytes.[54-56] Xu et al. showed that during incubation of human umbilical vein endothelial cells (HUVEC) with AGE-modified albumin without high glucose medium, NOS endothelial isoform was supressed by concentration and time dependent.[57] Naser et al. showed that 5 min incubation with AGE in bovine aortic endothelial cells causes depletion of intracellular $Ca^{2+}$ stores.[58] When angiogenesis is impaired in the peripheral vascular system, it contributes to delayed wound healing, exacerbation of ischemia in the peripheral extremities, and exacerbates cardiac morbidity with reduced collateral vascular development.[59] The mechanism lying under impared angiogenesis induced by AGEs is explained by Liu et al.[60] Researchers have shown that using *in vitro* endothelial cells and mouse aortas, methylglioxaline (highly reactive AGE precursor) decreases endothelial angiogenesis through RAGE-mediated, peroxynitrite-dependent, and autophagy-induced vascular endothelial growth factor receptor 2 (VEGFR2) degredation. VEGFR2 is the main receptor that causes vasodilatation, endothelial cell migration and proliferation in vascular endothelial growth factor signaling.[60,61] Extracellular AGEs are responsible for impaired cell proliferation and adhesion and inhibition of growth by cross-linking with extracellular proteins.[62] The major extracellular proteins targeted for glycation are long-lasting matrix proteins such as collagen Types I, III and IV, elastin, cartilage proteoglycan aggregate, and short-lived plasma proteins such as ApoB, LDL, albumin, immunoglobulins. Glycation of intracellular and extracellular proteins changes protein function and disrupts cellular metabolism. RAGE is an AGE receptor that accumulates in diabetes, the aging process and neurodegenerative processes in Alzheimer's. RAGE is abundant during embryonic development and is thought to be involved in cell migration.[63] Binding of RAGE with different ligands results in activation of the transcription factor NFkB.[64] Binding proteins for RAGE or AGE moieties have been shown in monocyte/macrophages, endothelial cells, pericides, T-lymphocytes, mesengial cells and Type I pneumocytes and osteoblast-like cells.[65–68]

## Materials and Methods

We used Glyoxal 40% solution (Cat No: 820610-1L, Merck) in our study. Glyoxal is used as a biocide and disinfecting agent in pharmacy and paint production, is released to the environment, air and water with emissions. Furthermore, glyoxal is produced endogenously in non–enzyme-mediated pathways in intracellular metabolism which can be detected frequently in fermented food and beverages. In human blood plasma the concentration of glyoxal is 0.1–1 $\mu$mol/L, of course it has higher levels in patients with diabetes or renal failure. Glyoxal, is considered to be an important intermediate in the formation of advanced glycation end products (AGEs) by attacking amino groups of proteins, nucleotides, and lipids. Skin irritation, allergic skin reaction and serious eye irritation are the effects of glyoxal; and causing genetic defects also. In animal models, the acute toxicity of glyoxal is low to moderate, depending on the actual concentration of glyoxal in the tested product. In rats, for 40% glyoxal, the LC50 for a single 4-h inhalation of aerosol is 2440 mg/m$^3$, the oral $LD_{50}$ value ranges from 3000 to 9000 mg/kg body weight (with higher sensitivity in females), and dermal $LD_{50}$ values are >2000 mg/kg body weight. Exposure with inhalation causes local irritations of the eyes and respiratory organs. After oral exposure to glyoxal, macroscopic observations include irritations of the gastrointestinal tract and congestion in the gastrointestinal tract, lung, kidney, and adrenal glands are seen.[70]

We used HUVECs in our study. When the cells were examined with inverted microscope, it was seen that the cells spread and multiplied by holding on the bottom of the culture chamber. The cultured cell medium was changed every 72 hours. The culture of confluent exhibited a typical endothelial cell characteristic of cobblestone morphology with large and dark nuclei inside the cell (Fig. 1). Appearance of HUVECs in light microscopy (X400). Cell proliferation and viability were evaluated by MTT method by culturing from HUVEC cell line. The MTT method is a frequently used cell proliferation test based on the measurement of metabolic activity used to assess cell proliferation, viability and cytotoxicity. With this method, the proportion of living cells in the cell population can be determined quantitatively. When the dehydrogenase enzymes in the mitochondria of intact cells reduce the MTT stain and break down the tetrazolium ring a color change occurs. This change can be evaluated by this method. This reaction depends on the activity of succinate dehydrogenase, a fragile mitochondrial enzyme. As a result of the degradation of tetrazolium ring in living vascular smooth muscle cells, the pale yellow MTT dye becomes dark blue-purple formazone product.[71–73] The method is mainly based on the principle of colorimetric measurement of the absorbance value of the color change resulting from the conversion

of proliferating cells to violet formazone using yellow water soluble tetrazolium with increased dehydrogenase activity. Since proliferating cells are more metabolically active than non-proliferating cells, not only cell viability and cytotoxicity but also cell activation and proliferation are evaluated with this method. The tetrazolium salt is converted to a water-insoluble formazone ring in the presence of a living cell and formazone crystals dissolve and turn purple by the addition of detergent-active solutions such as DMSO. In the proliferation study, HUVECs were seeded in a 96-well culture dish with $5 \times 10^3$ – $10^4$ cells per well. After cells were incubated for 24 hours in an incubator containing 5% $CO_2$ – 95% air at 37°C, glyoxal at doses of 320, 16, 0.8, $4 \times 10^{-2}$, $2 \times 10^{-3}$, $1 \times 10^{-4}$, $5 \times 10^{-5}$, $2 \times 10^{-5}$, $1 \times 10^{-6}$, $6 \times 10^{-7}$, $3 \times 10^{-7}$ $\mu$M were added to each well and incubated again for 24 hours. At the end of the incubation, the solution on the cells was removed. Cells were incubated for 4 hours by adding 100 $\mu$L of MTT (5 mg/mL MTT) solution to each well. At the end of this period, chemicals were removed from the wells by pipette and 100 $\mu$L of DMSO was added. The cells were incubated for 20–30 minutes to dissolve the formazone crystals. It was observed that the pale yellow MTT dye formed as a result of the degradation of the tetrazolium ring turned into a dark blue-violet formazone product. DMSO was used to dissolve MTT and reduction products. After incubation of ninety-six-well cell culture dish with DMSO for 20–30 minutes, color change was evaluated as absorbance at 570 – 630 nm wavelength with spectrophotometer or plate reader. The mean absorbance of each group was calculated by subtracting the absorbance value of each well at 570 nm wavelength from the absorbance value at 630 nm (A570–A630 nm).

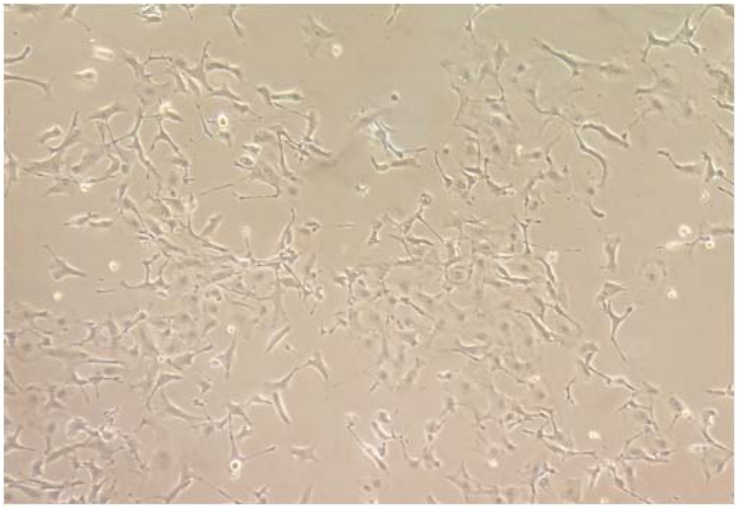

**Fig. 1.** Appearance of HUVECs in invert microscopy (X400).

### *Statistical analysis of data*

SPSS 21.0 package program was used for statistical analysis of the data. Data were expressed as mean ± standard error. In the statistical analysis, after examining whether the variances were homogeneously distributed or not, the parametric test ANOVA was used. In case of difference, post hoc Dunnett test was performed to show which groups were significant among the groups. The statistical significance level was taken as 0.05 in all tests.

## Results

The HUVECs in the cell culture dishes were spread on the surface of 75 $cm^2$ flask and the proliferation and cell morphology were evaluated with an inverted microscope (Fig. 1). Appearance of HUVECs in light microscopy (X400).

Glyoxal at doses of 320, 16, 0.8 $\mu$M significantly decreased cell proliferation compared to control group ($p = 0.000$, $p = 0.000$, $p = 0.000$, repectively). Doses of $4 \times 10^{-2}$, $2 \times 10^{-3}$, $1 \times 10^{-4}$, $5 \times 10^{-5}$, $2 \times 10^{-5}$, $1 \times 10^{-6}$, $6 \times 10^{-7}$, $3 \times 10^{-7}$ $\mu$M, significantly increased cell proliferation ($p = 0.001$, $p = 0.001$, $p = 0.000$, $p = 0.003$, $p = 0.002$, $p = 0.000$, $p = 0.000$, $p = 0.000$, respectively), (Fig. 2), Glyoxal dose proliferation study in HUVEC culture. Data are expressed as mean ± SD, * $p < 0.05$ compared to control. Oneway ANOVA Post hoc Dunnett test ($n = 8$–16).

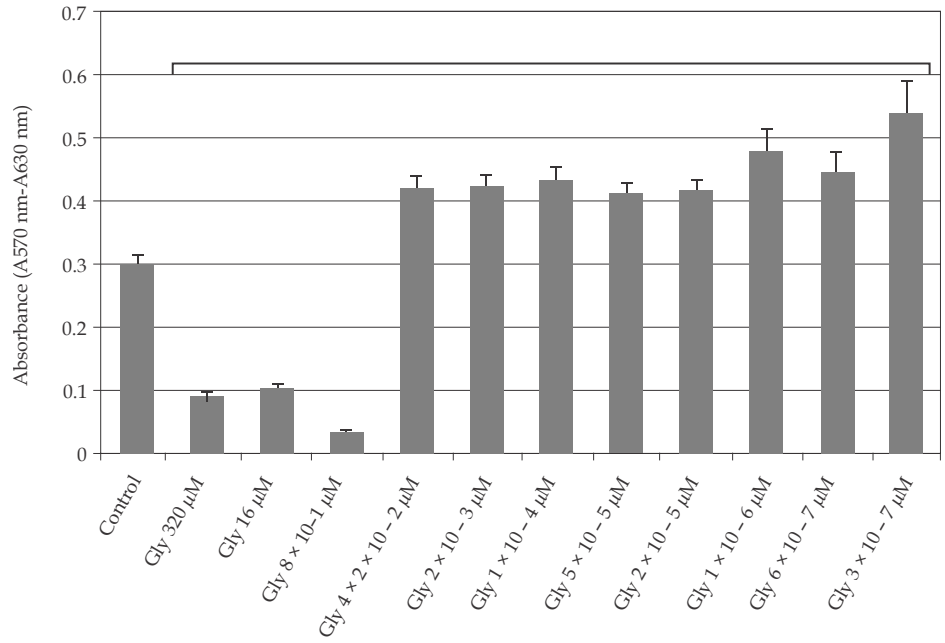


**Fig. 2:** Glyoxal dose proliferation study in HUVEC culture. Data are expressed as mean ± SD
* $p < 0.05$ compared to control. Oneway ANOVA Post hoc Dunnett test ($n$ = 8–16).

## Discussion

Our findings showed that, when compared with human plasma glyoxal level (0.1–1 $\mu$M), doses of glyoxal above 0.8 $\mu$M had cytotoxic effect on cells, but doses below 0.8 $\mu$M increased the proliferation of endothelial cells. Glyoxal, causes cytotoxicity, changes in cell morphology, mitochondrial dysfunction, cell skeleton rearrangements, barrier dysfunction, inhibition of DNA synthesis and cell replication and inhibition of vascular endothelial cells. It is accepted as an important intermediate in the formation of advanced glycation end products (AGEs) by binding to the amino groups, nucleotides and lipids of the glyoxal proteins entering the cell.[74] During diabetes, AGEs are paired with a number of complications such as the pathogenesis seen in cardiovascular diseases like vascular damage including macrovascular and microvascular complications. Cross-linking with AGE has been reported to cause vascular stiffening and endothelial dysfunction.[74] Glyoxal reacts with arginine to form imidazolium and with lysine to form N-carboxymethyl lysine (CML).[75] 40–50% of AGEs are estimated to form Schiff's base originating from glyoxal.[76] Glyoxal adducts with nucleic acids (DNA and RNA) causing mutations. It has been reported that α-oxoaldehydes-mediated glycation plays a role in diabetic vascular damage.[75,77] It is known that vasculopathy during diabetes is the cause of various cardiovascular diseases.[78] Generally, it is attributed to diabetic vascular damage, glycative, glyoxidative, carbonyl and oxidative stress due to increased glucose levels.[79,80] Methylglyoxal exposure revealed diabetes-like microvascular changes and damage in rats.[81] Increased serum levels of AGE in patients with Type 2 diabetes are associated with endothelial dysfunction.[82] High levels of intracellular AGE formation in endothelial cell culture has shown to be induced by heypergylcemia due to ROS.[83] AGEs show their effect on vascular ECs by interacting with RAGE.[84] AGEs also mediate oxidative stress (lipid peroxidation) by altering gene expression and vascular abnormalities by interacting with RAGE in vascular ECs.[85,86] Interacting with RAGE, AGE causes increased EC permeability and vascular hyperpermeability *in vitro*.[55] In the mean while AGE-modified human serum albumin (HSA) has been shown to induce hyperpermeability and actin cytoskeletal reorganization in ECs.[87,90] Both glucose-derived oxoaldehydes and AGE-modified proteins cause EC cytoskeletal rearrangement and hyperpermeability. Albumin-derived AGEs have been shown to disrupt vascular EC junctions associated with increased EC permeability, such as cadherins and catenins.[89] Sliman et al. showed that in BPAECs, glyoxal induces reorganization of tight junction protein ZO-1 and suggested that glucose-derived α-oxoaldehyde is effective in the alteration of EC tight juntions that would cause EC barrier dysfunction and hyperpermeability.[90] Healthy ECs are very important for angiogenesis. The results of

Sliman's study show that glyoxal inhibits *in vitro* angiogenesis (tube formation) in BPAECs culture. As a result glyoxal causes cytotoxicity, changes in cell morphology, mitochondrial dysfunction, inhibition of cell replication and cytoskeletal changes. Glycated basic fibroblast growth factor (FGF-2), similar to AGEs in hyperglycemia, is shown to activate signal transduction pathways[91] also activate specific signaling pathways including oxoaldehydes in vascular ECs including PTyKs, p38 MAPK, extracellular signal-regulated kinase and JNK. shown.[92] Glycation and oxidative stress mediated lipid peroxidation in hyperglycemic conditions contributes to the formation of reactive carbonyl species and induction of carbonyl stress.[93] Glyoxal cytotoxicity in hepatocytes is associated with GSH depletion, oxidative stress and mitochondrial damage.[75] In another study by Shangari et al. 10 mM glyoxal concentration causes oxidative stress.[93] In Sliman's study it was accepted that oxidative stress mediated glyoxal cytotoxicity in BPAECs.[89] Kasper M et al. reported that up to 0.4 mM, glyoxal causes apoptosis in human embryonic lung epithelial cells.[94] In immortalized E1A-NR3 retinal cells, up to 0.8 mM glyoxal causes cytotoxicity, alterations in cell morphology, mitocondrial and DNA damage.[95] Cervantes-Lauren et al.[96] and Sliman's studies in BPAEC model, high concentrations of glyoxal (1–10 mM) could have been drastically lowered due to the formation of Schiff's base adducts, AGEs, and also enzymatic and non-enzymatic degradation at the target sites.

Glyoxalase has shown to metabolically detoxify glyoxal by converting glyoxal to glycolic acid. However, high concentrations of glyoxal have been shown to inhibit glyoxal-metabolizing enzymes. High concentrations of glyoxal were also detected in diabetic conditions (~27.2 $\mu$g/ml). Sliman et al.[89] showed for the first time glyoxal related changes such as cytotoxicity, cytoskeletal changes and barrier dysfunction in vascular ECs. Glyoxal may activate cell proliferation pathways at low doses by altering protein function in endothelial cells and influencing intracellular signaling pathways, while at high doses it may affect repair mechanisms and apoptotic processes, leading to cell damage. These effects are due to the fact that glyoxal activates different receptors or due to disturbance on certain receptors. AGEs are known to play a major role in vascular cell injury, and microvascular and macrovascular complications associated with hyperglycemia increase the cost of treating diabetes. In addition, it causes additional organ damage and extends the spread of the disease in the body, causing damage to many organs and systems. Therefore, it seems to be necessary to plan and produce treatment regimens that inhibit AGE formation and receptor binding.

When it is considered that glyoxaline is also an AGE variant, it seems necessary to clarify its effects at cellular level and the pathological mechanisms it elicits. It is also important to clarify in which signaling pathways glyoxal plays a more active role on receptors. Each new mechanism to be discovered is important in finding solutions for the treatment of diabetes and developing new treatment strategies. For this purpose, additional studies are needed to place the results of our study on a more meaningful basis.

***Conflict of Interest:*** No

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*Original Article*

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# Toxicology of the Various Elements in the Decoction Samples of Lemon Balm and Sage Species

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**Abstract**

Plants are used for many purposes from past to present. One of them is consumed by people for therapeutic purposes. Some compounds or excessive heavy metal intake during consumption can be harmful to human health. In this study, the amount of Aluminum (Al), Nickel (Ni) and Cobalt (Co), which are toxic elements in the decoction samples obtained with different sample amount (g) and decoction time (min) of *Melissa officinalis* L. (lemon balm) and *Salvia officinalis* L. (sage) were investigated. All elements were analyzed by Inductive Coupled Plasma-Mass Spectrophotometer (ICP-MS) at Yozgat Bozok University Science and Technology Application and Research Center. The calibration curve was plotted with 11 points. In both species, the amount of Al was observed to be higher than the other elements. The highest amount of Al was observed in sage plant (1155.6 ppb), while the highest amount of Ni was found in lemon balm (771.3 ppb). In contrast, the maximum amount of Ni and Al was found in the decoction samples of the lemon balm. There was no statistically significant difference between the amounts of Co element in lemon balm and sage decoction samples. In general, increased amount and increased application time of lemon balm plant were found to cause an increase in Al and Ni amounts. As a result, toxicological evaluation of such plants used for their therapeutic properties is important for human and public health.

**Keywords:** Sage; Lemon balm; Toxicology; ICP-MS; Decoction.

## Introduction

Medicinal sage (*Salvia officinalis* L.) and lemon balm (*Melissa officinalis* L.) plants are among the most consumed herbal teas. Furthermore, they are medicinal and aromatic plants rich in rosmarinic acid, an important flavonoid compound in both species.[10,8] While the therapeutic and antioxidant components of bioactive compounds are rosmarinic acid, caffeic acid and derivatives, chlorogenic acid, ferulic acid, gallic acid, quercetin, quercitrin and routine in lemon balm plant,[5,11,12] in medical sage, it has been found that compounds such as rosmarinic acid, ellagic acid, caffeic acid, routine, chlorogenic acid and quercetin are present.[4,8] Many scientists have reported that these compounds exhibit numerous biological activities.[7,2,13,9] Therefore, parts of these species such as herba, leaf are actively used in many fields such as traditional medicine, cosmetics, medicine, food and perfumery.[1] In particular, the use of herbal medicines to alleviate or treat human disease is increasing day by day in many parts of the world due to its low side effects.[6] The most common forms of drug preparation and consumption of herbal products can be listed as powder, pill, infusion, decoction, ointment, tincture, medicinal oil and scented oil.[3] During the preparation of the drug, the elements in the plant pass to the preparation.

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## Materials and Methods

Plant samples of sage (*Salvia officinalis* L.) and lemon balm (*Melissa officinalis* L.) which was cultivated in Yalova were used in the preparation of decoction samples.

### *Preparation of plant samples*

The leaves of medicinal sage and lemon balm were dried in the shade and herbal teas were obtained by decoction method. For this method, different amounts (2g and 3g) and different periods (5 min and 10 min waiting) were applied (Table 1). At the end of the application, the tea samples were filtered and stored in a refrigerator at 4°C until analyzed.

### *Al, Ni and Co analyzes*

Decoction samples digested with 5 mL Suprapur (Merck, Darmstadt, Germany) nitric acid ($HNO_3$), 2 ml hydrochloric acid (Merck, Darmstadt, Germany) and 3 ml ultrapure water (Direct-Q; Millipore, Darmstadt, Germany)in the Teflon tubes using a microwave digestion system (Start D; Milestone, Maryland).

The digested samples diluted with ultrapure water to the total volume of 20 ml in a 50 ml polypropylene tube. Al, Ni and Co standards (Multi-Element Standard Chem-Lab, Zedelgem, Belgium) used for calibrations. 11-point calibration curve (0.5–1000 ppb) was used to measure the level of each element.

**Table 1:** Preparation of decoction samples of lemon balm and sage

| Decoction Samples | Amount of samples (g) | Waiting time (min) |
|---|---|---|
| D1 | 2 | 5 |
| D2 | 3 | 10 |
| D3 | 2 | 5 |
| D4 | 3 | 10 |

The $r^2$ values of the calibration curves of all parameters were calculated, and the minimum value was 0.9998. Al, Ni and Co were measured using inductively coupled plasma-mass spectrometry (ICP-MS, ICAPQc, Thermo Scientific, USA). The results of these measurements showed that the relative standard deviations did not exceed 5%. Certified Reference Material (CRM-Seronorm Trace Elements Whole Blood L-2, Sero AS, Billingstad, Norway) was used for the validation method. Internal standard (Hf) was used to check the stability and sensitivity of the instrument.

### *Statistics*

All data was statistically analyzed using one-way ANOVA, and comparison of the means was carried out by Duncan's multiple range tests at a significance level of 0.05 and the data were given as the mean ± standard error. The data matrix of the plant samples and their decoction samples was obtained from hierarchical cluster analysis.

## Results and Discussion

In this study, the effects of different amounts and time application on the heavy metal contents such as Al, Ni, and Co which are found in the decoction samples of sage and lemon balm plants and affect human health were investigated. In both species, the highest amounts were Al, Ni and Co, respectively.

For sage decoction samples, the highest amount of Al was statistically highest in AD1 and AD4 samples at 331.8 and 319.6 ppb, respectively.

The highest Ni content was observed in the AD4 decoction sample. The amount of Co was found to be statistically insignificant in all decompression samples (Table 2).

**Table 2:** Heavy metal contents in sage and its decoction samples (ppb)

| Sample | | Al | | Ni | | Co | |
|---|---|---|---|---|---|---|---|
| **Dry sample (A)** | | 1155.6 | a | 692.3 | a | 75.6 | a |
| **Decoction** | AD1 | 331.8 | b | 69.3 | c | 7.6 | b |
| | AD2 | 212.6 | d | 60.8 | d | 7.2 | b |
| | AD3 | 276.6 | c | 74.8 | c | 8.0 | b |
| | AD4 | 319.6 | bc | 86.0 | b | 10.2 | b |

The highest Al and Ni content was found in the MD4 decompression samples. Co contents were reported to be statistically insignificant in decoction samples similar to sage (Table 3). However, the maximum amount of Co in the decoction samples of both species was observed in D4 decoction samples.

When the sage and lemon balm were compared, the amount of Al was observed in the highest sage with 1155.6 ppb in herba samples, but in all the decoction samples of the lemon balm plant (Fig. 1). The amount of Ni was determined both in herba samples and in the highest lemon balm plant in terms of decoction samples. When Fig. 2

**Table 3:** Heavy metal contents in lemon balm and its decoction samples (ppb)

| Sample | | Al | | Ni | | Co | |
|---|---|---|---|---|---|---|---|
| **Dry sample (M)** | | 862.1 | a | 771.3 | a | 91.6 | a |
| **Decoction** | MD1 | 384.9 | d | 105.4 | d | 8.4 | b |
| | MD2 | 485.7 | c | 136.8 | c | 10.2 | b |
| | MD3 | 490.6 | c | 146.7 | c | 9.5 | b |
| | MD4 | 664.5 | b | 178.2 | b | 11.4 | b |

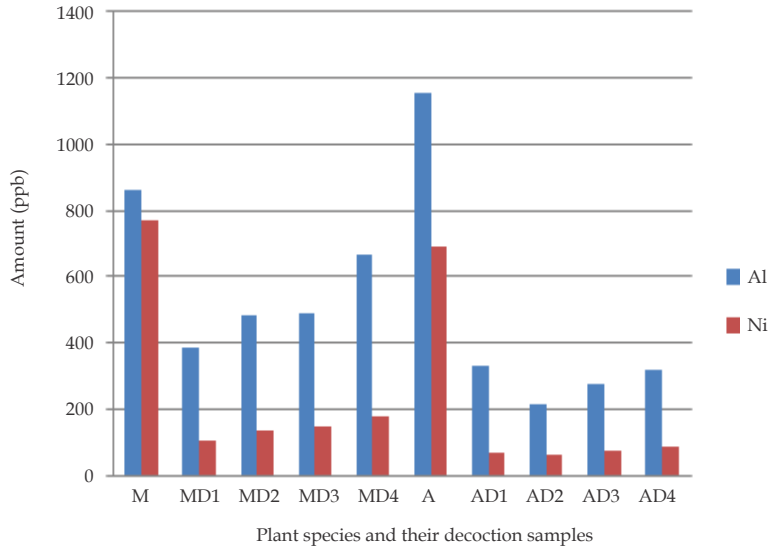


**Fig. 1:** Al and Ni distribution in lemon balm, sage and their decoction samples (ppb).

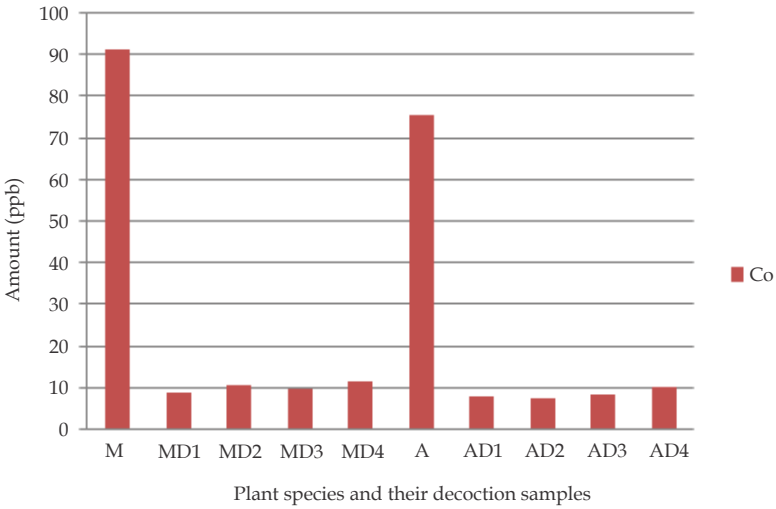


**Fig. 2:** Co distribution in lemon balm, sage and their decoction samples (ppb).

was examined, Co was also observed in both herba and decoction samples (MD4) of the highest lemon balm species.

Table 4 shows how many percent of the Al, Ni and Co micatars contained in the plant are transferred to the decoction samples. It was determined that Al was the most transitive element among the heavy metals in both species (Average, M: 58.7% and A:24.7%). The highest Al transition to the decoction samples was observed in the D4 decoction application of lemon balm plant with 77.1%. This shows that the amount of transition is very high. This situation is important for human and public health. In terms of Co and Ni, Ni was found to be the highest element transition percentage (18.4%) in lemon balm and Co element (10.9%) in sage. However, in the sage and lemon balm Co element has been observed to have similar values on average (10.9% and 10.8%, respectively). When the effect of different plant sample and decoction time on the amount of Al, Ni and Co in the preparation of the decoction samples were examined, it was found that the amount and transition time of these heavy elements increased as the amount and application time increased. However, it was observed that the amount of 2 g substance in sage was more effective than 5 min application (AD1) than 10 min application (AD2).

**Table 4:** Percentage of the elements in their decoction samples compared to the plant species

| | Al | Ni | Co |
|---|---|---|---|
| **Lemon Balm** | | | |
| MD1 | 44.7 | 13.7 | 9.1 |
| MD2 | 56.3 | 17.7 | 11.2 |
| MD3 | 56.9 | 19.0 | 10.4 |
| MD4 | 77.1 | 23.1 | 12.4 |
| Average | 58.7 | 18.4 | 10.8 |
| **Sage** | | | |
| AD1 | 28.7 | 7.2 | 10.1 |
| AD2 | 18.4 | 6.3 | 9.6 |
| AD3 | 23.9 | 7.8 | 10.5 |
| AD4 | 27.7 | 8.9 | 13.5 |
| **Average** | 24.7 | 7.5 | 10.9 |

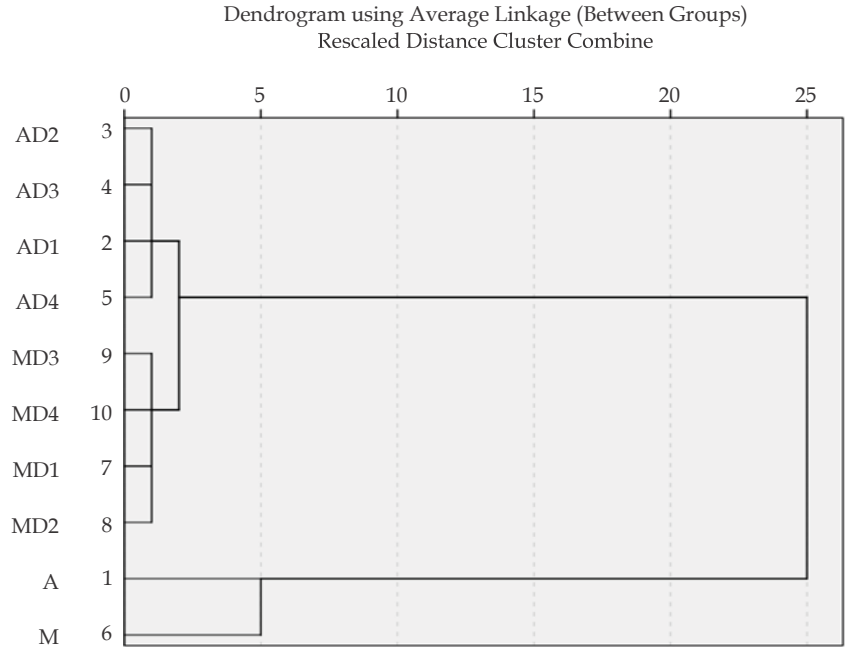


**Fig. 3:** Dendrogram of sage, lemon balm and their decoction samples (versus each of the four elements under study) obtained by hierarchical cluster analysis using square euclidean distance.

In the application of 3 gr, it was recorded that the amount of transition of the elements increased with increment time.

A hierarchical clustering by applying the Between-groups linkage method, which uses the squared euclidean distance as a similarity measure, was applied using the SPSS package. In dendrogram in Fig. 3, according to the elements tested, the decoction samples of sage were in the same group (Group 1), and the decoction samples of lemon balm were in the same group (Group 2), as well as dry samples of sage and lemon balm were in the same group (Groups 3). Group 1 was in the group close to Group 2 while Group 3 alone formed a separate entity from other groups.

## Conclusion

As a result, toxicological evaluation of such plants used for their therapeutic properties is important for human and public health.

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# Fetuin A: Is a New Biomarker for Growth?

**Esra Domur[1], Filiz Tubas[2], Vugar Ali Turksoy[3], Lütfiye Tutkun[4], Mujgan Ercan[5], Fatma İnci Arısoy[6]**

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**Abstract**

Vitamin D deficiency is common in the World and Turkey due to changing lifestyles. The association with autoimmune diseases such as obesity, metabolic syndrome, diabetes, cardiovascular diseases and important diseases such as cancer has made it important to prevent vitamin D deficiency. Fetuin-A is a glycoprotein produced in the liver and it is effective in the inhibition of bone mineralization, insulin resistance, obesity and calcification in smooth muscle. In the study conducted, it was aimed to investigate the relationship between the two substances in growth retardation, considering that Vitamin D and Fetuin-A are acting through similar mechanisms. The study was conducted with 50 healthy children with growth retardation. In short patients and underweight patients, vitamin D levels were found to be lower than control group (respectively $p$ = 0,011 $p$ = 0,036). As vitamin D value increased one unit, it was found that the risk of growth retardation decreased by 1,071 times ($p$ = 0,21). Fetuin-A levels were higher in those with growth retardation ($p$ = 0,035). In the preschool group, fetuin-A was higher than middle childhood ($p$ < 0,001). In short patients, fetuin-A levels were higher than control groups ($p$ = 0,043). There was a poor correlation between vitamin D and fetuin-A levels in those with growth retardation (rho: 0,366 $p$ = 0,009). Fetuin-A levels were higher in short children and there was a positive relationship between vitamin D and fetuin-A levels in those with growth retardation. Vitamin D levels were lower in those with growth retardation.

**Keywords:** Fetuin-A; Vitamin D; Growth retardation; Underweight, Short stature.

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## Introduction

Low height and/or weight measurements of children who have not completed growth compared to their peers is defined as growth retardation.[1] Growth retardation is common in patients with malnutrition, food intake problems, absorption disorders, chronic diseases such as chronic lung disease and cardiovascular disease.[2] Growth and height of the child primarily depends on many variable factors such as normal bone structure, proper nutrition, tissue oxygenation, pH of the environment, hormones, environment

and additional diseases. The relationships between growth hormone (GH), thyroid hormones, insulin-like growth Factor-1 (IGF-1), sex hormones in the pubertal period and their receptors are effective in achieving normal height growth.[3] Although the main factor in the development of bone mass is the genetic structure, various factors such as activity, nutrition and lifestyle also affect the genetic structure. The most important factors that play a role in the genetic structure are collagen Type 1, Vitamin D receptors (VDR), IGF-1 and estrogen related genes.[4] The prevalence of growth retardation may vary depending on the definition of the term and the participant and therefore, it has been stated that it is between 1.3% and 20.9% in various sources.[5] Growth retardation may have many different causes, however irrespective of the reason, it is known that a child with growth retardation may have many mental, physical and psychological problems in the future.[2] Growth retardation in the world and in Turkey decreased over the years, though, it remains important because of the advanced stage effects.[1,6] Nutritional disorders and deficiency of vitamins and minerals are seen in children with growth retardation.[7] Although treatment and follow-up are important in the approach, early diagnosis and prevention efforts are more effective to reduce cost and comorbidity.

There are different parameters depending on the type of percentile curve used to express growth retardation. These are listed below:

1. Body mass index is below the 5$^{th}$ percentile
2. Height-for-age is below the 5$^{th}$ percentile
3. Weight-for-age is below the 5$^{th}$ percentile
4. Weight deceleration that crosses two major percentile lines on a growth chart

It is accepted that there is growth retardation in the presence of at least one of the mentioned criteria.[7]

Vitamin D is a fat-soluble vitamin and has been recognized as a hormone when its functions and structure are better understood.[8] It can be taken with diet, it can also be synthesized in the body by the effect of sun rays. Its main function is on calcium and phosphorus metabolism.[7] If the calcium level in the body is sufficient, calcium and phosphorus are absorbed from the intestines by the effect of 1.25 $(OH)_2D$ while bone mineralization is provided.[7] Vitamin D metabolism is tightly regulated by calcium, phosphorus, PTH serum levels, fibroblast growth factor 23 (FGF 23) and 1,25 $(OH)_2D_2$.[7] While vitamin D is necessary to maintain normal calcium levels in adults, calcium homeostasis and bone development are not dependent on vitamin D levels in the fetal period.[9] Dark skin color, insufficient sunlight, absorption disorders, the use of anticonvulsants and some drugs increase the risk of Vitamin D deficiency.[10] It is commonly seen in Turkey and worldwide.[11–13] Vitamin D deficiency is the most common cause of osteomalasia and if the bone has not completed its growth, rickets is seen and as a result the bone growth is disrupted.[7] In today's world lifestyles are changing in many ways and increased amount of time spent indoors reduces sun exposure.

However, when examined in more detail, it is understood that vitamin D deficiency causes problems clinically even without rickets. Vitamin D has been shown to be associated with autoimmune diseases such as cancer, metabolic syndrome, cardiovascular diseases and diabetes.[10]

To define Vitamin D status in healthy children and adolescents, the following standards of the 2016 Global Consensus Guidelines,[14] based on the measurement of 25 (OH) D serum concentrations, similar to the recommendations of the Pediatric Endocrine Society, are used.

- Vitamin D sufficiency: 20 to 100 ng/mL (50 to 250 nmol/L)
- Vitamin D insufficiency: 12 to 20 ng/mL (30 to 50 nmol/L)
- Vitamin D deficiency: <12 ng/mL (<30 nmol /L)

Fetuin-A is a glycoprotein produced mainly in the liver and is produced abundantly in multiple tissues during embryogenesis and the amount decreases after birth.[15–17] This suggests that Fetuin-A may be related to organ development and growth, and also acts as a negative acute phase reactant.[15] It is one of the most common non-collegenous proteins in bone and teeth.[18] It prevents calcification in smooth muscles.[19] In addition to being an ectopic calcification inhibitor, it has been shown to have many different functions.[15] Fetuin-A acts as an inhibitor of transforming growth factor-$\beta$ (TGF-$\beta$), insulin-like growth factor (IGF) and hepatocyte growth factor [liver growth factor (HGF)].[20] With this aspect, it acts as a regulator in tissue regeneration and has roles in bone metabolism.[20,21] Metabolic syndrome has been associated with insulin resistance and diabetes.[22–24]

Fetuin-A plays a role in calcium and phosphate balance. Although its effect on bone formation and mineralization is not fully understood, it has strong affinity to hydroxyapatites and plays a role in bone formation.[25]

It was found that Fetuin-A level is inversely related to cardiovascular disease risk.[26] Fetuin-A deficient mice showed resistance to weight gain.[27] Only normal Fetuin-A levels are useful for humans.[28] However, not all functions of Fetuin-A have been fully elucidated.

The effects of Fetuin-A on bone metabolism and its relationship with metabolic syndrome and diabetes brings in mind vitamin D which has similar effects.[7,10,16,20,22,23,25,27] As a result of the literature search, no article examining Fetuin-A relationship with children with growth retardation was found.

The aim of this study was to evaluate the level of Fetuin-A in patients with growth retardation and to determine its relationship to weight or height, and to assess its association with Vitamin D, if any.

## Materials and Methods

The study was started after the approval of the ethics committee of Bozok University Research and Application Hospital numbered 2017–04/02. The research was supported by Bozok University Scientific Research Projects Unit with the project number of 6602b-TF/17–106.

The study was conducted between February 2017 and May 2018 by informing the children and their families who applied to our outpatient clinic and fulfilling the inclusion criteria, and obtaining an informed consent form. Detailed medical history was taken and physical examinations of the subjects were performed and those who were in accordance with the study criteria after anthropometric measurements were included in the study. Participants were divided into two groups as healthy and growth retarded. Each group was divided into play-age children (1–6 years) and school-age children (7–11 years) according to age groups.

Study Group Acceptance Criteria:

1. Being between the ages of 1–11.
2. Height-for-age and weight-for-age measurements which are below 3 percentile according to Olcay Neyzi's percentile curves or height-for-age and weight-for-age measurements at -2SD and/or 2SD growth deviation (growth pause) during follow-up.
3. Having no chronic or acute disease in terms of genetic, metabolic, liver, congenital, cardiovascular, renal, respiratory or other systems.
4. Not taking medication for any reason
5. Having not taken vitamin D supplements in the last 3 months.
6. Voluntarily participating the study and signing the inform consent form.
7. Venous blood samples will be taken on the day of admission for any reason.

In the control group, the following conditions were requested: To have a 3–97 percentile curve according to the percentile curves of the same age range, to have no disease, not to use medication, not to use vitamin D in the last 3 months, voluntarily participating the study and having venous blood sample taken on the admission day.

The children included in the study were duly measured and weighed by trained personnel. Centimeters (cm) were used as units of length. Kilograms (kg) were used as a weighing unit. All of the measurements were marked on the growth charts prepared by Olcay Neyzi et al.[29] according to the age and gender. Afterwards, detailed anamnesis of the participants was taken according to their chronic or acute diseases, medications, nutritional history and family history and detailed physical examinations were performed.

Venous blood samples were taken to vacuum gel flat tubes for the tests required by the participants. No additional intervention was performed for the study purpose. On the same day the samples analyzed, each of the serum samples were taken into two eppendorf and frozen at –20°C. Samples were then transferred to Bozok University Science and Technology Application and Research Center Laboratory in the cold chain equipment and stored at –20°C up to the analysis time. Afterwards, the samples were thawed and Vitamin D and Fetuin-A levels were analyzed.

Olkowski et al. method[30] was used for Vitamin D analysis. Human (FETU-A) ELISA Kit (Sun Red, catalog number: 201-12-1387) was used for Fetuin-A analysis. Subjects were not given any special treatment for the study. However, necessary follow-up and treatments were performed according to the results of medical examination and accomplished tests.

### *Statistical Method*

Data were evaluated by IBM SPSS Statistics 25.0 (IBM Corp., Armonk, New York, USA) statistical package program. Descriptive statistics were given as number of units (*n*), percentage (%), median (M), first quartile (Q1) and third quartile (Q3) values. The normal distribution of the numerical variables was evaluated by the Shapiro Wilk normality test

and Q-Q plots. Mann-Whitney U test was used for comparison of two groups and Kruskal-Wallis test for more than two group comparisons. Dunn-Bonferroni test was used as a multiple comparison test if there was a difference in Kruskal Wallis test. The relationships between numerical variables were evaluated by Spearman correlation analysis. The relationship between categorical variables was examined by the exact method of Pearson chi-square test in 2 × 2 and rxc tables. If the Pearson chi-square test result is significant in the rxc tables, two proportion *z* test with Bonferroni correction were used as the sub-analysis. Backward elimination Wald method of binary logistic analysis was used to determine the factors affecting the dependent categorical variable (study control). $p < 0.05$ was considered statistically significant.

## Results

The clinical and demographic characteristics of the patient and control groups are shown in Table 1.

In the study, no difference was found between the groups in terms of age, age group and gender distributions. Fetuin-A levels were found to be statistically higher in the study group than the control group ($p = 0.035$). Vitamin D was found higher in control group ($p = 0.01$). When the percentile evaluation of the children was made according to their weight, it was found that 38% of the control group were between 10 and 25 percentile and 28% were between 25 and 50 percentile and 66% of the patients in the study group were under 3 percentile. When the percentile was evaluated according to height, it was also seen that 26% of the control group were between 10 and 25 percentile and 24% were between 25 and 50 percentile, while 64% of the patients in the study group were below 3 percentile.

When the patients in the study group were classified according to their gender and age groups, they were similar in terms of height and weight percentiles ($p > 0.05$).

The subjects in the study group were grouped according to their anthropometric measurements and gender. Those whose weight is below 3 percentile are grouped as thin, those who were under 3 percentile in length were short, and those who were both thin and short were classified as thin + short (Table 2).

Calcium, phosphorus and ALP levels were found to be within normal range in both study and control

**Table 1**: Demographic and clinical characteristics of participants

| Gender | Groups | | *p* |
|---|---|---|---|
| | Healthy Group *n* (%) | Study Group *n* (%) | |
| Boy | 28 (56.0) | 34 (68.0) | 0.303 |
| Girl | 22 (44.0) | 16 (32.0) | |
| School-age | 21 (42.0) | 15 (30.0) | 0.298 |
| Play-age | 29 (58.0) | 35 (70.0) | |
| Age (years) | 5.5 (3.0 – 8.3) | 5.0 (2.0 – 8.0) | 0.289 |
| Fetuin-A (mg/L) | 1132.14 (793.31 – 1265.95) | 1240.11 (920.97 – 1317.85) | 0.035 |
| Vitamin D (ng/mL) | 19.63 (15.52 – 27.53) | 16.53 (13.17 – 19.49) | 0.01 |

**Table 2**: Classification of children in the study group according to anthropometric measurements and age groups

| | Age Groups | | *p* |
|---|---|---|---|
| | School | Play | |
| | *n* (%) | *n* (%) | |
| Thinness status | | | |
| Normal | 6 (40.0) | 11 (31.4) | 0.746 |
| Thin | 9 (60.0) | 24 (68.6) | |
| Shortness status | | | |
| Normal | 4 (26.7) | 14 (40.0) | 0.523 |
| Short | 11 (73.3) | 21 (60.0) | |
| Thinness and shortness | | | |
| Normal | 9 (60.0) | 23 (65.7) | 0.754 |
| Thin and short | 6 (40.0) | 12 (34.3) | |

groups (Respectively; Calcium mean value: 9.8 mg/dL; Phosphorus mean value: 4.6 mg/dL; ALP mean value: 163.1 IU/L; Calcium mean value: 10.8 mg/dL; Phosphorus mean value: 4.1 mg/dL; ALP mean value: 175.1 IU/L). In the study and control groups, vitamin D level was found to be 17.69 (14.21–23.41) ng/mL in males and 16.58 (12.86–22.19) ng/mL in girls ($p$ = 0.28). Moreover, Fetuin-A levels were found to be 1218.21 (881.19–1311.06) mg/L in males and 1195.20 (824.11–1273.53) mg/L ($p$ = 0.23) in girls.

Vitamin D deficiency was found in 18% and vitamin D insufficiency was found in 49% of all participants. Furthermore, vitamin D deficiency was found in 22% and vitamin D insufficiency was found in 58% of the study group.

When grouping according to vitamin D levels (deficiency, insufficiency, sufficiency), no difference was found in Fetuin-A levels according to groups ($p$ > 0.05) (Table 3). When the control and study groups were grouped according to vitamin D levels, no difference were found in Fetuin-A levels according to the groups (respectively $p$ = 0.823, $p$ = 0.067).

A weak positive relationship was found between Fetuin-A and vitamin D only in the study group ($p$: 0.009 $\rho$: 0.366). Further statistical analysis showed that as vitamin D level increases by one unit, growth retardation decreases by 1.071 (1/0.934) times. No such association was found with Fetuin-A.

**Table 3**: Vitamin D and Fetuin-A levels by groups

| | **Control School** | **Study School** | **Control Play** | **Study Play** | |
|---|---|---|---|---|---|
| | ***n* = 21** | ***n* = 15** | ***n* = 29** | ***n* = 35** | ***p*** |
| | ***M* ($Q_1$ – $Q_3$)** | ***M* ($Q_1$ – $Q_3$)** | ***M* ($Q_1$ – $Q_3$)** | ***M* ($Q_1$ – $Q_3$)** | |
| Fetuin-A (mg/L) | 831.52 (724.56–1043.40)[a] | 839.94 (660.60–1235.24)[a] | 1227.03 (1114.32–1274.19)[b] | 1301.17 (1190.67–1334.28)[b] | <0.001 |
| Vitamin D (ng/mL) | 18.62 (15.79–29.22)[a] | 14.13 (11.59–16.54)[b] | 20.3 (13.70–24.78)[a] | 17.44 (14.25–22.25)[ab] | 0.005 |

The superscripts *a, b* indicate differences between groups. Groups with the same letters are similar.

Fetuin-A levels were lower in school groups than in play groups ($p$ < 0.001). Vitamin D levels were found to be lower in the school group compared to the other groups ($p$ = 0.005). There was no statistical difference found in vitamin D levels in other groups. Vitamin D and Fetuin-A levels according to thinness and shortness are shown in Table 4.

Vitamin D levels of the thin ones were lower than normal ones ($p$ = 0.036). Fetuin levels were higher in the short group ($p$ = 0.043) and vitamin D levels were higher in the normal group ($p$ = 0.011). Fetuin-A levels were found to be statistically different in play and school groups ($p$ < 0.001). The play group Fetuin-A levels were found to be higher in the shorter patients compared to the school groups ($p$ < 0.001). In comparison with school-normal group and play-normal group, vitamin D levels were found to be lower in the school-short group ($p$ = 0.019). Moreover, Fetuin-A levels in the play group were higher than school groups ($p$ < 0.001).

**Table 4**: Fetuin-A and Vitamin D levels according to age group and thinness

| | **School Normal** | **School Short** | **School Thin** | **School Thin/ Short** | **Play Normal** | **Play Short** | **Play Thin/ Short** | **Play Thin** |
|---|---|---|---|---|---|---|---|---|
| | ***n* = 21** | ***n* = 11** | ***n* = 9** | ***n* = 6** | ***n* = 29** | ***n* = 21** | ***n* = 12** | ***n* = 24** |
| | **M ($Q_1$ – $Q_3$)** | **M ($Q_1$ – $Q_3$)** | **M ($Q_1$ – $Q_3$)** | **M ($Q_1$ – $Q_3$)** | **M ($Q_1$ – $Q_3$)** | **M ($Q_1$ – $Q_3$)** | **M ($Q_1$ – $Q_3$)** | **M ($Q_1$ – $Q_3$)** |
| Fetuin-A (mg/L) | 831.52 (724.56 – 1043.40) | 885.85 (768.92 – 1244.47) | 885.85 (698.78 – 1214.08) | 909.26 (785.36 – 1252.36) | 1227.03 (1114.32 – 1274.19) | 1296.19 (1201.26 – 1336.23) | 1292.44 (1185.73 – 1337.10) | 1292.44 (1174.90-1334.43) |
| Vitamin D (ng/mL) | 18.62 (15.79 – 29.22) | 13.95 (11.59 – 16.63) | 14.24 (12.97 – 16.73) | 15.33 (13.46 – 17.09) | 20.3 (13.70 – 24.78) | 16.94 (13.73 – 22.27) | 17.19 (12.20 – 23.32) | 17.75 (13.16 – 20.58) |

## Discussion

Growth retardation remains one of the serious problems of childhood. Although many factors cause growth retardation, malnutrition and arbsorption disorders are prominent.[1] Early diagnosis and treatment is important because of complications that may cause in the future.

In this study, when the weight was evaluated according to height in children who were shorter for their age, there was no child whose weight was below 3 percentile. This was attributed to chronic malnutrition of the patients participating in the study.

In previous studies, Fetuin-A levels in children were found to be 0.52 ± 0.009 mg/mL in children between the ages of 6 and 18 by Van Summeran et al.,[31] 0.3 (0.21–0.52) mg/mL in children between the ages of 5–12 years by Marhaug et al.[15] and 0.22–0.70 g/L (0.46 + 0.24 g/L) in healthy children by Wigger et al.[32]

In this study, it was found in [1132.14 (793.31–1265.95)] mg/L range in healthy subjects and in [1240.11 (920.97–1317.85)] mg/L range in patients with growth retardation. Fetuin-A levels were found to be higher in both groups in comparison to levels reported in some adults and pediatric studies of the literature.[15,24,31-34]

Similar to other studies in the literature, no difference was found between the genders.[32,34] Although the reason for these high levels could not be fully explained, it was thought to be related to race, geography or environmental factors. In some studies, a slight increase with age has been proven and in some other studies a decrease by time after prenatal period has been proven.[15,17,34,35] Most of these studies were done in people with comorbidities. When evaluated with this aspect, no comparative study on growth need was found in the literature. These variable data related to age suggest that release of Fetuin-A is affected by more than one factor and that there is no constant release rate of Fetuin-A. As a result of statistical analysis, Fetuin-A levels and gender and weight were not correlated in this study. When the patients were classified as play-age and school-age according to their growth stages, we observed that Fetuin-A levels were lower than that of play-age children during the school period where growth is slower ($p < 0.001$).

Fetuin-A is abundant in fetal bovine serum, fetal blood and tissues and this suggest that Fetuin-A may play a more general role in organ development.[18,19] In a study, when preterms, very low birth weight infants, infants, school-age children and adolescents were evaluated, the highest Fetuin-A levels were highest in preterms born at 24–30 weeks of gestation. When preterms, very low birth weight infants, infants, school-age children and adolescents were evaluated by a study, the highest Fetuin-A levels were measured in preterms born at the 24–30 weeks of gestation. Afterwards, Fetuin-A levels were found to be decreased and reached adult levels and this decrease was found to be related to biological age rather than chronological age.[17] In a study with sheep, a decrease with age was reported after high prenatal serum Fetuin-A.[35] These data support that Fetuin-A is high in organisms in need of growth and their concentration depends on biological age. In this case, Fetuin-A can be assumed to stimulate growth.[17] In our study, serum Fetuin-A levels were found to be higher in children with short stature than healthy control group ($p = 0.035$). This elevation in serum Fetuin-A level was thought to be related to the growth need. It should be remembered that children in our study group were not able to achieve age-appropriate growth and could not yet complete their chronological age-appropriate growth compared to their peers. As a result of this idea, the Fetuin-A level should be expected to increase during the periods when the biological age of the child who has not yet completed the growth remains behind the chronological age or when the catch-up growth is required (such as the catch-up growth of the premature). When the need for growth is reduced, namely, when the child reaches the characteristics of its chronological age, serum Fetuin-A levels should decrease to reach a stable age-appropriate level. In accordance with this study, Shroff et al.[34] showed that Fetuin-A levels were lower in healthy children aged between the ages of 12 and 18 who are longer than 50 percentile compared to their peers.

Fetuin-A should always remain stable in the body to a certain extent, given its non-bone functions. In a study conducted by Topsakal et al.[36] with 37 patients with acromegaly and 30 healthy participants, Fetuin-A levels were found to be very high and significant statistically in acromegalic patients. In addition, IGF-1 levels were found to be correlated with Fetuin-A levels. This study supports that Fetuin-A increases growth. Moreover, increase for any reason after the closure of the epiphyseal plate gives an impression that it may be related to pathological growth.

Newborns with intrauterine growth restriction (IUGR) were found to have defective glycosylation

of Fetuin-A.[37] However, concentrations of total Fetuin-A were found to be similar in healthy term newborns with growth restriction.[38] When these data are evaluated together with high fetuin levels in premature babies, it is suggested that the presence of Fetuin-A is not sufficient for proper growth and that its structure and receptor relations should be normal. In our study, the reason for finding high Fetuin-A levels in short participants might be the fact that the structure of Fetuin-A is not normal. Since the Fetuin-A structure could not be studied in this study, it was not possible to clarify this. Further studies are needed to confirm the possibility.

It has been proposed in a study conducted by Hausler et al.[17] that decrease in Fetuin-A serum concentrations to adult levels after intrauterine life and the presence of similar low Fetuin-A concentrations from early infancy to adulthood was not associated with a decrease in Fetuin-A synthesis. On the contrary, it has been suggested that Fetuin-A as a mineral chaperone is required and consumed during active skeletal mineralization and accumulate in the bone, as shown in an animal model.[25] The fact that Fetuin-A levels in shorter children are higher than healthy children can be evaluated as the accumulation of Fetuin-A in bone.

In contrast to high bone mass in healthy children and the inclusion of Fetuin-A in bone formation, it can be assumed that Fetuin-A is used less in short children because of its low bone mass compared to their peers and therefore serum Fetuin-A levels are higher. While Fetuin-A decreases in Paget's disease, the increase in Fetuin-A in osteogenesis imperfecta may be related to consumption of Fetuin-A, but may also be related to the need for growth and mineralization as discussed earlier.[39,40] However, this theory is based on the assumption that Fetuin-A release is relatively constant, although it explains the gradual decrease in Fetuin-A levels with age. Increased secretion in patients with acromegaly or changes in Fetuin-A levels in patients with osteoporosis indicate that Fetuin-A is not at a constant release rate and that the release rate may change.[36,41] Studies which are showing that the Fetuin-A levels increases with age contradicts the idea that Fetuin-A levels decreases because it accumulates in bone.[25] The risk of cardiovascular disease also rises as age increases.[42,43] However, the fact that Fetuin-A is lower at a younger age and increases with age also contradicts this information. Because low-level Fetuin-A will reduce the inhibition capacity of intravascular calcification, the risk of cardiovascular disease should increase as in the study of Ix et al.[26] It was found that the femurs of the mice whose Fetuin-A gene was genetically defected were extend more slowly between 3 and 18 months and especially the femur, one of the long bones, was found to be severely stunted. Bone composition, mineral and collagen properties of cortical bone were not affected by the absence of Fetuin-A. Mineralization of premature growth plate resulted in shortening of femoral length. In this context, it has been argued that Fetuin-A is a requirement for appropriate long bone growth, at least in mice.[16] This information supports the fact that Fetuin-A is not necessary for bone calcium accumulation and that mineralization occurs even if Fetuin-A is not present and that the effect of Fetuin-A on mineralization is more on stature. In addition to studies advocating that Fetuin-A should be increased in order to accelerate mineral formation *in vitro* and support collagen calcification, there are studies advocating that Fetuin-A should be low in terms of contributing to the procalcial environment for bone growth.[15,44]

Mathew's et al.[27] performed Fetuin-A gene ablation in mice causing complete deficiency of Fetuin-A and as a result of the study, it was found that mice did not gain weight despite fat feeding. Thinness can be expected in people with Fetuin-A deficiency due to this study, but complete deficiency of Fetuin-A has not been demonstrated in humans. Increased Fetuin-A levels have been associated with obesity.[22,45] In some studies, no correlation was found between Fetuin-A serum concentration and BMI-SDS.[32,46] Low serum Fetuin-A has been reported in infected and malnourished children.[21] These contradictory data indicate that the relationship between weight and Fetuin-A is not fully understood. In this study, we found that Fetuin-A was not associated with weight in thin or healthy children ($p = 0.064$). However, it should be remembered that the participants did not have heavy malnutrition.

In addition, vitamin D levels did not differ according to gender. Vitamin D levels were significantly lower in the group with growth retardation than healthy children ($p = 0.01$). Vitamin D levels were lower in both thin ($p = 0.036$) and short ($p = 0.011$) group than in the control group. In a study of mice examining inflammatory bowel diseases, IL-10 deficiency was induced in mice and a group of mice were specifically raised to lack vitamin D. It has been observed that mice with vitamin D deficiency started to eat less when

they were 9 weeks old and afterwards started lose weight rapidly. Control group mice with vitamin D deficiency were grew slower than vitamin D-sufficient/IL-10 deficient mice, but there was not found significant difference between the two groups at the week of 12.[47] Vitamin D deficient group was observed to grow more slowly but in the absence of additional disease, it was finally reached the target. Although it is not correct to adapt this information directly to human, we have found that vitamin D levels were lower in pediatric patients with growth retardation, it suggests that there may be a similar mechanism exist in humans. Although there are studies showing that there is no relationship between vitamin D and body mass index or weight, studies on weight gain and vitamin D in humans are usually related to obesity. In addition, there are no studies examining thinness and vitamin D in children.[48]

The group with the lowest vitamin D levels was school-age children with growth retardation. In this age group, increased time spent indoors such as schools and homes and increased veiling clothing style due to the region where we live may have caused vitamin D levels to decline as a result of decreased vitamin D synthesis in the skin.

Considering the relationship with calcium and studies on bone mineralization, it can be thought that vitamin D and Fetuin-A together affect bone mineralization. This suggests that vitamin D and Fetuin-A may be related. Both vitamin D and Fetuin-A have insulin resistance effect and are associated with obesity.[22,49] On the other hand, there are no studies in the literature that can clearly explain the relationship between the two. Hovewer, it has been shown in several studies that vitamin D administration affects Fetuin-A levels in animals and humans.[46,50,51] However, the possible outcome of interactions of Fetuin-A and vitamin D on bone mass is currently not fully known.

In a study of 112 children with chronic kidney disease, no correlation was found between Fetuin-A levels and vitamin D levels. In the same study, the annual cumulative dose of calcitriol associated with weight and a relationship between calcium and Fetuin-A was found.[46] It has been shown that 1.25 (OH) vitamin D levels correlate significantly positive with serum Fetuin-A in adults not receiving dialysis treatment with diabetic nephropathy and coronary artery calcification.[50] Moreover, in adult dialysis patients with secondary hyperparathyroidism, calcitriol has been shown not only to suppress PTH but also to stimulate serum Fetuin-A levels.[50] Keskin et al.[52] showed that Fetuin-A levels decreased after parathyroidectomy and Santos et al.[53] showed that vitamin D and Fetuin-A increased. Regardless of the triggering event, its effect on calcium homeostasis requires Fetuin-A, an important calcification inhibitor. Fetuin-A is required in order to prevent the formation of hydroxyapatite crystals and calcium chelation.[25] The effect of vitamin D treatment on Fetuin-A is partially achieved with serum calcium.[46] Nimitphong[54] showed that the relationship between Fetuin-A and bone mass varies according to DBP genotype and this effect is independent of vitamin D status. However, this observation requires further approval. In the study, the mean values of calcium were found to be normal in healthy and growth retarded group. When Fetuin-A levels were compared according to vitamin D levels in children with growth retardation, this difference was not statistically significant although there were higher levels of Fetuin-A in vitamin D-sufficient group compared to the other two groups but it was close to the level of significance ($p$ = 0.067). The difference is considered to be significant if the number of samples is increased. In the study, a weak positive correlation was found between Fetuin-A and vitamin D only in the study group ($p$ = 0.009 $\rho$: 0.366). There was no relationship between Fetuin-A and vitamin D in healthy group ($p$ = 0.97). In view of the fact that this group is children who have not completed their age-appropriate growth, it is expected that Fetuin-A will increase in parallel with vitamin D in order to increase the calcium absorption of vitamin D and to allow the deposition of increased calcium to the bone.

## Conclusion

To our knowledge, this is the first study examining the relationship between growth retardation and Fetuin-A. Patients with growth retardation compared with healthy patients, a correlation was found between vitamin D and Fetuin-A levels. As a conclusion, vitamin D and Fetuin-A levels were found to be correlated with patients with growth retardation compared to healthy patients but further studies are needed to explain this relationship.

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# Treatment in Scorpion Sting: Which is Correct?

**Feyza Hüsrevoglu Esen[1], Filiz Tubaş[2]**

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**Abstract**

*Background:* The scorpion sting is a condition that requires management in emergency department. Anti-allergic, analgesic and anti-venom administrations are commonly used, particularly in pediatric patients. In this study, it was aimed to present data from patients who presented with scorpion sting and underwent various treatments.

*Material and method:* We retrospectively reviewed 50 patients who presented to our hospital with scorpion sting. Demographic characteristics, severity of clinical presentation, treatments employed and anti-venom administration were evaluated. In patients underwent anti-venom treatment, we evaluated whether or not anti-venom caused systemic signs or additional pathology.

*Results:* Mean age was 9.88 ± 4.58 years in the study population. There were 18 girls (36%) and 32 boys (64%). It was found that electrocardiogram was performed in all patients and that there was sinus tachycardia in 6 patients (12%). No systemic sign was detected during follow-up. It was found that anti-venom treatment was used in only 7 patients (14%). No significant difference was detected between anti-venom treatment and other therapeutic modalities used in ED.

*Conclusion:* Scorpion sting is one of the leading causes of insect bites presenting to pediatric emergency departments in Turkey. It was observed that, even in tertiary care settings, anti-venom treatment is used in scorpion sting without systemic signs. Based on this study, no anti-venom indication was present in this patient group. Thus, anti-venom decision should be assessed by relevant unit or pediatric emergency clinicians in cases requiring anti-venom treatment. Unnecessary anti-venom use should be avoided by training clinicians in primary care.

**Keywords:** Scorpion; Anti-venom; Sting; Treatment.

## Introduction

Scorpion bite is one of the leading causes of emergency department presentations due to insect bite. Scorpion bite is more common in tropical regions.[1] The scorpion species in our country are generally non-toxic but scorpion bite is an important public health issue Aegean and Southeast regions where temperature is higher due to climatic characteristics.[2] Of 23 scorpion species found in Turkey, 8 were toxic. Among these, Leiurus abdullahbayrami (Yellow scorpion) and Androctonus crassicauda (Black scorpion) are extremely toxic and lethal. The Yellow scorpion can be encountered at the western areas of Southeast Anatolia region while Black scorpion at East Anatolia, Southeast Anatolia and Eastern Mediterranean regions.[3] The most common species is Mesobuthus gibbosus (Anatolian yellow

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scorpion) in Anatolia. Its venom can be lethal, particularly in children.[4]

Although majority of scorpion stings lead only localized pain without threatening life, one-third can cause intoxication resulting in dead. Children are more vulnerable to scorpion venom. The severity of scorpion sting depends on presence of neurotoxin in the venom. The neurotoxin causes acute release of neurotransmitters from autonomic nervous system, particularly from sympathetic system. In addition, a potent inflammatory response has direct influence on several vital functions including cardiovascular, respiratory and neuromuscular systems, resulting in worsening of symptoms.[5]

## Materials and Methods

We retrospectively reviewed 50 patients presented to Kayseri City Hospital with scorpion sting between July, 2018 and October, 2019 and treatments employed in these patients. In all patients, complete blood count, ECG, coagulation assays, muscle enzyme levels, systemic signs and anti-venom administration were evaluated. The patients were assigned into two groups according to presence of neurological signs. The patients with preponderance of neurological signs were classified into 4 stages: Stage 1, localized pain and paresthesia; Stage 2, proximal progression of pain and paresthesia; Stage 3, cranial nerve involvement or somatic neuromuscular dysfunction; Stage 4,5 cranial nerve involvement together with somatic neuromuscular dysfunction. Again, the patients with preponderance of neurological signs were stratified as mild, moderate or severe: mild, localized signs; moderate, localized signs with progression to proximal and/or mild systemic signs; severe, life-threatening systemic signs. It is considered that anti-venom is indicated in cases with Stage 3 and 4 disease.[6]

### *Statistical analysis*

Statistical analyses were performed by SPSS version 22.0. Categorical variables are presented as count (%). Chi-square test was used to assess categorical variables. A *p*-value <0.05 was considered as statistically significant in all analyses.

## Results

There were 18 girls (36%) and 32 boys (64%) in our study. Mean age was 9.88 ± 4.58 years. Figure 1 shows age distribution in the study population.

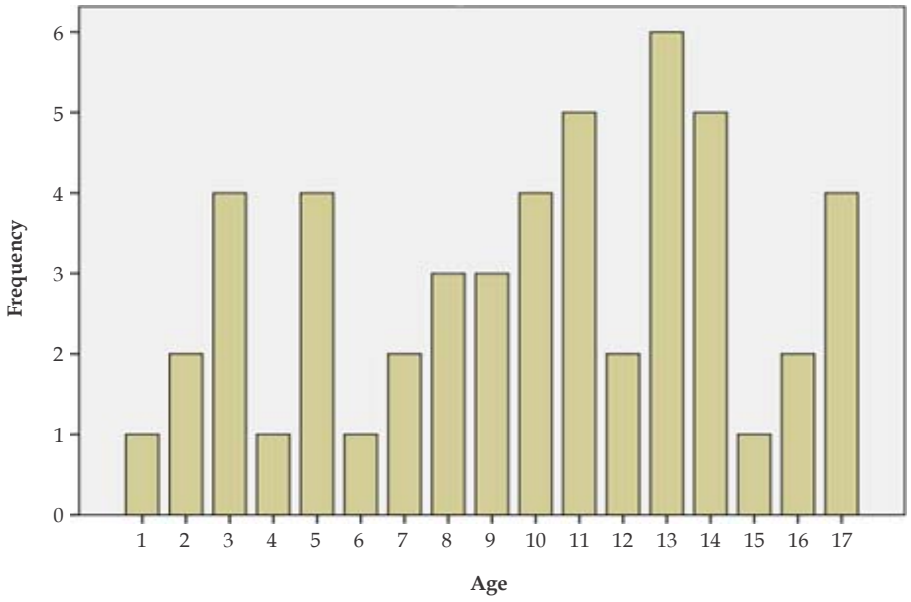


**Fig. 1:** Age distribution.

In all patients, complete blood count, ECG, coagulation assays, muscle enzyme levels, systemic signs and anti-venom administration were evaluated. There was no abnormal hemogram, biochemistry parameters. It was found that electrocardiogram was performed in all patients

and that there was sinus tachycardia in 6 patients (12%).

No systemic sign was detected during follow-up. It was found that anti-venom treatment was used in only 7 patients (14%).

The treatment modalities used at presentation to ED were classified as anti-allergic plus analgesic, analgesic and other treatments. Anti-venom administration (whether or not) was assessed using chi-square test; however, no significant difference was found ($p = 0.125$). Table 1 presents demographic and clinical characteristics of study population.

**Table 1:** Demographic and clinical characteristics

| Variable | Mean ± SD or *n* (%) |
|---|---|
| Gender | |
| Male | 32 (64) |
| Female | 18 (36) |
| **Age** | 9.88 ± 4.58 |
| **ECG finding** | |
| Positive | 6 (12) |
| Negative | 44 (88) |
| **Treatment modalities** | |
| No treatment | 2 (4) |
| Anti-allergic | 18 (36) |
| Analgesic | 17 (34) |
| Other | 13 (26) |
| **Anti-venom** | |
| Given | 7 (14) |
| Not given | 43 (86) |

## Discussion

Although majority of scorpion species in our country are known to be non-toxic, several treatment modalities are used at presentation to pediatric emergency department. The severity of clinical presentation should be considered during decision-making about treatment modalities. Staging is guiding for identifying clinical severity.

Local cold compresses are applied if there is pain at wound site. It is more effective when applied within 2 hours. It relieves pain and decreased passage of venom into systemic circulation via local vasoconstriction. In our study, local compresses were used in 14% of patients.

Venom absorption can be delayed by fixing bite site below heart level. In our cases, it was seen that no such order was given.

As increased heart rate and blood pressure facilitate venom spread, the patient should be calm down in order to maintain heart rate and blood pressure within normal range. However, it seems not possible to employ this measure given the patient volume in pediatric emergency department.

The most common symptom is local pain at wound site in scorpion bite.[6] If pain is severe, oral or intramuscular analgesic can be used. In our study, analgesics were used in 34% of patients. A topical anesthetic can be applied in order to reduce localized paresthesia. In our study, topical anesthetics were used in 2 patients.

In a study comparing pain medication for scorpion sting, Aksel et al. found that topical lidocaine administration significantly decreased pain when compared to cold compress plus paracetamol.[7] Tetanus prophylaxis was considered in 3 patients; however, 2 of these patients were on routine vaccination program and no additional vaccination was performed.

The prazosin use can be life-saving in cases with sympathetic signs such as tachycardia, pallor-cold in hands and feet, hypertension, hypersalivation or sweating. It acts by blocking sympathetic hyper-activation. It also activates potassium channels inhibited by venom. In addition, it reduces blood pressure without affecting heart rate. It must be used in all cases with signs of autonomic storming; however, it should not be used in patients having pain but no other sign. In our study, none of the patients received prazosin. The scorpion anti-venom can be given to severe patients with systemic signs. The effectiveness of available anti-venom is controversial as a specific treatment in scorpion sting. Although it is recommended that anti-venom can be used in the presence of systemic signs and symptoms, one should be careful during and after anti-venom administration as standard anti-venom can cause anaphylaxis and serum sickness.[8]

In our study, it was found that scorpion anti-venom was given to 7 patients despite lack of systemic signs. In a study on adult patients in 2018, high rate of anti-venom use was detected in the absence of indication.[5] In a study from Çukurova region, 189 patients were retrospectively reviewed between 2007 and 2013. It was found that scorpion anti-venom was given to 18 of 88 patients with Stage 1 disease although systemic symptoms were lacking.[9]

In our study, the finding that there was no significant difference between anti-venom administration (whether or not given) and other treatments in ED were attributed to fact that there was no anti-venom indication as the patients were

considered as mild or Stage 1 disease. Again, in an adult study by Şahin et al., it was shown that anti-venom was used without indication in scorpion stings.[10]

## Conclusion

In this study, it was found that anti-allergic and analgesic agents were commonly used in the treatment of scorpion sting presented to pediatric emergency department. In addition, it was seen that there was considerable anti-venom use. As it is known that anti-venom itself can cause severe allergic reactions such as anaphylaxis, we intended to emphasize that anti-venom should not be used unless there is an appropriate indication.

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*Original Article*

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# Investigation of the Biogeochemical Anomalies of *Euphorbia cyparissias* plant in Gümüshacıköy – Amasya Pb-Zn-Ag Deposits, Turkey

**Gullu Kirat**

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**Abstract**

The study area is located in Çorum G34 a3 and a4 section in Gümüşhacıköy (Amasya). 17 samples of *Euphorbia cyparissias* plant grown in this region and related soil samples were collected and analyzed. The metal content of the investigated soil samples is as follows: Fe > Zn > As > Mn > Pb > Cu > Sb > Cr >> Ag > Ni > Co > Cd. The soil pH controlling the metal transfer in the soil, the pH values of the examined soil samples are between 4.66 and 8.22. This range indicates that the soil samples are in acidic and basic conditions. Metal pollution (toxicity) due to increased mining activities may affect the environment and public health. The correlation coefficient was a strong positive correlation between Ni with Co and Ag, As with Sb (0.89); Ni (0.88) and Cu (0.86), Pb with Co (0.84), Cu (0.81), Ni (0.85) and Sb (0.81), Ni with Cu (0.81) and Sb (0.86), while there is a negative correlation between Mn with Ag, As, Cd, Co, Cr, Ni, Sb and Pb, and Fe with Cd, Co, Cr, Ni and Pb. The bioaccumulation factor (BAC) values of *Euphorbia cyparissias* have been found between not accumulate– high accumulator or hyperaccumulator plants in both root/soil and leaf/soil. The translocation factors (TF) of all metals (10 in location), Cr and Ni (3 in location), Co, Cu, Mn and Zn in the (9 in location), Ni (11 in location), and Cd, Co, Cr, and Ni values (16 in location) are less than 1. TF values calculated outside these locations are greater than 1. TF values greater than 1 showed that the metal concentration in the leaves was higher than the roots.

**Keywords:** *Euphorbia cyparissias*; Translocation; Bioaccumulation; Metal pollution (toxicity).

## Introduction

Metals caused by increased mining activities cause soil, water and air pollution.[1-6] If the pollution caused by metals is not taken immediately, it can affect the environment and public health.[2,4,7] The toxicity of the heavy metals can be a serious threat to human health due to its persistence and failure.[8,7]

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Remediation of soils contaminated by metals is one of the important issues of environmental restoration today.[5] The present application for the treatment of soils contaminated with heavy metals is the decontamination of the soil.[9] Since the extraction with chemicals is expensive and applied only for small areas, it is necessary to apply decontamination.[9–11] This process often causes adverse effects on biological activities, soil structure and fertility.[9] Recently developments in the field of environmental improvement have revealed the phytoremediation technique.[12] This method requires a low technology, can be performed in situ and used for decontamination of a specific area. It also protects the biological properties and physical structure of the soil, it is an inexpensive method.[13] An effective phytoremediation depends on the selection of local plant species and the hyperaccumulative properties such as the age,

texture and structure of the soil contaminated by metals.[2,9,11,14]

Soil is the most important source of trace metals (such as cadmium, chromium, lead, nickel, silver and zinc) that reach living beings (humans, plants and animals).[15] The trace elements content in the soil varies according to the composition of the rocks, slag, waste water, industrial wastes and the amount of fertilizer used in agriculture.[16] Although living things need some metals to live well, they can have negative effects on their health when they take these metals excessively.[15–19]

Lead (Pb), cadmium (Cd) and zinc (Zn) can occur simultaneously in high concentrations as a result of mining and smelting operations.[20] Zn, an important metal for the development of plants Zn, has a toxic effect in high concentrations. Zn usefulness for plants is quite narrow.[21,22] Pb and Cd are not essential elements for the growth of plants. These elements may cause negative effects on the prevention of photosynthesis and chlorophyll synthesis of plants, and finally plants may death.[23,24]

Investigation of the biogeochemical anomalies of *E. cyparissias* plants was the aim of this study. It is also aimed to determine the accumulation and distribution of metals in the roots and leaves of *Euphorbia cyparissias* plant growing in Pb-Zn-Ag deposits was of Gümüshacıköy region in Amasya, Turkey. In addition, TF and BAC values were calculated to determine if there was an accumulator in the study area of this plant.

### *Geology of the Study Area*

Pb-Zn-Ag deposits are located on the west of Gümüşshacıköy district of Amasya (Fig. 1), Corum G34, a3 and a4 section. Gümüshacıköy (Amasya) Pb-Zn-Ag deposits offer extrusion in many units from Permo-Triassic to Quaternary (Fig. 2). These beds in the study area show three different bedding patterns in carbonated rocks within the Karaali Complex (Upper Jurassic-Lower Cretaceous). These: (a) in the form of irregular pockets and scatterings in siliceous carbonated travertines deposited along the limestone—sandstone contact, (b) fracture zones in limestone blocks and vein—type occurrences in dissolution cavities, (c) in the limestone blocks, parallel to the plate planes.[25]


**Source:** Google map
**Fig. 1:** Location map of the study area.

As a result of the geochemical investigations, the Pb and Zn contents of the tuffs in the volcanic rocks in Karaali Karlukı and the Cu contents of the metabasalts were higher than the other rock types. Although Ag minerals are low in the ore samples, the high content of Ag in chemical analyzes shows that this metal is taken to replace Pb in galena. In addition, although Cd minerals were not detected, the high Cd content in the samples was considered to be enriched in sphalerites in this metal. All the data were evaluated together and it was concluded that the mineralizations in the region were due to the formation of siliceous-carbonate travertines, and they were probably formed by hydrothermal processes in recent days. The properties of the ore-forming solutions and the origins of the water and the metals in the hydrothermal solutions could not be obtained (Fig. 2).[25]

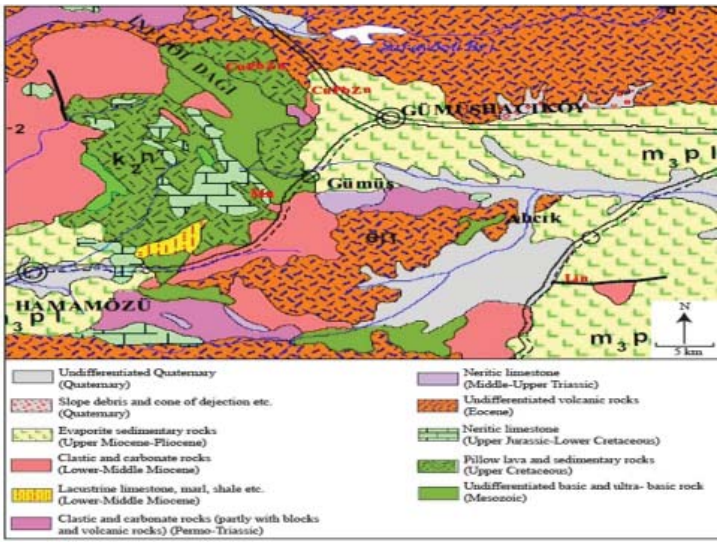


**Fig. 2**: Geological map of the study area (modified from[55]).

## Sampling and Analysis Methods

### *Plant*

Seventeen *Euphorbia cyparissias* plant samples were randomly collected from the study area and placed in pre-numbered plastic bags and brought to the laboratory (Fig. 3). After washing with pure water, these samples were separated into leaves and roots and dried at room temperature. These dried plant bodies were incinerated in flames at temperatures from 50°C to 550°C at intervals of one hour (Fig. 3).

**Fig. 3:** Burning and ashing of dried plant organs in the furnace.

### *Soil*

A total of 17 samples were collected from the soil of 10–15 cm depth where *Euphorbia cyparissias* grown on the plant. All soil samples were stored in plastic bags and numbered and brought to the laboratory. These samples brought to the laboratory were dried at room temperature and –80 mesh sieves to remove them from the coarse rock fragments and plant roots.

Sample tubes were prepared by mixing 4 g of soil with 10 ml of pure water to measure the pH values of soil samples. pH values were measured by using pH meter.[26,27] (Fig. 3).

0.1 g of the obtained plant ash and soil samples were taken into solution by adding 3 ml $HNO_3$ and 6 ml HCl at 200°C in closed environment.[28] Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sb and Zn were analyzed by ICP-MS. The analyzes were conducted at Yozgat Bozok University Technology Application and Research Center. LOD values (ppb); Al: 0.3687, Ag: 0.0155, As: 0.0386, Au: 0.0027, Ba: 0.0108, Cd: 0.0049, Co: 0.0084, Cr: 0.0474, Cu: 0.0252, Fe: 0.7846, Mg: 0.0211, Mn: 0.0307, Mo: 0.0097, Ni: 0.2135, Pb: 0.0138, Sb: 0.0068, Se: 0.1192, Th: 0.0042, Ti: 0.0406, U: 0.0011, V: 0.0275 and, Zn: 0.0904

### *Statistical analysis*

Correlation coefficient ($r$) measures linear dependence between two data sets or two variables. $r = 1$ indicates an excellent positive correlation and $r = -1$ indicates a negative correlation.[29]

The calculated probability ($p$) is considered as statistically significant when $p < 0.05$ (2-tailed).[30,31] Sperman correlation analyses were made using SPSS 15.0 for Windows Evaluation Version.

### *Translocation factor (TF)*

Metal translocation from leaf to root was measured by the following Translocation factor (TF):

$$TF = C_{leaf}/C_{root}$$

Where $C_{leaf}$ and $C_{root}$ are metals concentration in the leaf (mg/kg) and root of plant (mg/kg), respectively. TF > 1 indicates that the translocation of metals is effectively performed from the root to the leaf.[31–36]

### *Bioaccumulation factor (BAC)*

The bioaccumulation factor (BAC) of the metals was calculated as follows:

$$BAC=C_{root}/C_{soil} \text{ or } BAC=C_{leaf}/C_{soil}$$

$C_{root}$, $C_{leaf}$ and $C_{soil}$ are the metal concentration in the root (mg/kg) and leaf (mg/kg) of the plant and in the soil (mg/kg), respectively.[35,37,38]

Metal accumulation is described under four categories. These:

(1) <0.01, plants without accumulator,

(2) 0.01–0.1, low degree of accumulator plants,

(3) 0.1–1.0, medium accumulator plants,

(4) 1–10, highly accumulating or hyper-accumulating plants. By using this ratio, the absorption of the metals in the soil can be shown and the magnitude of the metal transition from soil to plant can be estimated quantitatively.[39]

## Results and Discussion

Table 1 shows the minimum, maximum and mean ± standard deviation descriptive statistics of the metal concentrations in the samples collected in the study area. The metal contents in the soil are as follows: Fe > Zn > As > Mn > Pb > Cu > Sb > Cr > Ag > Ni > Co > Cd (Table 1). Soil pH is an indicator of various chemical activities and the pH values of the soil samples examined are given in Table 1. pH values range from 4.66 to 8.22. This range shows that the soil samples are in acidic, neutral and basic conditions. Suitable for plant growth, pH values are between 5.2–7.3. Soil pH is a parameter that controls metal transfer in soils. The decrease in pH in soil increases competition between $H^+$ and dissolved metals such as $CO_3^{2-}$, $SO_4^{2-}$, $Cl^-$, $OH^-$, $S^{2-}$ and phosphates.[5] This increased competition reduces the metal adsorption capacity in the soil and increases the mobility of metals. This increase in mobility increases the bioavailability of metals in soil.[14]

**Table 1:** The minimum, maximum and mean ± standard deviation descriptive statistics of the metal concentrations

| Metals | Minimum | Maximum | Median ± St. Deviation |
|---|---|---|---|
| Ag | 0.07 | 0.93 | 0.205 ± 0.21 |
| As | 7.65 | 258.42 | 68.94 ± 73.65 |
| Cd | 0.00 | 0.09 | 0.013 ± 0.03 |
| Co | 0.01 | 0.12 | 0.052 ± 0.04 |
| Cr | 0.18 | 0.64 | 0.36 ± 0.14 |
| Cu | 0.78 | 13.18 | 3.58 ± 2.88 |
| Fe | 846.20 | 2483.7 | 1659.81 ± 492.7 |
| Mn | 26.64 | 257.14 | 56.51 ± 61.93 |
| Ni | –0.01 | 0.54 | 0.16 ± 0.17 |
| Sb | 0.42 | 7.18 | 2.94 ± 2.19 |
| Pb | 34.74 | 141.45 | 68.19 ± 28.84 |
| Zn | 21.38 | 503.37 | 69.4 ± 108.17 |

While there is a positive correlation between all metals examined (Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Sb, Pb and Zn, $p < 0.05$ significant), there is a negative correlation between Mn with Ag, As, Cd, Co, Cr, Ni, Sb and Pb, and Fe with Cd, Co, Cr, Ni and Pb (Table 2).

The correlation coefficient between Co and Ni was 0.93, indicating a strong positive correlation at the level of significance of 0.01 and a commen origin of these metals. Arsenic showed strong positive correlations with Sb (0.89) Ni (0.88) and Cu (0.86). Pb showed strong positive correlation

with Co (0.84), Cu (0.81), Ni (0.85) and Sb (0.81). Ni showed strong positive correlation with Cu (0.81) and Sb (0.86). Ag and Ni suggest that it is probably due to a common origin and formed a pair with a highly positive correlation with 0.81 correlation coefficient (Table 2).

**Table 2:** Sperman correlation coefficient values of metal concentrations in soil.

| | Ag | As | Cd | Co | Cr | Cu | Fe | Mn | Ni | Sb | Pb | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ag | 1 | | | | | | | | | | | |
| As | .787(**) | 1 | | | | | | | | | | |
| Cd | 0.395 | .545(*) | 1 | | | | | | | | | |
| Co | .647(**) | .779(**) | .636(**) | 1 | | | | | | | | |
| Cr | 0.48 | 0.323 | 0.263 | .618(**) | 1 | | | | | | | |
| Cu | .674(**) | .860(**) | .580(*) | .796(**) | 0.179 | 1 | | | | | | |
| Fe | –0.458 | –0.458 | –.663(**) | –.683(**) | –.747(**) | –0.36 | 1 | | | | | |
| Mn | –.608(**) | –.581(*) | –.687(**) | –.625(**) | –.611(**) | –0.414 | .672(**) | 1 | | | | |
| Ni | .814(**) | .880(**) | .549(*) | .926(**) | .589(*) | .811(**) | –.634(**) | –.635(**) | 1 | | | |
| Sb | .790(**) | .893(**) | .647(**) | .771(**) | 0.253 | .798(**) | –0.38 | –.656(**) | .856(**) | 1 | | |
| Pb | .799(**) | .752(**) | .783(**) | .840(**) | 0.459 | .806(**) | –.694(**) | –.686(**) | .845(**) | .808(**) | 1 | |
| Zn | –0.353 | –0.382 | –0.114 | –0.401 | –.642(**) | –0.142 | 0.424 | .618(**) | –0.358 | –0.276 | –0.24 | 1 |

Show that it is correlation between these metals is related to the presence of primary minerals such as sphalerite, galena, fahlerz, pyrite, chalcopyrite and pyrrhotite and secondary minerals occurring under surface conditions such as covelline, pyroluzite, marcasite, sericite, anglezite, calcophanite-aurorite, goethite, limonite and gypsum in the study area.[25]

The bioaccumulation factors of the metals in the plant of *Euphorbia cyparissias* are shown in Table 3–4. In this table, the bioaccumulation factor values of Cd (1, 4, 10, 14, 15 and 17 locations), Ni (2 and 13 in locations) and Sb (4 in locations) in the roots, and As (2, 3, 4 and 13 in locations), Cd (1-4 and 13-17 in locations), Co (2-4, 13 and 15-17 in locations), Cr (2 in locations), Ni (2-4, 13 and 15-17 in locations, Sb (2–4, 13 and 17 in locations) and Pb (3 in locations) in the leaves of *Euphorbia cyparissias* plant are greater than 1.

*Euphorbia cyparissias* plant BAC values (root/soil), Ag (0.01-0.30), Cu (0.01–0.78) and Pb (0.01–0.42) metals in class of low accumulator— medium accumulator plants; Co (0.00–0.73), Fe (0.00–0.14), Mn (0.00–0.16) and Zn (0.00–0.31) metals, in class of non-accumulator—medium accumulator plants, and Ni (0.01–1.10) metal are found in the class of low accumulator— high accumulator or hyperaccumulative plants (Table 3).

**Table 3:** Bioaccumulation factor (BAC) of metal accumulations in soil (root/soil)

| | Ag | As | Cd | Co | Cr | Cu | Fe | Mn | Ni | Sb | Pb | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.02 | 0.02 | 1.25 | 0.08 | 0.08 | 0.02 | 0.02 | 0.02 | 0.06 | 0.03 | 0.13 | 0.04 |
| 2 | 0.10 | 0.19 | 0.91 | 0.21 | 0.15 | 0.07 | 0.01 | 0.00 | 1.00 | 0.18 | 0.08 | 0.02 |
| 3 | 0.02 | 0.02 | 0.33 | 0.03 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.07 | 0.03 | 0.01 |
| 4 | 0.30 | 0.52 | 6.67 | 0.73 | 0.26 | 0.38 | 0.08 | 0.05 | 0.73 | 1.10 | 0.42 | 0.17 |
| 5 | 0.15 | 0.12 | 0.32 | 0.22 | 0.14 | 0.20 | 0.14 | 0.16 | 0.26 | 0.21 | 0.23 | 0.20 |
| 6 | 0.07 | 0.05 | 0.16 | 0.16 | 0.08 | 0.14 | 0.03 | 0.05 | 0.11 | 0.05 | 0.07 | 0.04 |
| 7 | 0.05 | 0.03 | 0.72 | 0.13 | 0.03 | 0.18 | 0.01 | 0.03 | 0.13 | 0.04 | 0.06 | 0.04 |
| 8 | 0.05 | 0.11 | 0.24 | 0.15 | 0.19 | 0.19 | 0.12 | 0.15 | 0.30 | 0.12 | 0.12 | 0.20 |
| 9 | 0.17 | 0.04 | 0.96 | 0.08 | 0.02 | 0.78 | 0.04 | 0.12 | 0.05 | 0.04 | 0.06 | 0.31 |
| 10 | 0.17 | 0.06 | 1.57 | 0.17 | 0.13 | 0.29 | 0.07 | 0.11 | 0.12 | 0.06 | 0.15 | 0.27 |
| 11 | 0.03 | 0.01 | 0.19 | 0.02 | 0.00 | 0.19 | 0.01 | 0.02 | 0.00 | 0.03 | 0.03 | 0.12 |
| 12 | 0.07 | 0.04 | 0.29 | 0.04 | 0.02 | 0.09 | 0.02 | 0.02 | 0.03 | 0.04 | 0.07 | 0.06 |
| 13 | 0.13 | 0.25 | 0.40 | 0.16 | 0.05 | 0.09 | 0.01 | 0.01 | 1.00 | 0.12 | 0.08 | 0.00 |
| 14 | 0.07 | 0.25 | 1.67 | 0.26 | 0.02 | 0.13 | 0.01 | 0.02 | 0.17 | 0.12 | 0.15 | 0.04 |
| 15 | 0.14 | 0.20 | 2.29 | 0.43 | 0.07 | 0.22 | 0.02 | 0.01 | 0.75 | 0.10 | 0.21 | 0.06 |
| 16 | 0.01 | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.00 |
| 17 | 0.04 | 0.05 | 2.00 | 0.43 | 0.03 | 0.23 | 0.00 | 0.01 | 0.00 | 0.08 | 0.07 | 0.08 |

In terms of metal accumulation, Pb (41, 42) and Cd are a risky heavy metal for the environment and living organisms.[26] Cd (0.00–6.67) are included in the class of non accumulator—high degree accumulator or hyperaccumulative plants. In the study area, Cd (0.00–6.67), has a high accumulation of accumulators or hyperaccumulators at 6 sampling locations (Table 3).

In the study area, the BAC of As metal is between 0.01–0.52 mg/kg and it is in the class of accumulator in the low - medium level (Table 3).

*Euphorbia cyparissias* plant BAC values (leave/soil), Ag (0.05-0.96), Fe (0.03-0.29), Mn (0.03-0.39) and Zn (0.03-0.84) metals in class of low accumulator— medium accumulator plants; As (0.02–2.56), Co (0.07–4.57), Cr (0.02–1.05), Ni (0.02-12.0), Sb (0.03–4.71) and Pb (0.05–1.07) metals in class of low accumulator— high accumulator or hyperaccumulative plants; Cd (0.19–10.0) and Cu (0.11–2.05) metals in class of medium accumulator —high accumulator or hyperaccumulative plants (Table 4).

**Table 4:** Bioaccumulation factor (BAC) of metal accumulations in soil (leave/soil)

| | Ag | As | Cd | Co | Cr | Cu | Fe | Mn | Ni | Sb | Pb | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.14 | 0.30 | 8.75 | 0.79 | 0.63 | 0.20 | 0.28 | 0.27 | 0.50 | 0.31 | 0.71 | 0.43 |
| 2 | 0.80 | 2.36 | 2.09 | 1.33 | 1.05 | 0.53 | 0.10 | 0.04 | 12.00 | 1.79 | 0.52 | 0.16 |
| 3 | 0.69 | 1.89 | 10.00 | 1.88 | 0.32 | 0.81 | 0.17 | 0.14 | 1.83 | 4.71 | 1.07 | 0.38 |
| 4 | 0.59 | 1.31 | 8.00 | 1.25 | 0.26 | 0.75 | 0.18 | 0.13 | 1.55 | 2.15 | 0.74 | 0.33 |
| 5 | 0.51 | 0.34 | 0.79 | 0.65 | 0.26 | 0.60 | 0.29 | 0.39 | 0.74 | 0.55 | 0.72 | 0.57 |
| 6 | 0.28 | 0.23 | 0.40 | 0.48 | 0.19 | 0.34 | 0.11 | 0.18 | 0.52 | 0.27 | 0.43 | 0.19 |
| 7 | 0.26 | 0.23 | 0.94 | 0.50 | 0.10 | 0.32 | 0.07 | 0.10 | 0.63 | 0.25 | 0.40 | 0.13 |
| 8 | 0.15 | 0.23 | 0.28 | 0.57 | 0.39 | 0.38 | 0.24 | 0.35 | 0.63 | 0.29 | 0.27 | 0.40 |
| 9 | 0.09 | 0.09 | 0.19 | 0.08 | 0.02 | 0.29 | 0.08 | 0.10 | 0.05 | 0.09 | 0.08 | 0.17 |
| 10 | 0.05 | 0.02 | 0.29 | 0.07 | 0.03 | 0.11 | 0.03 | 0.05 | 0.02 | 0.03 | 0.05 | 0.09 |
| 11 | 0.10 | 0.07 | 0.29 | 0.09 | 0.04 | 0.52 | 0.05 | 0.06 | 0.13 | 0.07 | 0.09 | 0.23 |
| 12 | 0.44 | 0.37 | 0.82 | 0.29 | 0.11 | 0.64 | 0.19 | 0.20 | 0.38 | 0.39 | 0.39 | 0.40 |
| 13 | 0.96 | 2.56 | 1.70 | 1.26 | 0.32 | 0.55 | 0.06 | 0.07 | 9.00 | 1.21 | 0.61 | 0.03 |
| 14 | 0.14 | 0.82 | 2.67 | 0.74 | 0.09 | 0.45 | 0.04 | 0.06 | 0.58 | 0.35 | 0.31 | 0.14 |
| 15 | 0.50 | 0.74 | 4.71 | 1.35 | 0.21 | 0.74 | 0.07 | 0.03 | 2.50 | 0.32 | 0.63 | 0.24 |
| 16 | 0.50 | 0.51 | 2.08 | 1.05 | 0.33 | 0.48 | 0.06 | 0.07 | 3.00 | 0.54 | 0.42 | 0.13 |
| 17 | 0.33 | 0.99 | 7.33 | 4.57 | 0.24 | 2.05 | 0.05 | 0.15 | 11.00 | 1.26 | 0.81 | 0.84 |

As a result, the plants analyzed in this study can be said to be contaminated with Cd and Sb. As a result, these metals can be transferred to the food chain.[26,30,41]

Arsenic (As) is an important pollutant in the world. The BAC for As is about 5 mg/kg in uncleaned soils,[26,42,43] however, this value is between 1400 and 2700 mg/kg in polluted soils.[26,45]

Manganese is one of the most essential nutrients for plants.[40] Although the known botanical function of nickel is not known, copper, zinc and iron are essential metals for plants, but they may be toxic in high concentrations.[45]

The metals are usually found in roots and leaves.[46] Plant metals take place with metals accumulation of metals. Therefore, the roots of the *Euphorbia cyparissias* plant can prevent the spread of heavy metals into the environment. Metal contaminants in high concentrations in soils can be stored in the leaves of this plant.[47,48]

The Translocation Factor (TF) was calculated as the ratio of the total metal concentration in the leaves to the metal content in the roots.[45]

The TF of metals in *Euphorbia cyparissias* plants are shown in Table 5. In this table, the translocation factors of all metals in the location 10, Cr and Ni in the location 3, Co, Cu, Mn and Zn in the location 9, Ni in the location 11, and Cd, Co, Cr, and Ni values in the location 16 are less than 1. The TF of the metals examined in all other locations are greater than 1. The fact that this factor is greater than 1 indicates that it can carry the metals from the roots to the leaves.[48]

**Table 5:** Translocation factor (TF) of metal accumulations in soil

| | Ag | As | Cd | Co | Cr | Cu | Fe | Mn | Ni | Sb | Pb | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.40 | 15.78 | 7.00 | 9.44 | 8.00 | 8.45 | 14.42 | 11.41 | 9.00 | 10.24 | 5.62 | 10.31 |
| 2 | 8.33 | 12.62 | 2.30 | 6.33 | 7.00 | 7.96 | 9.27 | 10.10 | 12.00 | 9.92 | 6.18 | 9.28 |
| 3 | 40.0 | 94.5 | 30.0 | 64.0 | 0.00 | 66.3 | 92.1 | 74.7 | 0.00 | 66.0 | 42.0 | 64.9 |
| 4 | 1.97 | 2.49 | 1.20 | 1.72 | 1.00 | 1.97 | 2.19 | 2.65 | 2.13 | 1.95 | 1.75 | 2.02 |
| 5 | 3.44 | 2.76 | 2.50 | 2.96 | 1.88 | 2.94 | 2.08 | 2.39 | 2.88 | 2.64 | 3.18 | 2.85 |
| 6 | 4.17 | 5.14 | 2.57 | 3.00 | 2.33 | 2.45 | 4.36 | 3.28 | 4.67 | 5.04 | 6.11 | 4.47 |
| 7 | 5.20 | 7.23 | 1.31 | 3.71 | 3.00 | 1.75 | 6.18 | 3.23 | 5.00 | 6.77 | 6.44 | 3.10 |
| 8 | 3.03 | 2.09 | 1.14 | 3.92 | 2.00 | 2.02 | 1.93 | 2.41 | 2.11 | 2.30 | 2.27 | 2.06 |
| 9 | 0.52 | 2.51 | 0.20 | 0.90 | 1.00 | 0.37 | 2.16 | 0.85 | 1.00 | 2.14 | 1.22 | 0.55 |
| 10 | 0.29 | 0.45 | 0.18 | 0.39 | 0.25 | 0.40 | 0.41 | 0.41 | 0.20 | 0.47 | 0.34 | 0.35 |
| 11 | 4.00 | 4.80 | 1.50 | 4.00 | 0.00 | 2.79 | 4.37 | 3.25 | 0.00 | 2.33 | 3.07 | 1.96 |
| 12 | 5.94 | 9.96 | 2.85 | 6.80 | 7.00 | 6.76 | 8.86 | 8.36 | 13.00 | 8.79 | 5.81 | 7.08 |
| 13 | 7.22 | 10.20 | 4.25 | 8.00 | 6.00 | 6.33 | 10.34 | 10.15 | 9.00 | 10.25 | 7.78 | 7.92 |
| 14 | 1.95 | 3.28 | 1.60 | 2.83 | 4.00 | 3.56 | 2.97 | 3.68 | 3.50 | 2.81 | 2.11 | 3.92 |
| 15 | 3.45 | 3.64 | 2.06 | 3.10 | 3.00 | 3.38 | 3.71 | 3.96 | 3.33 | 3.33 | 3.08 | 4.11 |
| 16 | 35.5 | 84.6 | 0.00 | 0.00 | 0.00 | 52.0 | 94.1 | 88.7 | 0.00 | 73.0 | 40.9 | 58.9 |
| 17 | 7.6 | 20.6 | 3.7 | 10.7 | 8.00 | 8.9 | 18.8 | 12.3 | 11.0 | 16.0 | 12.0 | 11.0 |

The translocation of metals from the roots to the upper organs is a very important physiological process to remove the contaminated areas. [45,49–53]

The TF for metals in plants must be more than one to be considered as bioaccumulators.[47] Accumulators plants can accumulate metals in high concentrations in the above ground organs.[32] The *Euphorbia cyparissias* plant has high leaf/root translocation factors, except for some sample locations. The leaf/root translocation values greater than 1 showed that the metal concentration in the leaves was higher than in the roots. The translocation of metals in plants occurs with vascular system or xylem tissue. For plants, metal translocations of the essential metals (Cu, Fe, Mn and Zn) from roots to leaves were higher compared with the non-essential metals (As, Cd, Sb and Pb). The low translocation factors of non-essential metals show that *Euphorbia cyparissias* use these metals for both metabolic activity and growth, whereas the essential metals have higher mobility to the leaves. Lead is a toxic metal for the synthesis of photosynthesis of leaves, chlorophyll and antioxidant enzymes. Most of the time, the roots prevent the transport of non-essential metals to accumulate metals in the roots.[20,48]

## Conclusion

In this study, the biogeochemical anomalies of metal/trace metals in soils and in leaves and roots of Euphorbia cyparissias plant were investigated in Pb-Zn-Ag deposits was of Gümüshacıköy region in Amasya, Turkey. In this study, concentrations of metals such as: Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sb and Zn, and the accumulation and distribution of these metals in the soil contents and in leaves and roots of *Euphorbia cyparissias* plant were determined. The order of metals in the soil; Fe > Zn > As > Mn > Pb > Cu > Sb > Cr > Ag > Ni > Co > Cd.

In the *Euphorbia cyparissias* plant (root/soil), Ag, Cu and Pb are classified as low accumulator-medium accumulator plants; Co, Fe, Mn and Zn were found in the class of non-accumulator-medium accumulator plants, Cd in the class of non– accumulator-high accumulator or hyperacumulative plants, and Ni in the class of low accumulator-high accumulator or hyperacumulative plants. In the *Euphorbia cyparissias* plant (root/soil), Ag, Fe, Mn and Zn are classified as low accumulator– medium accumulator plants; As, Co, Cr, Ni, Sb and Pb in the class of low accumulator - high accumulator or hyperacumulative plants, Cd and Cu in the class of medium accumulator-high accumulator or hyperacumulative plants.

Ag, As, Cu, Mn and Zn metals (2 in the different located), Fe, Pb, Sb Cd and Co metals (1 in the located), and Cr and Ni metals (4 in the different located) TF values 1 is smaller than. Other metal values outside these locations are greater than 1. The TF value greater than 1 indicates that the metal concentration in the leaves is higher than the roots and that the metals are transfered from the roots to the leaves.

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*Original Article*

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# Analyzes of the Patients Admitted to an Academic Emergency Department with Acute Toxic Exposure

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**Abstract**

*Background:* The aim of the study was to report the pattern of patients with acute intoxication in an academic emergency department (ED).

*Method:* The study included 180 patients with acute intoxication who admitted to the Research Hospital of Yozgat Bozok University in Yozgat. Turkey between January 2018 and January 2019. The demographic features, arrival mode, intoxication type, intent status, requested consultations, and disposition forms were retrospectively evaluated based on the patient files.

*Results:* Acute toxic exposure was detected in a total of 180 patients during the study period. The male/female ratio was 0.68 (73/107). The median age was 26 ranging between 3 and 89. The most common acute toxic exposure presentations were due to drugs (57.2%, *n* = 103), venomous animal/insect bites (16.1%, *n* = 29) and toxic gas inhalation (13.9%, *n* = 25). Toxic gas inhalation included inhalations of carbon monoxide (11.7%, *n* = 21) and vapor of hydrochloric acid (2.2%, *n* = 4). The rest of the presentations (19.4%, *n* = 35) were due to food poisoning (7.8%, *n* = 14), alcohol intoxication (3.9%, *n* = 7) and ingested toxic agents (1.1%, *n* = 2). No illicit drug use was observed. Almost half of the toxic exposures (51.7%, *n* = 93) were considered as unintentional whereas 77.7% (*n* = 93) of drug intoxication cases were intentional suicidal attempts. At least one consultation was requested for 126 patients. Two or more consultations were performed for 19 patients. The most consulted division was Internal medicine (48.9%, *n* = 115). The most common drug intoxications were due to analgesics (24.3%, *n* = 25) and antidepressants (23.3%, *n* = 24).

*Conclusion:* Acute toxicity is not limited to drug poisoning. It is not a rare reason for ED admission with its many sub-headings.

**Keywords:** Intoxication; Poisoning; Overdose; Environmental; Drug.

## Introduction

Acute toxic exposure can be described as being affected by a substance or substances adversely in a short period of time. The time period is usually less than 24 hours but can last up to 2 weeks.[1] The exposed agents are generally classified into chemical, biological, physical or radiation. The exposure route can be dermal, oral, parenteral or inhalation. Most of the acute toxicity studies in the literature focus on specific subgroups like drug, chemical or industrial exposures.

World Health Organization declared 5.4 deaths per 100.000 inhabitants in 2004.[2] Acute toxicity cases are mostly encountered in emergency departments (ED). The knowledge of ED physicians regarding the acute presentations of common toxic agents and mechanisms of action is critical to identify and meet

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the patient's initial and further treatment needs. The study was conducted to describe the pattern of the patients admitted to an academic emergency department with acute intoxication.

## Materials and Methods

The study included all acute intoxication cases without any age limitation who admitted to the Research Hospital of Yozgat Bozok University in Yozgat, Turkey during a one-year period between January 2018 and January 2019. A total of 180 patients were treated for acute toxic exposure.

The demographic features, arrival mode, intoxication type, intention status, requested consultations, and disposition data were obtained from electronic patient files and were retrospectively evaluated. The ethical approval was obtained from the Ethical Committee of Yozgat Bozok University Medical Faculty.

The following intoxication types were included: ingested caustic and irritating agents, alcohol intoxications, biological intoxications due to food, plants or animals, toxic inhalations, radiation exposures and intoxications with medications. Ingested non-toxic foreign bodies (coins, plastics, or toys) or vaccination were excluded.

Analyses were done using SPSS version 25.0, At 95% CI, a $p$-value <0.05 was considered as significant. In statistical analysis, we used the chi-square test for comparing proportions. Means were compared by using the Mann–Whitney $U$ test.

## Results

Acute toxic exposure was detected in a total of 180 patients during the one-year study period. The study group represented 0.4% of all ED visits (180/41850). The study population included 73 males (40.6%) and 107 females (59.4%) with a male/female ratio of 0.68. The total number of pediatric and geriatric patients was 31 (17.2%). The median age was 26 ranging between 3 and 89 with a standard error of 1.3. The intentional exposures consisted of 48.3% of the study population. The most common admission season was autumn (34.4%) as represented in Table 1.

**Table 1**. Demographic data of the patients

| Parameters | *n* | % |
|---|---|---|
| **Sex** | | |
| Male | 73 | 40.6 |
| Female | 107 | 59.4 |
| **Age groups** | | |
| 18 | 18 | 10.0 |
| 18–65 | 149 | 82.8 |
| 65 | 13 | 7.2 |
| **Intention status** | | |
| Intentional | 87 | 48.3 |
| Unintentional | 93 | 51.7 |
| **Admission season** | | |
| Spring (March–May) | 33 | 18.3 |
| Summer (June–August) | 44 | 24.4 |
| Autumn (September–November) | 62 | 34.4 |
| Winter (December–February) | 41 | 22.8 |

The mean ages were 34 (SE = 2.2) and 24 (SE = 1.5) for males and females, respectively. The female patients were significantly younger than males, $z$ = –3.678; $p$ < 0.01. The intoxication with medication subgroup included 103 patients with 25 males (median age = 33) and 78 females (median age = 23), females being significantly younger than males $z$ = –3.28. $p$ < 0.01. The single/married ratio was 45/55%. Almost half of the patients (50.5%) arrived in the ED by ambulance. The ambulance service use was significantly higher in intentional group ($p$ < 0.05) and the ambulatory arrivals were significantly higher in unintentional group ($p$ < 0.05). The mean time between the incident and the admission to ED was 103 ± 93 minutes and was significantly lower in the intentional group (86 vs 129 min) ($p$ < 0.01) (Table 2).

**Table 2.** The subanalysis of the patients with acute toxic exposure by the intention status

| Total 180 patients | Intention status | | | | | | *p* |
|---|---|---|---|---|---|---|---|
| | Intentional 87 (48.3%) | | | Unintentional 93 (51.7%) | | | |
| | Male | Female | Total | Male | Female | Total | |
| ***n*, (%)** | **24** | **63** | **87** | **49** | **44** | **93** | |
| Age (years ± SE). 26 | 26.5 ± 2.1 | 23 ± 0.5 | 23 ± 0.7 | 25 ± 2.9 | 35.5 ± 3.2 | 39 ± .2.1 | z (–5.37) $p < 0.01$ |
| **Marital status** | | | | | | | |
| Single, *n* = 81 (45%) | 11 (22.0%) | 39 (78.0%) | 50 (61.8%) | 11 (35.5%) | 20 (64.5%) | 31 (38.2%) | |
| Married, *n* = 99 (55%) | 13 (35.1%) | 24 (64.9%) | 37 (37.3%) | 38 (61.3%) | 24 (38.7%) | 62 (62.7%) | |
| **Admission type** | | | | | | | |
| Ambulance, *n* = 91 (505%) | 14 (21.9%) | 50 (78.1%) | 64 (70.3%) | 15 (55.6%) | 12 (44.4%) | 27 (29.7%) | *z* (–3.68) $p < 0.05$ |
| Ambulatory, *n* = 89 (49.5%) | 10 (43.5%) | 13 (56.5%) | 23 (25.8%) | 34 (51.5%) | 32 (48.5%) | 66 (74.2%) | *z* (-2.79) $p < 0.05$ |
| Incident-Admission Time (Minutes), 103 ± 93 min | 105 ± 90 | 79 ± 65 | 86 ± 73 | 163 ± 126 | 102 ± 92 | 129 ± 112 | *z* (–2.60) $p < 0.05$ |

Almost half of the toxic exposures (51.7%, *n* = 93) were considered as unintentional whereas 77.7% (*n* = 93) of drug intoxication cases were intentional suicidal attempts. The most common acute toxic exposure presentations were due to drugs (57.2%, *n* =103). Venomous animal/insect bites (16.1%, *n* = 29) and toxic gas inhalation (13.9%, *n* = 25) (Fig. 1). Toxic gas inhalation included inhalation of carbon monoxide (CO) (11.7%, *n* = 21) and vapor of hydrochloric acid (HCl) (2.2%, *n* = 4). The rest of the presentations (19.4%, *n* = 35) were due to food poisoning (7.8%, *n* = 14), alcohol intoxication (3.9%, *n* = 7) and ingested toxic agents (1.1%, *n* = 2). No illicit drug use was observed. The most common drugs involved in intoxication with medications were analgesics (24.3%, n=25), antidepressants (23.3%, *n* = 24). benzodiazepines (12.6%, *n* = 13), salicylates (12.6%, *n* = 13) and neuroleptics (9.7%, *n* = 10), representing 82.5% of all intoxication with medications.

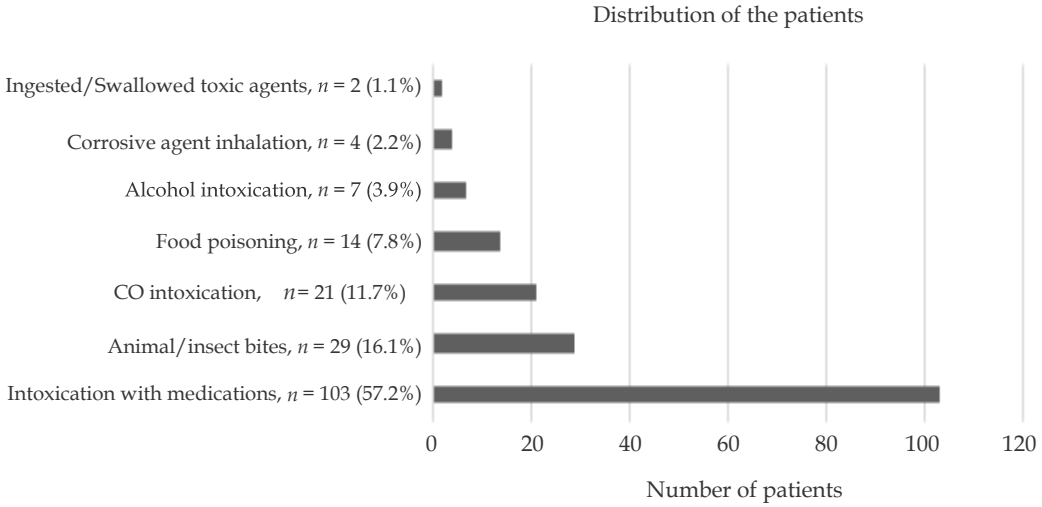


**Fig. 1.** The distribution of acute toxic exposure patients.

A positive history of depression was present in 88.5% of the intentional cases. The female subjects had a significantly higher rate of history of depression compared to male subjects ($p < 0.01$). There was a history of suicide attempt in 13 patients who had intentional exposures. There was no suicide attempt history in the group of unintentional cases.

More than one-third of total patients (35%) were discharged after the initial treatment from ED,

61.1% were hospitalized for further treatment and observation, and 2.8% were transferred to another medical center. No patient died during the study period due to acute toxic exposure. Only one patient left the ED without permission and medical advice (Table 3).

At least one consultation was requested for 126 patients. Two or more consultationswere performed for 19 patients. The most consulted division was Internal Medicine (48.9%, $n$ = 115).

**Table 3:** Exposure type. history of depression. history of previous suicide attempt and disposition state of the patients by intention status

| $n$ (%) | Intention status | | | | | | $p$ |
|---|---|---|---|---|---|---|---|
| | Intentional 87 (48.3%) | | | Unintentional 93 (51.7%) | | | |
| | Male | Female | Total | Male | Female | Total | |
| | 24 | 63 | 87 | 49 | 44 | 93 | |
| **Exposure type** | | | | | | | |
| Intoxication with medications | 17 (21.3%) | 63 (78.8%) | 80 (77.7%) | 8 (34.8%) | 15 (65.2%) | 23 (22.3%) | |
| Alcohol intoxication | 7 (100%) | 0 | 7 (100%) | 0 | 0 | 0 | |
| Toxic inhalation | 0 | 0 | 0 | 12 (48.0%) | 13 (52.0%) | 25 (100%) | |
| Food poisoning | 0 | 0 | 0 | 8 (57.1%) | 6 (42.9%) | 14 (100%) | |
| Animal and insect bites | 0 | 0 | 0 | 19 (65.5%) | 10 (34.5%) | 29 (100%) | |
| Positive history of Depression $n$ = 26 (14.4%) | 2 (8.7%) | 21 (91.3%) | 23 (88.5%) | 2 (66.7%) | 1 (33.3%) | 3 (11.5%) | $z$ (–4.41) $p$ < 0.01 |
| Positive history of Suicidal Attempt. $n$ = 13 (7.2%) | 3 (23.1%) | 10 (76.9%) | 13 (100%) | 0 | 0 | 0 | |
| **Disposition status** | | | | | | | |
| Discharge | 7 (53.8%) | 6 (46.2%) | 13 (20.6%) | 31(62.0%) | 19 (38.0%) | 50 (79.4%) | |
| Hospitalization | 16 (23.2%) | 53 (76.8%) | 69 (62.7%) | 17 (41.4%) | 24 (58.6%) | 41 (37.3%) | |
| Exitus | 0 | 0 | 0 | 0 | 0 | 0 | |
| Transfer | 0 | 4 (100%) | 4 (80.0%) | 0 | 1 (100%) | 1 (20.0%) | |
| Leave without permission | 1 (100%) | 0 | 1 (50.0%) | 1 (100%) | 0 | 1 (50.0%) | |

## Discussion

We foresee that a current and detailed profile of acute toxic exposure cases will play a role in the preparation, labor, and needs of the EDs. Acute toxic exposure has very broad subheadings, although even health care professionals focus on a narrow group "intentional drug poisoning" when acute toxicity termis on the table. Our study showed that 51.7% of the cases were unintentional. Unintentional acute toxic exposures are generally under diagnosed and/or under reported groups. The toxicity studies show low rates of intentional cases.[3,4] Therefore, this study can be seen as a comprehensive retrospective study including so many subgroups as possible.

ED admissions due to acute toxicities have an increasing trend worldwide.[5-7] The acute toxic exposure-related annual admissions represented 0.4% of all ED visits in our study which was close to the lower limits reported in the literature (0.39–7.8%).[5–10] As mentioned earlier, a variety of the acute toxicity ratios reported in the literature may stem from the number of the subgroups included in the studies, under-reporting of the cases, andthe included age range of patients.[7,10] It is obvious that the broader inclusion criteria are, the higher the epidemiologic rates would be got.

The second and third most common causes are generally differed but "intoxication with medication" is the leading cause of poisoning which is similar to the results of previous reports.[5,7,10,11] The median age was 26 (3–89) indicating that acute toxic exposure mainly an adolescence and adulthood problem. Intentional exposures constitute almost half of the study population (48.3%). They are mostly young female adults who abused pharmaceutical drugs—especially antidepressants—during

autumn. This common profile can be seen in many studies.[6,8,11,12] Another typical intoxication type with a seasonal pattern is CO intoxications. As expected, our cases mostly admitted to ED in the autumn and winter seasons.[13] In case of venomous insect bites, the expected seasons are generally spring and autumn.[8] Geographically speaking, the venomous insect/animal species living in Turkey are limited yet but we seasonally encounter tick bites and occasional snake, scorpion and spider bites in the clinical routine.[14]

Food poisoning was another common cause of intoxication in our patients (7.8%). The cases with food poisoning were all due to mushroom intoxication, which is compatible with toxicity literature in Turkey stating that mushroom intoxication is the most common reason of toxic liver failures both for adults and children in Turkey.[15]

A total of 35% of patients were discharged after the initial treatment from the ED, 61.1% were hospitalized for further treatment and observation, and 2.8% were transferred to another medical center. No patient died during the study period due to acute toxic exposure. The reported mortality rates can be up to 27% but it is generally associated with severely intoxicated ICU patients.[16] When this subpopulation is excluded, the mortality rate ranges from 0.24 to 9%.[17,18]

The pediatric and geriatric patients represent only a small part of the study population (10% and 7.2%, respectively). Despite the limitations in pediatric cases, most of them were young children with unintentional exposures and battery ingestions emerged as an important problem in pediatric groups, which were consistent with the literature.[13,19]

As suggested by the previous literature, some precautions should be taken to decrease acute toxic exposures such as increasing control of prescribed medications, psychological and/or psychiatric support in cases of depression and/or suicide attempts, promotion of public awareness via information campaigns especially among young population for intoxication with medication, effective protection of children from potentially harmful agents for pediatric toxic exposures, controlling of indoor heating systems for CO intoxications before winter use, and standardized personal protection during pesticide use.[11,20]

Being a descriptive study conducted at the ED of a university hospital located in a small town, our study is not free from some limitations. To begin with, our findings are likely to underestimate the current state due to the limited nature of the groups included in the study: our hospital does not have a pediatric ED so that we could not observe any drug exposures in children. As our city is a small, non-industrialized residential area in the central Anatolia where agriculture is not a prime way of living, we did not encounter any sea-origin, industrial, or agricultural (pesticide) intoxications. We excluded bee-sting and dog bite cases as parts of venomous animal/insect bites and detected no illicit drug abuse case during the study period. Moreover, we probably had an underreporting problem for acute toxic exposures, as well. To conclude, more representative studies conducted within broader range of time periods, based on national databases and of multi-centered nature are needed for more accurate data.

## Conclusion

Current analysis of acute toxic exposures plays a key role in the regulation of EDs and the creation of holistic health care strategies. Although it appears to contain a small proportion of ED admissions, acute toxicity will always be an important medical topic due to its morbidity and mortality potential.

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*Original Article*

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# Embryotoxic Effects of Para Substituted Chloro-Nitro Phenol Compounds on Zebrafish

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**Abstract**

*Background:* Found on the priority organic pollutants list and mono substituted derivatives of phenol, 4-chloro and 4-nitrophenol compounds are organic-origin micropollutants with toxicologic research performed due to recalcitrant properties, endocrine disrupting and lipophilic character, and tendency for bioaccumulation. As both pollutants are used in many industrial and other applications (from paper, pesticides, dye, pharmaceutical and explosives industries to chlorination of drinking water), they rapidly accumulate at all trophic levels of the ecosystem and are known to cause negative effects on organisms in the ecosystem led by aquatic systems. As a result, there are many methods developed for toxicity research of these types of priority organic pollutants, though studying the small freshwater fish species of zebrafish (embryo and adult) is chosen due to some advantages compared to other methods.

*Aims:* To our knowledge, there are no studies performed on the embryotoxic effects of 4-nitrophenol and 4-chlorophenol derivatives on fish species. With this aim, the lethal and teratogenic effects of the phenol derivatives on zebrafish (*Danio rerio*) were evaluated by means of FET (fish embryo toxicity) test.

*Materials and methods:* According to FET test, embryos at 1.5 hpf (hour post-fertilization) were exposed to 4-nitrophenol and 4-chlorophenol solutions in concentrations 1.0, 2.0, 4.0, 8.0 and 16.0 mg/L during 96 h. Every 12 hours, the embryos were observed and scored for lethal and teratogenic effects. Also, LC50 values were calculated for 48 h.

*Results:* 48 h LC50 values were 2.409 mg/L for 4-chlorophenol and 4.581 mg/L for 4-nitrophenol. With increasing phenol concentrations, lethality and malformations increased. Teratogenic malformations more frequently produced by phenol derivatives were: incomplete body, chorda deformity, tail curvature, lordosis, scoliosis, delayed development and hatching, weak or non-pigmentation.

*Conclusion:* 4-chlorophenol was found to be more toxic than 4-nitrophenol. Results firstly showed that the phenol derivatives caused embryotoxicity in zebrafish.

**Keywords:** 4-chlorophenol; 4-nitrophenol; Zebrafish; Embryotoxicity, LC50

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## Introduction

Currently, in parallel with developments in industry and technology, the amount of waste polluting the environment in the form of solid-liquid-gas has rapidly increased, whether due to the numbers of chemicals entering production or linked to the production capacity. These pollutants (organic/inorganic) have toxic and ecotoxic

effects linked to their origin. In addition to direct toxic effects on humans and animals, the ecotoxic effects caused linked to distribution and variation processes within the ecosystem (water-soil-air-sediment) have reached dimensions that cannot be ignored.[1] Additionally, it is a known reality that the split in the correlation between ecological footprint and industrial production has opened to a degree that cannot be compensated for and this is an important criterion showing the degree to which toxic chemicals have polluted the ecosystem.

Intensely used in agriculture, industry and even disease control, the important chemicals of chlorinated and nitrated phenol compounds are known to cause both health and environmental problems at serious dimensions.[2] Especially 4-chloro/nitro phenol compounds, with high logKow (bioaccumulation marker) value and resistant to biodegradation, accumulate in aquatic environments and negatively affect aqueous organisms.[3]

Chlorinated phenol compounds are organic phenol derivatives with many alternative derivatives containing from one (mono) to five (penta) chlorine atoms. Chlorophenols (CP) are listed among the priority toxic pollutants according to the US EPA, and are organic chemicals that have the ability to change bacterial activity, phytotoxicity and bioaccumulation capability linked to the number and position of chlorine atoms and which can be found in surface waters, groundwater and even drinking water (disinfected with chlorine).

As a result, the mixing and accumulation percentage of chlorophenols in the environment is very high.[4] In addition to use in wood, dye, tanning and disinfectant, they are commonly encountered in many industrial processes and byproduct synthesis.

These include the use of chlorophenols as raw materials in production of herbicides, fungicides, insecticide, pharmaceuticals and dyes, and their use as organic solvents. They may also be released from burning of waste with high organic content, chlorine paper-bleaching processes and dechlorination of drinking waters.[5]

Among chlorophenols, the most toxic mono-chlorophenol derivative of (para) 4-chlorophenol (4-CP) is a congener of this group and in addition to being included on the priority pollutant list, it is an organic pollutant known to be teratogenic, carcinogenic, and mutagenic for organisms and very toxic.[6]

Nitrophenols, similar to chlorophenols, are other substituted phenols with alternative derivatives from mono to penta. Nitrophenol (NP) compounds are commonly used in production of pesticides, dyes and dye material, plastic, rubber, explosive materials, tanning and pharmaceuticals. NPs present in industrial wastewater have high refractivity, high stability and water-soluble characteristics, in addition to being released from diesel exhausts.[7]

Like other substituted phenol derivatives, nitrophenols have abundant sources in the environment and are pollutants with high probability of being found in surface water, groundwater (rain water, snow melt, rivers and sediments) and waste water.[8] There are 3 different nitrophenol derivatives (2-NP, 4-NP and 2,4-DNP) present on the Environmental Protection Agency (EPA) priority pollutant list.[9]

Among these NP compounds, the very toxic mono nitrophenol of 4-NP is resistant to biological degradation, and rapidly accumulates at trophic levels in aquatic and terrestrial organisms. 4-NP was identified in the urine of people exposed to organophosphate pesticides, while it displays EDC (Endocrine Disruptive Compound) effects in rodents.[10] There are many studies showing this compound, resistant to biodegradation, is toxic.[11]

Some substituted phenol derivatives are known to have toxic effects on invertebrate and vertebrate animal species. Many studies have shown that these compounds have known genotoxic effects in both humans and animals, while the studies related to embryotoxicity are very limited.[12,13]

Zebrafish (*Danio rerio*) are an important model organism used in toxicity research. The embryos are very commonly used to determine the developmental toxicity of many chemicals.[14–17] The embryos are transparent, and every embryonic stage is easily visible.

Also, they have high fecundity, and can be produced by external fertilization so they are appropriate for *in vitro* studies. They have homologous gene structure to humans (xenobiotic metabolism phase I and phase II enzyme systems) making zebrafish a model organism with perfect compliance for FET (Fish Embryo Toxicity) tests. The aim of the present study was to determine the probable embryotoxic effects of phenol derivatives with substitution in the para position of 4-CP and 4-NP by using zebrafish embryos with the FET test.

## Materials and Methods

### *Chemicals*

4-nitrophenol (4-NP) and 4-chlorophenol (4-CP) compounds were obtained from Sigma. Stock solutions were separately prepared for each compound. 4-CP was dissolved in water, while 4-NP was dissolved in 1% DMSO. Stock solutions were stored at 5°C until the start of experiments.

### *Fish culture and eggs production*

Adult zebrafish were obtained from Atatürk University, Faculty of Aquaculture, Aquarium Fish Research Center. Before experiments, fish were left to adjust to the new environment for 14 days. Twenty healthy adult fish were placed in the aquarium and photoperiod was set to 14:10 light/dark cycles. Dechlorinated water was placed in the aquaria, and the water temperature was set to 27 ± 1°C.

The aquarium water had 1/3 of the water changed 1 time per week. Fish were fed with flake food 2 times per day. One week before obtaining eggs, the separated female and male fish were placed in a breeding aquarium. With fertilization occurring in the early hours of the morning, fertilized eggs were removed from the aquarium and washed twice in distilled water. The fertilized eggs were later placed in petri dishes containing Hank's solution and placed in an incubator set to 27 ± 1°C.

### *Embyrotoxicity Test*

The FET test is a standard test coded OECD TG236 based on monitoring of embryo development at certain intervals after contact of fertilized fish embryos with a probable toxicant.[18] 4-NP and 4-CP phenol derivatives were analyzed according to this test. Firstly, previous studies were used to determine the experimental concentrations.[16,19] The control group used Hank's solution, while the negative control group used a solution containing 1% DMSO.

At approximately 1.5 hours post-fertilization (hpf), embryos in the incubator were placed in petri dishes containing 1.0, 2.0, 4.0, 8.0 and 16.0 mg/L substituted phenol derivatives, and the 96 hours exposure was begun after the fertilized eggs were placed into the test solutions. Each petri dish contained 20 fertilized fish embryos and the experiment had 3 repeats ($n$ = 60).

The solutions in the petri dishes were changed at 24-hour intervals. Every 12 hours, the embryos were observed. Dead embryos were immediately removed and recorded.

The fish embryos in 48-hpf; especially, were used to determine the median lethal dose (LD50) of the compounds. Teratogenic abnormalities like coagulation, edema, lordosis, etc. observed in fish embryos and larva were identified and photographed with a digital camera device. Teratogenic abnormalities were classified according to the grading reported by Padilla et al.[20] Using this grading, the total malformation index (MI) was calculated. The group with MI values from 0 to 3 were normal, the group with values from 4 to 6 were slightly abnormal, and the group with values of 7 and above were accepted as completely abnormal larva.

### *Statistical Analysis*

Statistical analysis was performed using the SPSS 20.0 software programme. The 48-hpf LC50 value was calculated with Probit analysis. Evaluation of abnormal embryos and larvae used the one-way ANOVA test. All data are given as mean ± standard deviation. Multiple comparisons had the Dunnett test performed after variance analysis and statistical significance level was accepted as $p < 0.05$.

## Results

The 48-hpf LC50 values were calculated as 2.409 mg/L for 4-chlorophenol and 4.581 mg/L for 4-nitrophenol. As the phenol concentration increased, it was found the number of deaths and abnormal embryos increased. It was observed that 4-CP was more toxic than 4-NP. The calculated LC50 values are reported for the first time in this study (Fig. 1 and 2).

The embryo development slowed down in the phenol groups (Table 1). The developmental parameters such as gastrulation, somite formation, optical vesicle formation, spontaneous movement, tail separation, heartbeat, blood circulation, pigmentation and hatching regressed at sub-lethal phenol doses, while some appeared not to fully occur. Phenols additionally caused a reduction in the number of hatching larvae. The larvae which never hatched with high phenol concentrations at the end of exposure died after a while.

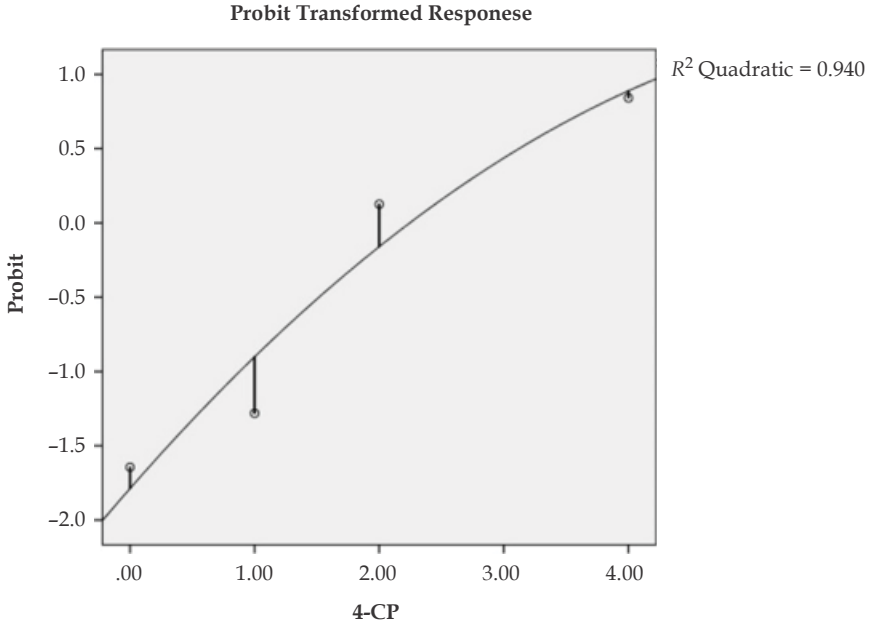


**Fig. 1:** Dose-response curve used for calculation of LC50 value for 4-CP (confidence interval 95%).

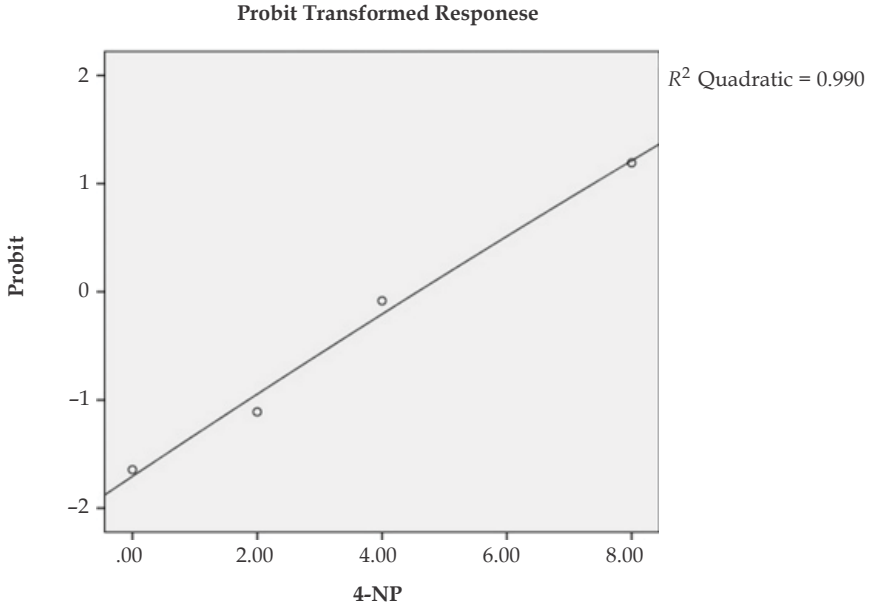


**Fig. 2:** Dose-response curve used for calculation of LC50 value for 4-NP (confidence interval 95%).

**Table 1.** Developmental rates of the embryos exposed to the phenols

| Developmental period[a] Hour[b] | Cleavage 1.5 hpf | 75% Epiboly 8 hpf | Pharyngula 24 hpf | Hatching 48 hpf | Larva 80 hpf |
|---|---|---|---|---|---|
| Control | 1.6 ± 0.10 | 8.7 ± 0.20 | 24.5 ± 1.20 | 49.3 ± 1.60 | 82.0 ± 1.30 |
| DMSO (%1) | 1.6 ± 0.05 | 9.0 ± 0.10 | 26.6 ± 0.80 | 47.2 ± 1.80 | 84.1 ± 1.90 |
| 4-CP (2 mg/L) | 1.7 ± 0.10 | 17.2 ± 1.10* | 39.4 ± 2.80* | 76.2 ± 6.30* | 120.2 ± 8.60* |
| 4-NP (4 mg/L) | 1.7 ± 0.10 | 16.0 ± 0.90* | 36.5 ± 2.20* | 69.4 ± 7.40* | 111.3 ± 8.80* |

[a]Stages given according to Kimmel et al. [21][b]hpf: hour post-fertilization. *shows differences at $p < 0.05$ level compared to control. Values given as mean ± standard error.

At high phenol doses, more than half of fish embryos died within the first 48 hours. The teratogenic effects observed over 96-hours duration in embryos and the mortality rates are given in Tables 2 and 3. As phenol concentrations increased, the number of affected embryos and larvae increased. With exposure to 8 and 16 mg/L 4-CP, all embryos died. At the end of 96 hours in the 2 and 4 mg/L the phenol groups, the majority of surviving embryos (76% and 86% respectively) were observed to have teratogenic abnormalities (Table 2).

**Table 2:** Adverse effects in embryos caused 4-CP at 96-hour exposure

| | Control | 1 mg/L | 2 mg/L | 4 mg/L | 8 mg/L | 16 mg/L |
|---|---|---|---|---|---|---|
| Number of teratogenic embryos | 2 | 4 | 13 | 6 | 0 | 0 |
| Number of dead embryos | 3 | 6 | 33 | 48 | 60 | 60 |
| Number of affected embryos | 5 | 10 | 46 | 54 | 60 | 60 |
| Number of normal embryos | 55 | 50 | 14 | 6 | 0 | 0 |
| % Teratogenic embryos | 3.3 ± 0.1 | 6.6 ± 0.5 | 21.6 ± 3.0 | 6.6 ± 0.9 | 0 | 0 |
| % Dead embryos | 5.0 ± 0.2 | 10 ± 1.0 | 55 ± 8.2 | 80 ± 5.6 | 100 ± 0 | 100 ± 0 |
| % Affected embryos | 8.3 ± 1.0 | 16.6 ± 2.0* | 76.6 ± 9.7* | 86.6 ± 6.7* | 100 ± 0* | 100 ± 0* |
| % Normal embryos | 91.7 ± 5.8 | 83.4 ± 5.0 | 23.4 ± 4.6 | 13.4 ± 1.4 | 0 | 0 |

*Shows differences at $p < 0.05$ level compared to control

All embryos died at 16 mg/L 4-NP dose. At the end of 96 hours, in the 4 and 8 mg/L the phenol groups, most of the surviving embryos (79% and 86%) were observed to have teratogenic abnormalities (Table 3).

**Table 3:** Adverse effects in the embryos caused 4-NP at 96-hour exposure

| | Control | DMSO | 1 mg/L | 2 mg/L | 4 mg/L | 8 mg/L | 16 mg/L |
|---|---|---|---|---|---|---|---|
| Number of teratogenic embryos | 2 | 1 | 3 | 7 | 20 | 2 | 0 |
| Number of dead embryos | 3 | 3 | 2 | 8 | 28 | 53 | 60 |
| Number of affected embryos | 5 | 4 | 5 | 15 | 48 | 55 | 60 |
| Number of normal embryos | 55 | 56 | 55 | 45 | 12 | 5 | 0 |
| % Teratogenic embryos | 3.3 ± 0.1 | 1.6 ± 0.1 | 5.0 ± 1.0 | 11.6 ± 1.5 | 33.3 ± 6.5 | 3.3 ± 0.2 | 0 |
| % Dead embryos | 5.0 ± 0.2 | 5.0 ± 0.3 | 3.3 ± 0.2 | 13.3 ± 2.6 | 46.6 ± 6.6 | 83.3 ± 5.3 | 100 ± 0 |
| % Affected embryos | 8.3 ± 1.0 | 6.6 ± 0.6 | 8.3 ± 0.7 | 24.9 ± 3.8* | 79.9 ± 8.5* | 86.6 ± 4.8* | 100 ± 0* |
| % Normal embryos | 91.7 ± 5.8 | 93.4 ± 6.0 | 91.7 ± 6.6 | 75.1 ± 8.4 | 20.1 ± 2.5 | 13.4 ± 2.1 | 0 |

*Shows differences at $p < 0.05$ level compared to control.

Fish embryos exposed to lethal and sub-lethal phenol doses within 48 hours were observed to have different teratogenic effects such as incompleted head, eye, tail and chorda (Fig. 3B), weak pigmentation, chorda defects and lack of body parts (Fig. 3D). In the same way, 72-hpf larvae had chorda abnormalities such as lordosis (Fig. 4B), tail curvature (Fig. 4C) and scoliosis (Fig. 4D) linked to increasing phenol concentrations. Additionally, non-hatching larvae and non-pigmentation embryos were identified (Fig. 5). These abnormalities were not observed (Fig. 3A, Fig. 3C, Fig. 4A) or observed less in fish embryos and larvae in the control and negative control groups. The most dominant abnormalities were detected as chorda malformations. All of teratogenic embryos and larvae died within 1–2 days after 96 hpf.

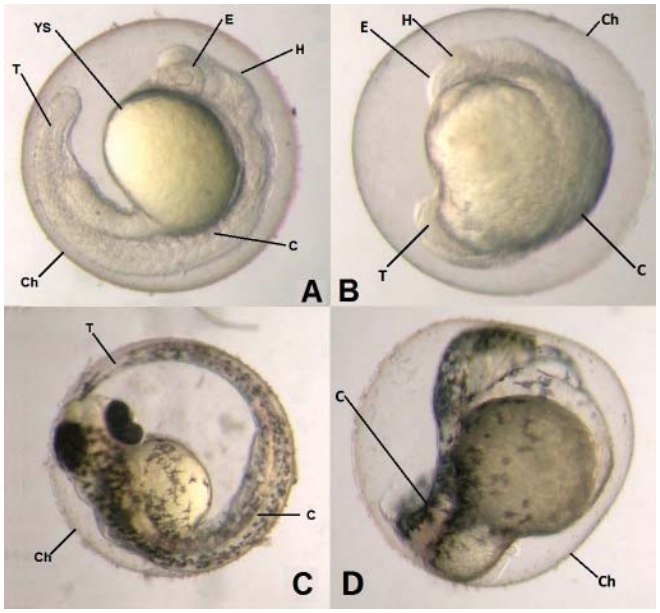


**Fig. 3:** Normal fish embryos at 24 and 48 hpf and abnormal embryos exposed to 4 mg/L 4-CP and 8 mg/L 4-NP. (A) 24-hpf normal embryo, (B) 24-hpf abnormal embryo; incompleted head, eye, tail and chorda, (C) 48-hpf normal embryo, (D) 48-hpf abnormal embryo; weak pigmentation, chorda defects and lack of body parts. Ch: chorion, C: chorda, E: eye, H: head, T: Tail, YS: yolk sac.

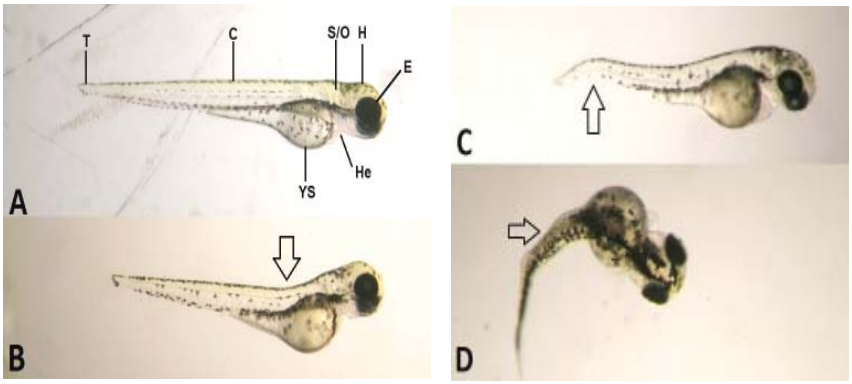


**Fig. 4:** 72-hpf normal fish larva and abnormal larvae exposed to 4 mg/L 4-CP and 8 mg/L 4-NP. (A) 72-hpf normal larva, (B) 72-hpf abnormal larva; lordosis (arrow), (C) 72-hpf abnormal larva; tail curvature (arrow), (D) 72-hpf abnormal larva: scoliosis (arrow). C: chorda, E: eye, H: head, He: heart, S/O: sacculus/otolith, T: Tail, YS: yolk sac.

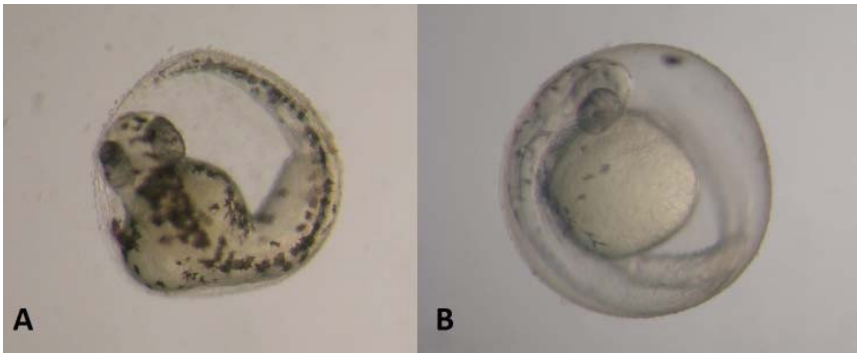


**Fig. 5.** Abnormal fish embryos exposed to 4 mg/L 4-CP and 8 mg/L 4-NP. (A) 72-hpf non-hatching larva, (B) non-pigmentation 48-hpf embryo.

The total malformation index (MI) values for the phenol compounds are presented in Table 4. The MI value for 1 mg/L phenol dose was not different to the control and DMSO groups. However, the MI values for 2 mg/L, 4 mg/L and 8 mg/L phenol concentrations increased. Especially for 4 mg/L 4-CP and 8 mg/L 4-NP doses, the MI values were above 7 indicating the most embryos and larvae were deformed. The MI values for 8 and 16 mg/L 4-CP and for 16 mg/L 4-NP could not be calculated.

**Table 4**. Total malformation index (MI) values calculated for 72-hour fish larvae exposed to the phenol derivatives

| | MI values | | | | |
|---|---|---|---|---|---|
| Control | | | 1.7 ± 0.2 | | |
| DMSO | | | 1.9 ± 0.2 | | |
| | 1 mg/L | 2 mg/L | 4 mg/L | 8 mg/L | 16 mg/L |
| 4-CP | 2.7 ± 0.7 | 5.6 ± 0.6* | 8.0 ± 1.0* | 0 | 0 |
| 4-NP | 2.3 ±0.6 | 4.5 ± 0.4* | 6.2 ± 0.8* | 7.6 ± 1.6* | 0 |

* Show differences at $p < 0.05$ level compared to control

## Discussion

Death and various teratogenic abnormalities were observed linked to increasing concentration with exposure of zebrafish embryos to 4-CP and 4-NP for 96 hours. For both compounds, the 48-hour LC50 values were calculated as 2.409 mg/L for 4-CP and 4.581 mg/L for 4-NP. These results comply with the results of other studies. The 96-hour LC50 value for 4-CP for Javanese ricefish (*Oryzias javanicus*) was found to be 4.1 mg/L.[22] Pentochloro-phenol (PCP) had 24- and 48-hour LC50 values of 196 and 130 $\mu$g/L for zebrafish,[23] with the 48-hour LC50 values for 2-CP and 2, 4-DCP identified as 8.378 mg/L and 6.558 mg/L for zebrafish embryos.[17] Zhang et al.[24] reported that the 120-hour LC50values for 2,4-DCP and 2,4,6-TCP were 2.45 mg/L and 1.11 mg/L for zebrafish embryos. For nitrophenols the situation is not different. Ceylan et al.[16] reported the 48-hour LC50 values for 2-NP and 2,4-DNP were 18.7 mg/L and 9.65 mg/L for zebrafish embryos. The 48- and 96-hour LC50 values identified for 4-NP for fresh and saltwater organisms varied from 11.0 mg/L to 62 mg/L. For rainbow trout (*Oncorhynchus mykiss*) this value was found to be 78.9 mg/L. For shellfish, this value was identified as 2.8 to 20 mg/L.[25] Nitrophenols have less deadly effect compared to chlorophenols and the USEPA[26] recommends they be present at maximum 3.5 mg/L levels in environmental waters and this is supported by our results.

The substituted phenol concentrations identified in aquatic systems are not yet at toxic levels. The highest identified chlorophenol concentration was 0.002–2 mg/L in river waters. In drinking water, this rate is lower.[27] The amounts of nitrophenols identified in fresh water do not pass 0.0057 mg/L.[28] However, comprehensive monitoring studies continue around the world.

In our study, sublethal doses of 4-CP and 4-NP caused developmental toxicity and a variety of teratogenic abnormalities in zebrafish embryos larvae. Similar results were obtained from previous studies. For example, Xu et al.[29] reported a reduction in neutrophil counts and endocrine system disruptions in 7-day zebrafish larvae with 5 mg/L 4-NP exposure. Osin et al.[19] exposed zebrafish to different doses of 4-NP (1.0, 5.0, 10.0, 15.0 and 20.0 mg/L) and reported that there was a dose-linked increase in embryo deaths and a reduction in the number of hatching larva. There are fewer studies showing the embryotoxic effects of chlorophenols. Xu et al.[30] stated there was developmental regression in 8-hpf zebrafish embryos with PCP exposure. Lopez-Romera et al.[31] observed that Na-PCP caused death of zebrafish embryos within 72 hours and revealed a variety of teratogenic effects.

There is limited information about the toxic effect mechanism of substituent phenols. Phenols are known to reduce the activity of some biochemical enzymes. For example, 2-CP triggers formation of reactive oxygen radicals in fish,[32] while 2,4-DCP causes oxidative stress.[33] Thus, the damage occurs in some important cell molecules such as DNA, protein and lipid.[34] Chlorophenols disrupt the mitochondrial membrane potential[35] and cause cell death leading to acute toxicity.[36] For nitrophenols, after exposure ATP levels in the organism reduce, and it is known that oxidative metabolism is disrupted especially in zebrafish.[37] The lethality and teratogenicity occurring in zebrafish embryos linked to 4-CP and 4-NP may be explained by

increased oxidative stress during embryonic cell development in fish and disruption of oxidative metabolism.

## Conclusion

This study showed the adverse effects of 4-CP and 4-NP on the embryonic development of zebrafish. 4-CP was found to be more toxic than 4-NP (nearly 2 times). Substituent phenol doses of 2.0, 4.0 and 8.0 mg/L are estimated to be potentially harmful for aquatic organisms. The level of 4-NP identified in aquatic systems is not yet at levels to affect aquatic organisms, while it has been revealed that 4-CP has reached the margins of critical levels in research. However, if precautions are not taken, it should not be forgotten that these two substituent phenol compounds will increase in natural environments and will affect organisms in the ecosystems.

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# Investigation of Metal Pollution in Soil Samples Between Akoluk, Mehmetbeyli and Temrezli Villages (Yozgat – Sorgun), Turkey

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**Abstract**

The study area is located in Kirsehir Turkey's blog from the tectonic units and Tertiary has been observed that epigenetic occur in the sedimentary units. Uranium mineralization is located between Akoluk, Mehmetbeyli and Temrezli Villages of Sorgun district of Yozgat. The unit consisting of conglomerate, sandstone and mudstone alternations (Paleson aged) is located on granites and the youngest unit in the region is Quaternary alluvium. 20 soil samples were taken to investigate the pollution dimensions, possible sources and spatial distribution of the elements, in the soil samples in the study area. In this study, the relationship between element values obtained by geochemical analysis (As, Cr, Cu, K, Mn, Ni, Pb, Th, U, V, Zn and Zr) was investigated statistically. A positive correlation was observed between Cr-Zn, Cu-Ni, K-Mn, Mn-Pb, Mn-Th, Mn-V, Mn-Zr, Pb-Th, Pb-V, Pb-Zn, Pb-Zr, Th-U, Th-V and U-V elements in soil samples ($p < 0.01$; $p < 0.05$). The average shale values were considered as background values in determining the metal pollution dimensions in soil samples. Enrichment factors (EF), geoaccumulation index (Igeo), Contamination factors (CF) and pollutant load index (PLI) were calculated to determine the pollution dimensions of the elements. According to the results of the analysis, the average values of Cr, Fe, U, V and Zr were below the average shale values of the world. For each element examined, the mean EF values K, Mn (in all samples), Ni (in samples 9, 16 and 17), and Zn (in samples 5, 8, 10, 14, 16 and 17) were extremely enrichement. Contamination factor values As (in all samples) elements in terms of considerably contamination and contamination were detected. Since the PLI values (except example 17) were > 1, all elements were found to contaminate the study area. According to the results obtained, the average values of the elements in the study area are K > Mn > Zn > Ni > Pb > V> Cu > As > Th > Zr > Cr > U.

**Keywords:** Enrichment factor; Geoaccumulation index; Pollution; Soil.

## Introduction

Since uranium (U) is widely used in the nuclear industry, global demand has increased. Uranium has become an important energy mineral in the world in the last sixty years.[1] However, harmful metals in uranium mining and mining wastes caused environmental pollution.[2] Plants from polluted soils pick up these metals and can pass through the food chain to humans and animals. This metal is toxic and can cause cancer in living things because it generates radiation.[3,4]

The uranium content of the soil has values ranging from 1 to 8 ppm and an average of about 1 ppm. It can be seen in uranium-rich areas over 100 ppm. In normal soils, the A horizon is the richest zone of uranium because it differs from humic materials, clay-humic complexes, humic-iron and humic-silicate complexes. In uranium-

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containing soils, however, the B horizon may be slightly enriched relative to the A horizon as it contains and/or separates, for example, hydroxides and aqueous oxides of iron, manganese, aluminum. The C horizon above the uranium deposits is the richest in mineral content.[5]

Soil pollution has been an important environmental issue in both developed and developing countries, particularly on land use.[6] Heavy metals are particularly important because they are toxic, carcinogenic and persistent in the environment. According to researches, heavy metals have high atomic weight and have a density of at least five times higher than water and are naturally occurring.[7] In the environment, heavy metals are distributed spatially in the form of mineralization.[8] Numerous domestic, industrial, medical, agricultural and technological applications have led to the spread of heavy metals into the environment, resulting in increased potential impacts on human health and the environment. Heavy metal accumulation in soil consists of both anthropogenic activities and lithogenic sources.[9] Two major sources of heavy metal pollution have been identified: natural resources such as erosion of rocks and thermal waters, or anthropogenic sources involving mining and related industries.[10,11]

Heavy metal deposits and accumulation studies have gained importance as heavy metals in soils can have negative effects on human health and environment.[12–15] While environmental pollution caused by heavy metals is caused by many activities, pollution caused by heavy metals in the soil system is mainly caused by natural processes such as decomposition of minerals, as well as anthropogenic activities related to industry, agriculture, combustion of fossil fuels, vehicle emissions and mining. In the environmental pollution study, some parameters are used to estimate how much soil is affected by heavy metals (either natural or anthropogenic).[16, 17]

Heavy metal pollution is a global problem that concerns all societies.[4] Methods such as enrichment factor (EF), geoaccumulation index (Igeo), contamination factor (Cf) and pollution load index (PLI) are widely used to assess the level of heavy metal pollution.[4,18–22,]

The aim of this study is to define the sources of pollution in the study area, and to determine the natural and anthropogenic pollution geochemically.

### *Geology of the Study Area*

The study area is located within the boundaries of Sorgun district of Yozgat province in the Central Anatolia Region and is bordered by Mehmetbeyli in the north, Şahmuratlı in the west, Temrezli in the south and Akoluk villages in the east (Fig. 1). When the work is discussed in terms of a wider regional scale geological, tectonic units of Turkey[23] shows that take place in Kirsehir Blog. It was identified as Yozgat Batolite and/or Yozgat Intrusive Complex in the previous studies and is located on the northern edge of the Crystalline Complex of Central Anatolia.[24–26]

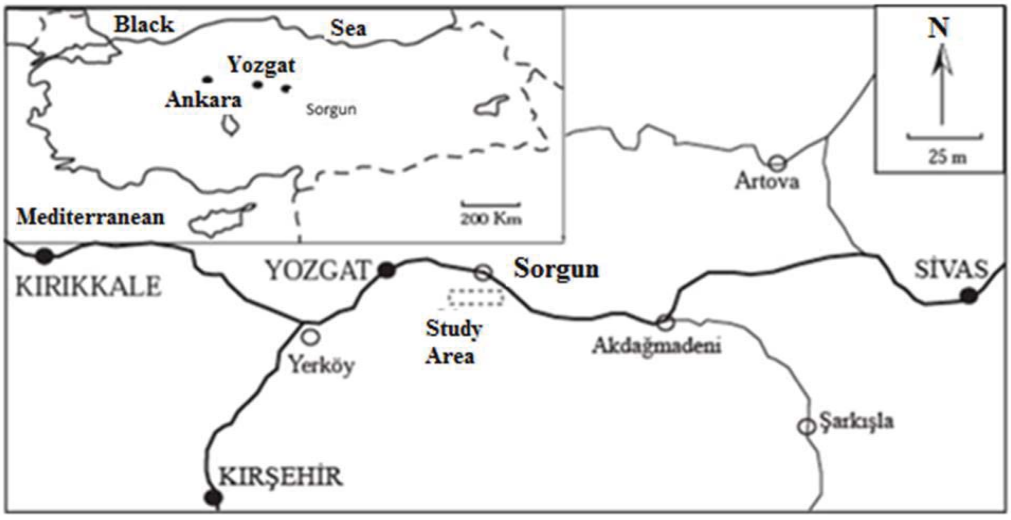


**Fig. 1:** Location map of the study area.

The uranium mineralization in the region occurred epigenetically in the Tertiary aged sedimentary units in the Sivas Basin. On the granite foundation, Paleocene aged conglomerate, sandstone, mudstone alternation is located. Eocene aged coarse and fine grained sandstone, siltstone and claystone are unconformably overlain by this unit. Andesitic and basaltic volcanics formed by the volcanism formed at the end of Eocene cover all these units in places. Pliocene aged limestones overlie the volcanics. Quaternary aged alluvial cover overlies all of these units (Fig. 2).[27]

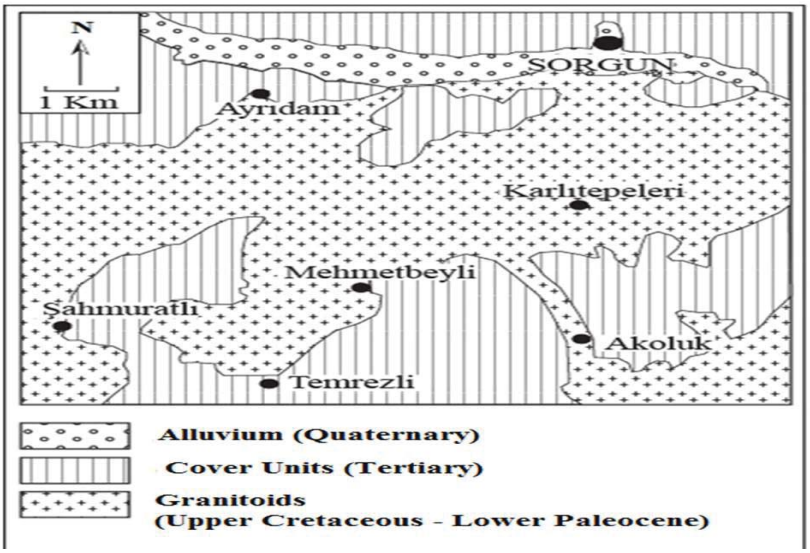


**Fig. 2:** Geological map of the study area.

### *Sampling and Analysis Methods*

In this study, soil samples taken from a depth of 25–30 cm were collected by sieving a 2 mm sieve first and cleared from coarse-grained rock fragments and plant remains. It was then brought to the laboratory and dried at room temperature (Fig. 3).

**Fig. 3:** A photograph of the soil samples drying in the laboratory.

For homogeneous sampling, all samples were milled to 10 micron size. 0.1 g of soil samples were taken and 2 ml of concentrated $HNO_3$ were added and dried on the heater. The solvent was prepared from a mixture of HCl + $HNO_3$ + $H_2O$ (1 : 1 : 1 from each acid). This solvent was diluted by adding distilled water to 500 ml until complete. 20 ml of this diluted mixture was added to each sample to dissolve the samples. These dissolved samples were filtered using filter paper and made ready for analysis.

Elemental analysis of soil samples were performed by Inductively Coupled Plasma—Mass Spectrophotometer (ICP-MS) and BILTEM (Yozgat Bozok University, Science and Technology Research and Application Center).

LOD values (ppb); As: 0.0414, Cr: 0.0426, Cu: 0.0149, K: 8.5567, Mn: 0.2822, Ni: 0.0106, Pb: 0.0136, Th: 0.0038, U: 0.0008, V: 0.1107, Zn: 0.6098 and Zr: 0.0158.

### *Statistical and Computational Analysis*

Various methods and factors are used to assess heavy metal pollution around the mineral deposits.[28] In this study, selected environmental pollution parameters are enrichment factor (EF), contamination factor (CF), geo-accumulation index (Igeo) and pollution load index (PLI).

### *Enrichment Factor*

Enrichment factor (EF) is an index commonly used to determine anthropogenic and natural effects on soils.[28–31] This factor is calculated based on a normalized element (Fe).[32,33] Since Fe is of lithogenic origin in this index, it has been used as a normalizing element and will not affect the importance of the metals to be investigated.[34–36] This factor compares with the concentration of one element in the samples to the concentration of the same element that is not contaminated.[28,37] The enrichment factor is calculated as follows to determine the anthropogenic and natural effects in the samples

$$EF = (Cn/Bn)\ sample/(Cref/Bref)$$

Cn: metal concentration in the sample

Cref: metal concentration in the reference sample

Bn: metal concentration of the Fe in the sample

Bref: metal concentration of the reference Fe in the reference sample

Five contamination categories are assigned on the basis of the enrichment factor;

EF < 2 minimal enrichment

EF = 2 – 5 Moderate enrichment

EF = 5 – 20 Significant enrichment

EF = 20 – 40 Very high enrichment

EF > 40 Extremely high enrichment.[36,38,39]

### *Geoaccumulation Index*

Since the geoaccumulation index (Igeo) is first calculated by Muller (1979), it is also called Muller index.[40] The Muller index is used to determine the amount of contamination caused by heavy metals in the soil. This index is divided into six classes using the following equation between polluted and non-polluted soils.[28,30]

$$Igeo = \log_2 (Cn/1.5\ Bn)$$

Cn: is the measured metal concentration,

Bn: is the background level (average shale)

1.5: a value used to minimize the impact of possible changes.

The degree of pollution is divided into seven different pollution classes.[41]

Igeo < 0 unpolluted

0 < Igeo < 1 unpolluted to moderately polluted

1 < Igeo < 2 moderately polluted

2 > Igeo < 3 moderately to strongly polluted

3 > Igeo < 4 strongly polluted

4> Igeo< 5 strongly to very strongly polluted

5 > Igeo very strongly polluted.[42]

### *Contamination Factor*

The contamination factor (CF) indicates the contamination rate of heavy metals in the soil.[28,43] This factor was calculated using the metal concentration studied with the world shale mean of the metal.[36,44]

This factor is calculated using the following equation.

$$CF\ metal = C\ metal/C\ background$$

C metal: Metal value in the sample

C background: Background value of the metal

Four grades of CF have been classified;

1 ≤ CF low contamination,

1 ≥ CF < 3 moderate contamination,

3 ≥ CF < 6 considerably contaminated,

6 ≥ CF highly contaminated.[36]

### *Pollution Load Index*

The pollution load index (PLI) is often used to evaluate and estimate the degree of pollution in soils.[45] PLI can be calculated as the geometric mean of all metal concentrations. If the PLI concentration is close to 1, it indicates that these concentrations are close to the background concentration, while PLI concentrations above 1 indicate soil pollution.[46,47] Total heavy metal pollution is obtained using this index and from the following equation.[28,48]

$$PLI = (CF1 \times CF2 \times CF3 \times ... CFn)^{1/n}$$

$0 \leq PLI < 1$ unpolluted

$1 \leq PLI < 2$ lightly polluted,

$2 \leq PLI < 3$ moderately polluted

$(3 \leq PLI < 4)$ moderately to highly polluted

$(4 \leq PLI < 5)$, highly polluted

$(PLI \geq 5)$ very highly polluted.[49]

## Results and Discussion

Table 1 shows the minimum, maximum, mean ± standard deviation (SD), median, mod, skewness and kurtosis of the statistical analysis results calculated for the element contents in soil samples in the study area. The concentration of uranium in uncontaminated soils ranges from 1.90–4.40 mg/kg.[49,50] Uranium concentrations found in this study were found in the range of 1.3 to 8.2.

**Table 1:** The statistical analysis results calculated for the element contents in soil samples in the study area

| | Minimum | Maximum | Mean ± St. Dev | Median | Mode | Skewness | Kurtosis |
|---|---|---|---|---|---|---|---|
| As | 33.6 | 56.1 | 43.89 ± 5.3 | 42.6 | 46.0 | 0.4 | 0.8 |
| Cr | –5.1 | 35.9 | 9.685 ± 8.8 | 8.2 | 4.9 | 1.4 | 3.3 |
| Cu | 30.5 | 57.0 | 46.81 ± 6.9 | 49.3 | 49.3 | –1.3 | 1.4 |
| K | 1588.7 | 8283.5 | 4279.7 ± 1461.7 | 4118.7 | 4247.8 | 0.9 | 2.0 |
| Mn | 217.0 | 1578.3 | 1064.9 ± 344.4 | 1054.6 | 983.4 | –0.7 | 0.3 |
| Ni | 58.0 | 279.2 | 116.2 ± 50.8 | 109.1 | 120.5 | 1.9 | 4.9 |
| Pb | 22.5 | 104.6 | 63.9 ± 21.9 | 62.1 | 62.0 | 0.2 | –0.2 |
| Th | 13.0 | 139.0 | 33.2 ± 27.7 | 26.7 | 15.1 | 3.3 | 12.1 |
| U | 1.3 | 8.2 | 2.7 ± 1.7 | 2.1 | 1.4 | 2.1 | 5.0 |
| V | 39.6 | 86.0 | 55.5 ± 12.3 | 53.5 | 45.1 | 1.1 | 0.8 |
| Zn | 70.9 | 236.0 | 143.1 ± 33.2 | 145.4 | 134.6 | 0.4 | 3.1 |
| Zr | 6.4 | 17.1 | 12.9 ± 2.9 | 13.6 | 13.5 | -1.1 | 0.8 |

It was observed that K and Mn elements had the highest concentrations in the soil samples taken. Th,[51,52] As, Mn, Ni, Pb and Zn element values were found to be higher than average earth crust values and As, Cu, Mn, Ni, Pb, Th, U[53] and K[54] element values were higher than average shale values (Table 2).

**Table 2:** Average earth crust and shale values of As, Cr, Cu, Fe, Mn, Ni, Pb, V, Zn and Zr.[51–57]

| Elements | As | Cr | Cu | Fe (%) | K | Mn | Ni | Pb | Th | U | V | Zn | Zr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Earth crust | 1.8 | 100 | 55 | 5 | 18400[a] | 950 | 75 | 13 | 2–20[c] | 0.5–5[c] | 135 | 70 | 165 |
| Shale | 13 | 90 | 45 | 4.7 | 2500[b] | 850 | 70 | 20 | 10[d] | 3[d] | 130 | 95 | 180 |

The correlation coefficient between elements in soil samples was calculated using Sperman correlation matrix to quantitatively analyze and confirm the relationship between metals. Significant positive correlations between Mn-Pb, Mn-V, Th-V and Th-U metal pairs indicate a common origin, while weak positive correlations (such as As-V, Cu-Zn, U-Pb, Th-Zn, Th-Zr and U-Zn) indicate different origins (Table 3).

**Table 3:** Correlations between metals in soil samples in the study area.

| | As | Cr | Cu | K | Mn | Ni | Pb | Th | U | V | Zn | Zr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| As | 1 | | | | | | | | | | | |
| Cr | –0.097 | 1 | | | | | | | | | | |
| Cu | –0.063 | 0.438 | 1 | | | | | | | | | |
| K | –.462(*) | 0.14 | 0.266 | 1 | | | | | | | | |
| Mn | –0.286 | 0.006 | 0.19 | .538(*) | 1 | | | | | | | |
| Ni | 0.075 | 0.286 | .501(*) | 0.001 | -0.059 | 1 | | | | | | |

| | As | Cr | Cu | K | Mn | Ni | Pb | Th | U | V | Zn | Zr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| As | 1 | | | | | | | | | | | |
| Cr | –0.097 | 1 | | | | | | | | | | |
| Cu | –0.063 | 0.438 | 1 | | | | | | | | | |
| K | –.462(*) | 0.14 | 0.266 | 1 | | | | | | | | |
| Pb | –0.299 | 0.083 | 0.061 | 0.429 | .878(**) | -0.196 | 1 | | | | | |
| Th | –0.165 | –0.152 | –0.115 | -0.038 | .558(*) | -0.116 | .591(**) | 1 | | | | |
| U | 0.111 | –0.265 | –0.313 | –0.291 | 0.291 | -0.083 | 0.298 | .670(**) | 1 | | | |
| V | 0.351 | –0.106 | 0.043 | 0.006 | .643(**) | 0.117 | .517(*) | .618(**) | .545(*) | 1 | | |
| Zn | -0.337 | .524(*) | 0.353 | 0.307 | 0.425 | –0.125 | .536(*) | 0.397 | 0.138 | 0.071 | 1 | |
| Zr | 0.196 | 0.22 | 0.384 | 0.378 | .540(*) | 0.243 | .575(**) | 0.239 | –0.022 | 0.436 | 0.256 | 1 |

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

EF values for heavy metals in the investigated soils are given in Table 4. EF is an instrument used for the assessment of anthropogenic metal pollution. Potassium was the extremely high enrichment. The highest EF value for K was 1051.6 and ranged from 573.4 to 2216.9. The highest EF value of K was observed in Example 11. On the other hand, the mean EF values of Mn was 253.5, with K and Mn indicating the extremely high enrichment. The mean EF values for Ni and Zn were 29.8 (in the range 11.0–83.2) and 34.5 (in the range 25.5–47.1), indicating very high enrichment. The mean EF values of As, Cu, Pb, Th and V show significant enrichment ranging from 5 to 20. Average EF values of Cr and Zr range between 2 and 5 and moderate enrichment is observed. Uranium has the lowest average Ef value (0.6), varies between 0.3 and 1.5 and this value indicates that there is non-enrichment. The mean EF values increased in the order of K > Mn > Zn > Ni > V > Pb > Cu > As > Th > Zr > Cr > U (Table 4).[39]

**Table 4:** Enrichment factor (EF) of metals in the study area

| S. No. | As | Cr | Cu | K | Mn | Ni | Pb | Th | U | V | Zn | Zr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 8.5 | 1.4 | 7.4 | 668.7 | 226.1 | 14.6 | 18.3 | 11.8 | 0.9 | 12.0 | 28.0 | 2.7 |
| 2 | 7.0 | 1.2 | 7.6 | 573.4 | 234.1 | 11.0 | 14.1 | 5.2 | 0.7 | 11.6 | 25.5 | 2.0 |
| 3 | 7.7 | 0.6 | 9.1 | 624.1 | 255.4 | 25.0 | 15.0 | 8.7 | 0.8 | 15.5 | 26.4 | 3.1 |
| 4 | 7.5 | 3.7 | 7.6 | 847.1 | 271.6 | 20.3 | 19.9 | 6.6 | 0.6 | 13.7 | 29.5 | 2.6 |
| 5 | 7.1 | 1.7 | 10.6 | 1336.3 | 273.4 | 16.8 | 18.6 | 5.9 | 0.4 | 10.0 | 32.2 | 3.0 |
| 6 | 6.9 | 2.3 | 9.8 | 769.5 | 209.1 | 16.4 | 12.7 | 5.1 | 0.3 | 8.4 | 45.5 | 2.6 |
| 7 | 7.6 | 2.0 | 8.4 | 579.0 | 174.4 | 18.0 | 10.1 | 25.9 | 1.5 | 8.9 | 30.0 | 2.0 |
| 8 | 10.0 | 2.4 | 12.3 | 1374.1 | 205.6 | 23.9 | 13.2 | 5.3 | 0.3 | 9.9 | 40.6 | 3.0 |
| 9 | 9.7 | 4.3 | 12.1 | 760.3 | 187.8 | 46.1 | 11.0 | 3.0 | 0.3 | 10.3 | 27.6 | 2.9 |
| 10 | 14.8 | 0.9 | 15.8 | 1530.1 | 476.9 | 23.9 | 24.5 | 10.8 | 0.5 | 20.8 | 47.1 | 4.8 |
| 11 | 11.1 | 1.3 | 13.2 | 2216.9 | 371.9 | 32.2 | 16.6 | 7.2 | 0.6 | 15.3 | 36.7 | 3.6 |
| 12 | 17.0 | 0.6 | 11.3 | 845.8 | 251.5 | 21.5 | 14.4 | 5.6 | 0.7 | 16.7 | 26.3 | 2.7 |
| 13 | 11.1 | 4.4 | 10.8 | 882.5 | 204.3 | 26.5 | 11.1 | 4.5 | 0.3 | 11.7 | 30.9 | 3.5 |
| 14 | 11.5 | 2.1 | 14.4 | 1299.4 | 258.5 | 33.3 | 15.7 | 4.4 | 0.4 | 12.6 | 40.2 | 3.5 |
| 15 | 10.3 | 1.2 | 12.3 | 1404.1 | 323.5 | 27.8 | 16.3 | 7.2 | 0.6 | 12.1 | 33.4 | 3.8 |
| 16 | 12.5 | 3.5 | 15.8 | 1481.5 | 401.0 | 83.2 | 19.2 | 7.9 | 0.7 | 18.1 | 43.2 | 4.1 |
| 17 | 21.8 | –2.4 | 22.8 | 742.7 | 101.4 | 67.8 | 10.5 | 14.6 | 1.4 | 25.7 | 45.3 | 3.0 |
| 18 | 14.2 | 1.7 | 12.5 | 1008.8 | 164.1 | 21.9 | 8.9 | 5.1 | 0.9 | 15.6 | 29.5 | 2.3 |
| 19 | 14.1 | 9.0 | 12.4 | 951.7 | 215.3 | 33.2 | 13.9 | 5.4 | 0.4 | 13.6 | 35.8 | 4.0 |
| 20 | 12.3 | 1.3 | 8.2 | 1136.9 | 263.2 | 32.2 | 16.6 | 4.1 | 0.5 | 12.1 | 36.0 | 3.6 |

The calculated Igeo values are shown in Table 5. Regarding the Igeo values, As was found to be the highest pollutions among the selected metals. The Igeo values of As, Cr, Cu, K, Mn, Ni, Pb, Th, U, V, Zn and Zr were found to be less than 1. Igeo values indicate that the study area is not polluted by these metals. The average Igeo values of the metals were As > Th > Pb > K > Ni > Zn > Mn > Cu > U > V > Cr > Cr (Table 5).

**Table 5:** Geoaccumulation index (Igeo) of metals in the study area

| S. No. | As | Cr | Cu | K | Mn | Ni | Pb | Th | U | V | Zn | Zr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.7 | 0.02 | 0.2 | 0.3 | 0.3 | 0.2 | 1.0 | 1.3 | 0.35 | 0.10 | 0.3 | 0.02 |
| 2 | 0.7 | 0.02 | 0.2 | 0.3 | 0.4 | 0.2 | 1.0 | 0.7 | 0.29 | 0.12 | 0.4 | 0.02 |
| 3 | 0.7 | 0.01 | 0.2 | 0.3 | 0.3 | 0.4 | 0.8 | 1.0 | 0.29 | 0.13 | 0.3 | 0.02 |
| 4 | 0.6 | 0.04 | 0.2 | 0.4 | 0.3 | 0.3 | 1.0 | 0.7 | 0.22 | 0.11 | 0.3 | 0.02 |
| 5 | 0.5 | 0.02 | 0.2 | 0.5 | 0.3 | 0.2 | 0.9 | 0.6 | 0.12 | 0.07 | 0.3 | 0.02 |
| 6 | 0.6 | 0.03 | 0.2 | 0.3 | 0.3 | 0.2 | 0.7 | 0.5 | 0.12 | 0.07 | 0.5 | 0.02 |
| 7 | 0.6 | 0.02 | 0.2 | 0.2 | 0.2 | 0.3 | 0.5 | 2.8 | 0.55 | 0.07 | 0.3 | 0.01 |
| 8 | 0.6 | 0.02 | 0.2 | 0.4 | 0.2 | 0.3 | 0.5 | 0.4 | 0.09 | 0.06 | 0.3 | 0.01 |
| 9 | 0.6 | 0.04 | 0.2 | 0.3 | 0.2 | 0.6 | 0.5 | 0.3 | 0.09 | 0.07 | 0.3 | 0.01 |
| 10 | 0.7 | 0.01 | 0.2 | 0.4 | 0.3 | 0.2 | 0.7 | 0.6 | 0.09 | 0.09 | 0.3 | 0.02 |
| 11 | 0.6 | 0.01 | 0.2 | 0.7 | 0.3 | 0.3 | 0.6 | 0.5 | 0.14 | 0.09 | 0.3 | 0.02 |
| 12 | 0.7 | 0.00 | 0.1 | 0.2 | 0.2 | 0.2 | 0.4 | 0.3 | 0.13 | 0.07 | 0.1 | 0.01 |
| 13 | 0.8 | 0.05 | 0.2 | 0.3 | 0.2 | 0.4 | 0.5 | 0.4 | 0.11 | 0.09 | 0.3 | 0.02 |
| 14 | 0.7 | 0.02 | 0.3 | 0.4 | 0.2 | 0.4 | 0.6 | 0.4 | 0.12 | 0.08 | 0.3 | 0.02 |
| 15 | 0.6 | 0.01 | 0.2 | 0.5 | 0.3 | 0.3 | 0.7 | 0.6 | 0.15 | 0.07 | 0.3 | 0.02 |
| 16 | 0.6 | 0.03 | 0.2 | 0.4 | 0.3 | 0.8 | 0.6 | 0.5 | 0.15 | 0.09 | 0.3 | 0.02 |
| 17 | 0.7 | –0.01 | 0.2 | 0.1 | 0.1 | 0.4 | 0.2 | 0.6 | 0.19 | 0.08 | 0.2 | 0.01 |
| 18 | 0.7 | 0.01 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 | 0.3 | 0.20 | 0.08 | 0.2 | 0.01 |
| 19 | 0.9 | 0.08 | 0.2 | 0.3 | 0.2 | 0.4 | 0.6 | 0.4 | 0.10 | 0.08 | 0.3 | 0.02 |
| 20 | 0.7 | 0.01 | 0.1 | 0.3 | 0.2 | 0.3 | 0.6 | 0.3 | 0.13 | 0.07 | 0.3 | 0.02 |

CF and PLI values for the metals examined are given in Table 6. As is the considerably contamination among the metals examined. The CF values of this metal varied between an average of 3.4 mg/kg and 2.6–4.3. The second largest values in the soil are Pb and Th and mean CF values are 3.2 and 3.3. Pb and Th were found to cause significant contamination in the study area. Similarly, the CF values of Cu, K, Mn, Ni and Zn indicate moderate contamination in the studied soils. U (0.91) has low pollution in the range of 1 ≤ CF. CF values are as follows: As > Th > Pb > K > Ni > Zn > Mn < C > U > V < Cr > Zr (Table 6). The calculated PLI values range from –1.1 to 2.1 (Table 6). A PLI value greater than 1 indicates pollution. The PLI values calculated in the study area were above 1 (except for example 17) and showed that there was metal pollution. Pollution ranking by location, 1 > 4 > 7 > 2 > = 16 > 3 > 19 > 13 > 11 > 5 > 6 > 14 > 15 > 8 > 9 > 10 > 20 > 18 > 12 > 17.

**Table 6:** Contamination factor (CF) and pollution load index (PLI) of metals in the study area

| S. No. | As | Cr | Cu | K | Mn | Ni | Pb | Th | U | V | Zn | Zr | PLI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3.6 | 0.1 | 0.9 | 1.5 | 1.5 | 1.2 | 5.0 | 6.5 | 1.7 | 0.5 | 1.6 | 0.1 | 2.1 |
| 2 | 3.6 | 0.1 | 1.1 | 1.5 | 1.9 | 1.1 | 4.7 | 3.5 | 1.5 | 0.6 | 1.8 | 0.1 | 1.9 |
| 3 | 3.3 | 0.0 | 1.1 | 1.4 | 1.7 | 2.0 | 4.2 | 4.8 | 1.4 | 0.7 | 1.5 | 0.1 | 1.8 |
| 4 | 3.1 | 0.2 | 0.9 | 1.8 | 1.7 | 1.5 | 5.2 | 3.5 | 1.1 | 0.6 | 1.6 | 0.1 | 2.0 |
| 5 | 2.6 | 0.1 | 1.1 | 2.5 | 1.5 | 1.1 | 4.4 | 2.8 | 0.6 | 0.4 | 1.6 | 0.1 | 1.7 |
| 6 | 2.8 | 0.1 | 1.1 | 1.6 | 1.3 | 1.2 | 3.3 | 2.7 | 0.6 | 0.3 | 2.5 | 0.1 | 1.6 |
| 7 | 3.1 | 0.1 | 1.0 | 1.2 | 1.1 | 1.4 | 2.7 | 13.9 | 2.7 | 0.4 | 1.7 | 0.1 | 2.0 |
| 8 | 3.1 | 0.1 | 1.1 | 2.2 | 1.0 | 1.4 | 2.7 | 2.1 | 0.5 | 0.3 | 1.7 | 0.1 | 1.5 |
| 9 | 3.2 | 0.2 | 1.2 | 1.3 | 0.9 | 2.8 | 2.4 | 1.3 | 0.4 | 0.3 | 1.2 | 0.1 | 1.5 |
| 10 | 3.3 | 0.0 | 1.0 | 1.7 | 1.6 | 1.0 | 3.5 | 3.1 | 0.5 | 0.5 | 1.4 | 0.1 | 1.5 |
| 11 | 3.2 | 0.1 | 1.1 | 3.3 | 1.6 | 1.7 | 3.1 | 2.7 | 0.7 | 0.4 | 1.4 | 0.1 | 1.7 |
| 12 | 3.5 | 0.0 | 0.7 | 0.9 | 0.8 | 0.8 | 1.9 | 1.5 | 0.7 | 0.3 | 0.7 | 0.0 | 1.0 |
| 13 | 4.1 | 0.2 | 1.2 | 1.7 | 1.2 | 1.8 | 2.7 | 2.2 | 0.5 | 0.4 | 1.6 | 0.1 | 1.8 |
| 14 | 3.5 | 0.1 | 1.3 | 2.1 | 1.2 | 1.9 | 3.1 | 1.8 | 0.6 | 0.4 | 1.7 | 0.1 | 1.6 |
| 15 | 3.2 | 0.1 | 1.1 | 2.2 | 1.5 | 1.6 | 3.3 | 2.9 | 0.8 | 0.4 | 1.4 | 0.1 | 1.6 |
| 16 | 3.2 | 0.1 | 1.2 | 2.0 | 1.6 | 4.0 | 3.2 | 2.6 | 0.8 | 0.5 | 1.5 | 0.1 | 1.9 |
| 17 | 3.6 | -0.1 | 1.1 | 0.6 | 0.3 | 2.1 | 1.1 | 3.1 | 1.0 | 0.4 | 1.0 | 0.0 | –1.1 |
| 18 | 3.7 | 0.1 | 0.9 | 1.4 | 0.7 | 1.1 | 1.5 | 1.7 | 1.0 | 0.4 | 1.1 | 0.0 | 1.3 |
| 19 | 4.3 | 0.4 | 1.1 | 1.5 | 1.0 | 1.9 | 2.8 | 2.1 | 0.5 | 0.4 | 1.5 | 0.1 | 1.8 |
| 20 | 3.5 | 0.1 | 0.7 | 1.7 | 1.2 | 1.7 | 3.1 | 1.5 | 0.7 | 0.3 | 1.4 | 0.1 | 1.4 |

## Conclusion

In this research, metal concentrations and pollution sources in soil samples in the area of Sorgun and surrounding uranium mineralization were interpreted using statistical techniques. In soil samples in the study area, the highest element concentrations were observed in K and Mn. In addition, Mn, Ni, Cu, Zn, Pb, As, Th values were higher than average shale values. For the accurate evaluation of heavy metal pollution results, enrichment factor, geoaccumulation index, contamination factor and pollution load index methods were applied. According to the classification, the extremely high enrichment in EF values is related to K and Mn elements. Among the studied elements, As with the highest Igeo value showed the unpolluted to moderately polluted. Igeo values of other elements were found to be less than 0.68. Among the calculated CF values, the considerably contamination was As, while the other studied elements showed moderate to low contamination. Igeo and CF values respectively; As > Th > Pb > K > Ni > Zn > Mn > Cu > U > V > Cr > Zr. Except for Example 17, all samples have PLI values above 1 and it can be said that these samples contain metal contamination.

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*Original Article*

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# Occupational Accident Frequency and Personal Protective Equipment Usage of Technical Staff Working in A State University

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**Abstract**

It is aimed to reveal the occupational accident situation, PPE usage situation and, accordingly, the difficulties that may be encountered in working and social life of the employees who work as a technical staff in a state university and the current job satisfaction, in this study. To calculate the findings of the study, percentage, frequency, mean and standard deviation have been used; to compare qualitative data chi-square test has been used and significance has been evaluated at $p < .05$ level. Spearman correlation analysis has been used to examine the relationship between variables. According to the results of the research, the majority of the employees do not receive any occupational safety training before and after they start work. Besides, according to the results of the study, it can be said that the expected and observed negativities will be minimized if the employees receive training on OHS materials.

**Keywords:** Occupational accident; Occupational disease; OHS.

## Introduction

Although people always want a healthy and safe environment in working life, it has been realized that occupational safety is accepted as a social need.[14] The development of industry has brought about the concept of occupational health and safety with economic contributions. Occupational health and safety aim to improve the conditions of employees in the workplace.[2] The issue of occupational health and safety is considered to have gained importance along with the industrial revolution, which created conditions that pose a danger to the health of workers.[5] In labor law, there is a condition of providing occupational safety as a result of the undertaking of the worker to undertake to work with labor.[14] The right granted to the employee as occupational safety ensures that the events that affect the life of the person being protected from the negativities that will be encountered during the work. Protecting the life and body integrity of the worker is also within the scope of job security.[7] Occupational safety can also be defined as a set of legal, technical and medical measures to be taken to ensure that the employee does not suffer physical and mental harm due to the hazards arising from the construction of the current job and the hazards arising both inside and outside the enterprise. From a wide-angle, occupational safety can be interpreted as the use of the state's facilities to the benefit of the employee in addition to the work related to the performance of the work. It covers the principle of social state and protection of workers on the basis of occupational safety which covers a wide range of

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subjects including wage security and job security.[8]

**Occupational Health and Safety**

In the globalization process, the flow of goods, services, and capital has accelerated. As a result of these developments, changes in the economy and the working life of countries have occurred. Under these circumstances, the concept of competition emerged and became compulsory. Struggling with competitors inside and outside the country, reaching more customers, production speed and capital development efforts are within the scope of competition. New conditions create new risks and hazards in enterprises. Measures are taken and implemented against this situation. Occupational health and safety measures have become compulsory in today's conditions. Although this situation is observed as an economic burden by most organizations, the experienced results may lead to more painful results over a wide period of time.[10]

The concept of occupational health is considered as minimized the hazards that may arise from the environment and equipment in working environment.[6] With the health and safety of workers, individuals also aim to develop a sense of protection and safety from risks from general to private.[16] Occupational safety also refers to the whole set of rules for eliminating the dangers that are parallel to worker health.[6] The concept of occupational health and safety is defined as the studies that will be carried out in order to create a human environment and eliminate the conditions that will pose a danger to the employee's work.[18]

Occupational health and safety is essential to ensure employee happiness. It can be thought that the employees will feel safe and the productivity level will increase in the environment where they feel comfortable. OHS, which aims to ensure the harmony of the employees with the work, also protects the employer and protects the treatment processes by identifying the possible work accidents and occupational diseases. OHS is an area of expertise that provides the social, spiritual and physical well-being of employees.[9]

The concept of Occupational Health and Safety, which is based on the material and moral well-being of the employee, is not a new phenomenon. As a matter of fact, human beings have been in working life since somehow to meet their needs. Based on this idea, it is possible to predict that the process of meeting the concepts of work, health and safety at the same denominator in history can go back to ancient times. It is known that the document, which is the first source related to OHS, was produced by Herodotus, who reveals the necessity of consuming high-calorie foods in order for employees to be productive.[12] The same style of work continued by Hippocrates and Dioscorides. The first study on a scientific basis was Ramazzini's book on measures in 1713.[21]

The developing world and technological activities turned into a race with the hard-working conditions of the countries and the conditions became more difficult for the employee. If we look at our own history, the guild activities of the Ottoman period are noteworthy for the craftsmen to protect their apprentices. The late industrial period we experienced led us to follow up on OHS developments compared to other countries. Dilaver Pasha's Regulation and the 1869 Maadin Regulation helped the workers in the coal mines provide some degree of convenience. On the other hand, the most important step of OHS developments can be thought to be the subject that came into force in 1937 in the history of the Republic.[12] Under the influence of modernism, improving work and working conditions, increasing the importance given to workers, providing employer supervision and so on formations have occurred. Thus, measures have been taken to protect the material and moral integrity of the employee and employer and laws have been established. One of the rights cited in this context is the right to social security.

Social security is an indispensable right for everyone without any discrimination. In using this right, it is applied regardless of age, sex, income level or any other characteristics of the person. It can also be considered as providing a life worthy of human dignity in relation to the state's right to social security. The social security right is also included in the Declaration of Human Rights and it is observed that everyone is determined to have the right to be a member of society. Social security is a natural right of people and in no way can be taken away.[23]

Social security is a duty assigned to the state and it is a right that can be demanded from a human perspective. The State will establish all the organizations necessary to meet this duty. Mainly the protection of the economic right of the person lies in protecting the person from social events but also affects the development of happiness and personality.[11] According to Article 60 of our Constitution; "Everyone has the right to social security. The state shall take the necessary

measures to ensure this security and establish the organization.

This article together with Article 2 of the Constitution stipulates that the Republic of Turkey is a social state of law if the assessment of the need to protect the safety and health of employees can be concluded that in our Constitution. The provision of the right to occupational safety to the person acts as a measure against compensation and occupational risks by the state.[20] It is also known that a person equipped with social rights will overcome the social and economic barriers to a dignified and healthy working life.[4]

In order to protect the right to social security, it is important to know the concepts that are closely related to the concept of occupational health and safety and to take necessary measures. Among these concepts, occupational accidents and occupational diseases come first.

### *Work Accident*

The accident is defined in the literature as a bad event that may cause loss of property and life.[22] The definition of the accident is considered from a legal dimension and from a narrow and wide-angle. While the accident in a narrow sense is the death of the person and the violation of the integrity of the body, in the broad sense; the damage is added to this definition. A work accident is a special case of the concept of accident in a narrow sense. The incident that caused the occupational accident was not requested by the person and should be caused by a sudden and external factor within a short time.[11]

The occupational accident is regulated in Article 13 of the Social Security and General Health Insurance Law no 5510.

Work accident; According to Article 3 (g) of Law No. 6331, it is defined as the event that occurs in the workplace or due to the execution of the work, causing death or rendering body integrity mentally or physically disabled.[19]

According to Article 13 of Law No. 5510, in order for an accident to be considered as a work accident, it must meet any of the following conditions:

a. While the insured is in the workplace,
b. If the insured is working independently on his/her behalf and account due to the work carried out by the employer,
c. In the period of time that the insured working under an employer is sent to another place as an official,
d. In the times allocated for breastfeeding the female insured to provide milk to her child in accordance with the labor legislation,
e. It is the event that occurs during the arrival of the insured by a vehicle provided by the employer to the place where the work is carried out and which immediately or subsequently makes the insured physically or mentally disabled.

In the absence of the above-mentioned factors, situations that may occur are not considered as occupational accidents.[15]

### *Occupational Disease*

The concept of occupational disease according to Law No. 5510; Occupational disease is defined as a temporary or permanent illness, physical or mental disability that the insured undergoes for a recurring reason due to the nature of the work that he or she works or due to the conditions of execution of the work. Considering the possibility that each insured person's illness is not an occupational disease, the situation is examined according to the Regulation on the Determination of the Loss Rate of Workforce and Earnings in the Profession. In this regulation, methods for determining occupational diseases are determined. In addition, it is stated how long it will take for the worker who has an occupational disease to start work.[19] In order to benefit from the rights determined by the law for the employee who has an occupational disease; In compliance with the procedures of the health service provider authorized by the institution, the health committee report and the medical documents obtained shall be determined by the health committee of the institution by examining the inspection reports and other necessary documents (Law no. 5510, Art: 14/2). In order to consider one disease as an occupational disease, the employee must first be considered as insured. The existing illness of the employee should eventually create disability in the physical or mental state of the person. The obstacle situation should be in a causal connection with the work being carried out by the person.[13] The causal link between occupational accident conditions is not as important as an occupational disease. However, there is a direct connection between occupational diseases and workplace conditions.[3]

### *Notification of Work Accident and Occupational Disease to the Institution*

In accordance with the law no 5510 in force in our country, the employer must notify the authorized law enforcement authorities immediately at the

place of the accident and at the latest within three working days after the accident. Within the scope of the same article, it is obligatory to notify the Institution directly or by registered mail by means of work accident and occupational disease notification within three working days after the insured's disability does not prevent him her from notifying his/her condition not to exceed one month.[1]

This situation is designed to vary in terms of employee diversity. Within the scope of Law No. 2925, agricultural workers make the notification within two days after the accident.[13] The period of notification can be submitted to the organization within three working days after the day it does not interfere in case of reporting the discomfort by the independent employees for a period not exceeding one month. If independent employees do not notify the institution within the required period, they will be deprived of disability allowances during those days.[19]

### *Assistance to the Insured or Rights Holders due to Occupational Accidents and Occupational Diseases*

It is known that the insured employee will suffer loss as a result of the problems. The depreciation of these losses and temporary incapacity allowance or permanent incapacity income, which is considered to be some support. If the insured dies, the funeral allowance, death income, and marriage allowance can be given to the employee by the institution. If these aids are examined under headings:

*Benefit for temporary incapacity*

As a result of an occupational accident or occupational disease, it is carried out by the institution with the aim of minimizing the material losses of the employee during the period of inability to work. This allowance is paid from the first day for each day of absence. In addition, the working time or the starting time is not taken into consideration for the benefit of the employee.[22] In order for the insured to benefit from the temporary incapacity benefit, if there are contractual or non-contractual health service providers, it is essential that he receives a rest report from the doctors authorized by the Ministry of Health and that the incapacity benefit is paid to the insured by the Authority.[13]

*Permanent incapacity income*

As a result of an occupational accident or occupational disease, the person suffers financial loss. The insured is given temporary disability benefits by the medical and institution in order to prevent loss. If it is found that the person's ability to earn in the profession decreases by 10% at the end of the treatment process, the person is paid disability by the institution continuously.[15]

Certain conditions for continuous disability payment include: in order to connect income to the insured, the insured has lost his or her earning power due to illnesses and obstacles caused by occupational accident or occupational disease, or has lost at least 10% of his earning ability by continuing to work in his profession.[11] Monthly earnings are determined primarily for the calculation of continuous disability income. This is the same method as calculating temporary disability income. The Authority notifies the result to the person within 3 months as of the examination of the documents required for payment. If the insured has previously benefited from a temporary disability allowance, the date of termination of this allowance is permanently incapacitated without the determination of temporary disability. If the person is not bound by the employment contract, the insured must pay all premiums and debts in order to benefit from the allowance.

*Funeral allowance*

It is made in case of death to the insured who lost their lives as a result of an occupational accident or occupational disease. In order to be able to receive this benefit, the employers who are working under the employer must have provided at least 360 days of long-term insurance premiums. 360-day premium payment is required for independent employees. The amount of payment to be made by the Authority is determined by the board of directors and made in accordance with the tariff approved by the related minister. Assistance can be paid first to the spouse, or else by following the order of the child, parents or siblings (Article 37/3 of Law no. 5510).

*Death income*

It is not necessary for the insured to have worked for a certain period or paid certain premiums in order to be able to attach death income to the relatives of the insured who died as a result of an occupational accident or occupational disease. The death income of the person who has been identified on the day of the insured's employment and the cause of the occupational accident will be attributed to the beneficiaries. For the benefit of the beneficiaries of independent employees, premiums and premium-related debts must be paid (Art. 19/3 of Law no. 5510).

*Marriage allowance for girls*

After the death of the insured, the income is deducted in the event of the marriage of the girl whose income has been tied to her.

## Materials and Methods

The research group consisted of 139 technical staff, 133 males and 6 females, working at Gazi University. This survey, which was conducted on 139 people, was developed by HakanSaraç in 2014 as an Occupational Health and Safety Survey for Employees at Republic of Turkey, Ministry of Education Course instruments center and State Books Revolving Fund. This questionnaire, which was applied to the participants, was carried out by the supervisors by going to the unit where the staff worked together with the determination of the content of the questionnaire and completely voluntarily. Percentage, frequency, mean and standard deviation were used to calculate the descriptive statistics of the participants; the chi-square test was used to compare qualitative data and significance was evaluated at $p < .05$ level. Spearman Correlation analysis was used to examine the relationship between variables. SPSS 23.0 Package Program was used for data analysis.

## Results

In this section, findings related to research problems are given

According to Table 1, 112 (81%) of the study group consisted of individuals in the 24-50 age range.

According to Table 2, 133 (95.7%) of the study group were male and 6 (4.3%) were female.

According to Table 3, the frequencies of the total sample are given as follows with respect to the education status: 53 (38.1%) high school students, 38 (27.3%) college, 24 (17.3%) universities, 16 (11.5%) and 8 (5.8%) are primary school students.

According to Table 4, 119 (85.6%) of the study group were married and 20 (14.4%) were single.

According to Table 5, the average age of participants is 42.97. The lowest participant is 24 years old and the largest participant is 63 years old. The average date of commencement of work-life is 24.17. Participants have been working for at least 1 year and maximum 49 years. The average date of starting the current job is 16.18. Participants have been working in their current job for at least 1 year and working for 41 years at most. The average starting date of the participants at the current workplace is 12.85. Participants have been working at the current workplace for at least 1 year and have been working for 33 years at most.

**Table 1:** Distribution of research sample in terms of age variable

| Age | *F* | % |
|---|---|---|
| 24 | 1 | 0.7 |
| 25 | 4 | 2.9 |
| 27 | 3 | 2.2 |
| 28 | 3 | 2.2 |
| 29 | 3 | 2.2 |
| 30 | 2 | 1.4 |
| 31 | 4 | 2.9 |
| 32 | 3 | 2.2 |
| 33 | 4 | 2.9 |
| 34 | 3 | 2.2 |
| 35 | 5 | 3.6 |
| 37 | 4 | 2.9 |
| 38 | 5 | 3.6 |
| 39 | 4 | 2.9 |
| 40 | 3 | 2.2 |
| 41 | 4 | 2.9 |
| 42 | 4 | 2.9 |

(Contd...)

| Age | *F* | % |
|---|---|---|
| 43 | 11 | 7.9 |
| 44 | 3 | 2.2 |
| 45 | 2 | 1.4 |
| 46 | 5 | 3.6 |
| 47 | 8 | 5.8 |
| 48 | 9 | 6.5 |
| 49 | 6 | 4.3 |
| 50 | 9 | 6.5 |
| 51 | 6 | 4.3 |
| 52 | 4 | 2.9 |
| 53 | 2 | 1.4 |
| 54 | 3 | 2.2 |
| 55 | 2 | 1.4 |
| 56 | 2 | 1.4 |
| 57 | 2 | 1.4 |
| 58 | 1 | 0.7 |
| 60 | 1 | 0.7 |
| 61 | 1 | 0.7 |
| 62 | 1 | 0.7 |
| 63 | 2 | 1.4 |
| **Total** | **139** | **100** |

**Table 2:** Distribution of research sample in terms of gender variable

| Gender | *F* | % |
|---|---|---|
| Males | 133 | 95.7 |
| Females | 6 | 4.3 |
| **Total** | **139** | **100** |

**Table 3:** Distribution of the research sample in terms of the state of education variable

| State of Education | *F* | % |
|---|---|---|
| Primary School | 8 | 5.8 |
| High School | 53 | 38.1 |
| Middle School | 16 | 11.5 |
| University | 24 | 17.3 |
| College | 38 | 27.3 |
| **Total** | **139** | **100** |

**Table 4:** Distribution of research sample in terms of marital status variable

| Marital Status | *F* | % |
|---|---|---|
| Married | 119 | 85.6 |
| Single | 20 | 14.4 |
| **Total** | **139** | **100** |

**Table 5:** The average values of the research sample in terms of the variable of starting work, starting the current job, and starting years at the current workplace

| Variables | *N* | Minimum | Maximum | Average | Std. Deviation |
|---|---|---|---|---|---|
| Birth Date | 139 | 24 | 63 | 42.97 | 9.193 |
| Date of Commencement of Work Life | 139 | 1 | 49 | 24.17 | 9.422 |
| Date of Starting The Current Job | 139 | 1 | 41 | 16.18 | 9.456 |
| Start Date of Work At The Current Workplace | 139 | 1 | 33 | 12.85 | 8.549 |

According to Table 6, 23 (16.5%) of the research sample group was in wood working unit, 5 (3.6%) in biomedical unit, 12 (8.6%) in dye unit, 1 (0.7%) in storage unit, 14 (10.1%) in other units, 33 (23.7%) in electrical units, 20 (14.4%) in administrative units, 5 (3.6%) in printing units, 10 (7.2%) in plastic units, 16 (11.5%) in the sanitary unit.

**Table 6:** Distribution of research sample in terms of worked department variable

| Department | *F* | % |
|---|---|---|
| Wood Working | 23 | 16.5 |
| Biomedical | 5 | 3.6 |
| Dye | 12 | 8.6 |
| Storage | 1 | 0.7 |
| Other | 14 | 10.1 |
| Electrical Units | 33 | 23.7 |
| Administrative Units | 20 | 14.4 |
| Printing Units | 5 | 3.6 |
| Plastic Units | 10 | 7.2 |
| Sanitary Unit | 16 | 11.5 |
| **Total** | 139 | 100 |

According to Table 7, 12 (8.6%) of the study group were workers, 1 (0.7%) engineers, 28 (20.1%) mechanic, and 98 (70.5%) technicians.

According to Table 8, 76 (54.7%) of the study group had knowledge of the relevant laws and regulations while 63 (45.3%) did not.

According to Table 9, 33 (23.7%) of the participants received occupational accident training while 106 (76.3%) did not.

**Table 7:** Distribution of research sample in terms of title variable

| Title | *F* | % |
|---|---|---|
| Worker | 12 | 8.6 |
| Engineer | 1 | 0.7 |
| Mechanic | 28 | 20.1 |
| Technician | 98 | 70.5 |
| **Total** | **139** | **100** |

**Table 8:** Distribution of research sample in terms of knowledge status variable, laws and regulations related to the work

| Knowledge of law | *F* | % |
|---|---|---|
| Yes | 76 | 54.7 |
| No | 63 | 45.3 |
| **Total** | **139** | **100** |

**Table 9:** Distribution of the research sample in terms of occupational accidents in life and occupational accidents in the institution

| | *F* | % |
|---|---|---|
| **Occupational accidents in life** | | |
| Yes | 42 | 30.2 |
| No | 97 | 69.8 |
| **Occupational accidents in the institution** | | |
| Yes | 33 | 23.7 |
| No | 106 | 76.3 |
| **Total** | **139** | **100** |

According to Table 10, 89 (64.0%) of the study group did not report any accidents at work, 17 (12.2%) were in the wood section, 8 (5.8%) in the paint section, 1 (0.7%) in the storage section, 2 (1.4%) in the other section, 14 (10.1%) in the electrical section, 2 (1.4%) in the administrative section, 6 (4.3%) in the plastic section of the accident.

According to Table 11, 71 (51.1%) of the study group did not specify the type of the last occupational accident, 3 (2.2%) were head trauma, 22 (15.8%) sinking of object and 1 (0.7%) other, 4 (2.9%) electric shock, 3 (2.2%) burring, 10 (7.2%) fractures, 2 (1.4%) explosion, 4 (2.9%) substance fall, 5 (3.6%) compression, 1 (0.7%) traffic, 2 (1.4%) limb rupture, 2 (1.4%) burning, 7 (5.0%) falling from high, 2 (1.4%) had an intoxication.

**Table 10:** The place/department where the latest work accident occurred in your workplace

| The latest work accident occurred in your workplace | *F* | % |
|---|---|---|
| NA* | 89 | 64.0 |
| Wood | 17 | 12.2 |
| Dye | 8 | 5.8 |
| Storage | 1 | 0.7 |
| Oher | 2 | 1.4 |
| Electric | 14 | 10.1 |
| Administrative | 2 | 1.4 |
| Plastic | 6 | 4.3 |
| **Total** | 139 | 100 |

**Table 11:** Distribution of the research sample in terms of the occupational accident type

| Work Accident Type | *F* | % |
|---|---|---|
| NA * | 71 | 51.1 |
| Head Trauma | 3 | 2.2 |
| Sinking of Object | 22 | 15.8 |
| Other | 1 | 0.7 |
| Electric Shock | 4 | 2.9 |
| Burring | 3 | 2.2 |
| Fractures | 10 | 7.2 |
| Explosion | 2 | 1.4 |
| Substance Fall | 4 | 2.9 |
| Compression | 5 | 3.6 |
| Traffic | 1 | 0.7 |
| Limb Rupture | 2 | 1.4 |
| Burning | 2 | 1.4 |
| Falling From High | 7 | 5.0 |
| Intoxication | 2 | 1.4 |
| **Total** | **139** | **100** |

According to Table 12, 77 (55.4%) of the study group stated that they did not get any injuries as a result of occupational accidents, 9 (6.5%) received minor wounds and 30 (21.6%) were away from work for 1/3 days; 12 (8.6%) stayed 3 days/1 week away from work, 2 (1.4%) 8 days/1 month away from work, 3 (2.2%) 2 months/1 year away from work, 4 (2.9%) stated that they were away from work for more than 1 year.

According to Table 13, 1 (.7%) of the study group did not specify the reason for working in the current job, 74 (53.2%) were job security, 6 (4.3%) were adequate wage, 49 (35.3%) social security, 3 (2.2%) social facilities/shelter-food-local, 2 (1.4%) good OHS conditions, 4 (2.9%) stated the reasons for working in the current job as other reasons.

**Table 12:** Distribution of the research sample in terms of variable as a result of occupational accidents

| Variables | *F* | % |
|---|---|---|
| * No injury | 77 | 55.4 |
| Got minor wounds | 9 | 6.5 |
| Away from work for 1/3 days | 30 | 21.6 |
| Away from work for 3 days/1 week | 12 | 8.6 |
| Stayed away from work for 8 days/1 month | 2 | 1.4 |
| Stayed away from work for 2 months/1 year | 3 | 2.2 |
| Aaway from work for more than a year | 4 | 2.9 |
| Other (……..……) | 2 | 1.4 |
| **Total** | **139** | **100** |

**Table 13:** Distribution of the research sample in terms of the reason for working in the current job

| The reason why that you are working current workplace | *F* | % |
|---|---|---|
| Na* | 1 | 0.7 |
| Job Security | 74 | 53.2 |
| Adequate Wage | 6 | 4.3 |
| Social Security | 49 | 35.3 |
| Social Facilities / Shelter-Food-Local | 3 | 2.2 |
| Good OHS Conditions | 2 | 1.4 |
| Other | 4 | 2.9 |
| **Total** | **139** | **100** |

According to Table 14, when the relationship between occupational accidents and satisfaction with the institution they work with is evaluated. It was seen that 33 (23.7%) of the participants who were satisfied with the institution they worked in had work accidents and 106 (76.3%) did not. There was no significant difference between being satisfied with the institution and working accident. ($\chi^2$ (2) 1.892[a], $p$ =0.388).

**Table 14:** Evaluation of satisfaction status of the worker who injured as a result of work accidents working at the institution

| Groups | Work Accident at the Institution | | | | | |
|---|---|---|---|---|---|---|
| **Job Satisfaction** | **Yes** | **No** | **Total** | **DF** | $\chi^2$ | *p* |
| Very Pleased | 6 | 21 | 27 | | | |
| | 22.2% | 77.8% | 100% | | | |
| Satisfied | 21 | 75 | 96 | 2 | 1.892[a] | 0.388 |
| | 21.9% | 78.1% | 100% | 2 | | |
| Not Satisfied | 6 | 10 | 16 | 2 | | |
| | 37.5% | 62.5% | 100% | | | |
| **Total** | 33 | 106 | 139 | | | |
| | 23.7% | 76.3% | 100% | | | |

According to Table 15, when the relationship between the reasons for working at the current workplace and the situation of having an accident is examined; it is seen that 33 (23.7%) of the participants who work in the current workplace have occupational accidents and 106 (76.3%) have not. There were no significant differences between working in current workplace and occupational accidents for various reasons. ($\chi^2$ (6) 5.633[a], $p$ = .466).

**Table 15:** Evaluation of the reasons for working in the current workplace and the situation of having an accident

| Groups | Work Accident at the Institution | | | | | |
|---|---|---|---|---|---|---|
| **The Reasons For Working In The Current Workplace** | **Yes** | **No** | **Total** | **DF** | $\chi^2$ | ***P*** |
| NA* | 0 | 1 | 1 | | | |
| | 0.0% | 100% | 100% | | | |
| Job Security | 17 | 57 | 74 | | | |
| | 23.0% | 77.0% | 100% | | | |
| Adequate Wage | 0 | 6 | 6 | 6 | | |
| | 0.0% | 100% | 100% | 6 | 5.633[a] | 0.466 |
| Social Security | 13 | 36 | 49 | 6 | | |
| | 26.5% | 73.5% | 100% | | | |
| Social Opportunities | 0 | 3 | 3 | | | |
| | 0.0% | 100% | 100% | | | |
| Good OHS Conditions | 1 | 1 | 2 | | | |
| | 50.0% | 50.0% | 100% | | | |
| Other | 2 | 2 | 4 | | | |
| | 50.0% | 50.0% | 100% | | | |
| **Total** | 33 | 106 | 139 | | | |
| | 23.7% | 76.3% | 100% | | | |

## Discussion

This study has been carried out with regard to occupational accident status, PPE use situations and the difficulties that may be encountered in working and social life of the employees working as technical personnel in a state university and the measurement of job satisfaction at present.

According to the results of the research, the majority of the employees do not receive training on occupational safety before and after they start.

While it is expected that there will be a meaningful relationship between the satisfaction of the persons who have had work accidents in the institution and the institutions they work with, this situation could not be caught in the analyses performed in this study. The reason for this situation can be explained by the fact that the study is carried out in one of the state institutions instead of the private sector.

## Conclusion

The conclusion stated in the study that 'no significant difference was found between working in the current workplace and occupational accidents for various reasons can be explained by the fact that there is a work guarantee in the public institutions. Furthermore, according to the results of the study, it can be said that the expected and observed negativities will be minimized if the employees receive training on OHS materials.

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*Review Article*

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# Fluoride in Waters and Toxic Effects

**Gullu Kirat**

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**Abstract**

Fluorine is the world's 13th most abundant element and constitutes 0.08% of the Earth crust. It has the highest electronegativity of all elements. Fluoride is found naturally in soil, water, and foods. It is also produced synthetically for use in drinking water, toothpaste, mouthwashes and various chemical products. Although fluoride is used industrially in a fluorine compound, the manufacture of ceramics, pesticides, aerosol propellants, refrigerants, glassware, and Teflon cookware, it is a generally unwanted byproduct of aluminum, fertilizer, and iron ore manufacture. This paper reviews the human health effects of fluoride. The authors conclude that available evidence suggests that fluoride has a potential to cause major adverse human health problems, while having only a modest dental caries prevention effect. As part of efforts to reduce hazardous fluoride ingestion, the practice of artificial water fluoridation should be reconsidered globally, while industrial safety measures need to be tightened in order to reduce unethical discharge of fluoride compounds into the environment. Public health approaches for global dental caries reduction that do not involve systemic ingestion of fluoride are urgently needed.

**Keywords:** Dental fluorosis; Skeletal fluorosis; Fluoride poisoning.

## Fluorine

Fluorine is the first element of the group of halogens, with an atomic number of 9 and an atomic weight of 19, with an odor of ozone, brownish, purple, green, white, semi-transparent and yellow. It was first discovered by Joseph Henri Moissan in 1886. Calcium is in the fluoride composition and when it is pure contains 51.3% calcium and 48.7% fluorine.

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The fluorine element is the most electronegative and most active of all elements. As it has a high electronegative property, it is present in salt composition by combining with other elements in nature. The compound that fluorine makes with another element is defined as fluoride (such as NaF and $CaF_2$). Fluorine element is found in soil, water, rock, air, plants and animals.[1] One of the essential elements of the body, fluoride, is found in bones and teeth.[2,3]

Florspar, cryolite, fluorapatite, mica, hornbled and tourmaline are the richest minerals in fluoride. Volcanic rocks, mica minerals (sirolite, fluorite, fluorine apatite) and thermal springs cause high fluoride concentrations in natural waters.[4] The level of fluoride in surface waters is generally below 1 mg/L. This amount can reach up to 20–53 mg/L in deep groundwaters that contact with fluoride-rich minerals or in hot spring waters.[1] Fluorides found in natural drinking waters are the largest source of fluoride taken into the body. Endemic fluorosis is a

major public health problem in individuals living in geographic areas with higher concentrations of fluorine in natural drinking water and resources than the optimal daily dose of fluorine.[5,3]

The amount of fluorine in the earth's crust is 950 ppm. Fluorine element is present in high amounts of fluoride apatite in carbonatites and alkali rocks. The amount of fluorine is up to 370 ppm in magmatic rocks, 850 ppm in acidic rocks, 500 ppm in schist, 330 ppm in carbonates, and 8500 ppm in fologophyte and kimberlite rocks. The presence of fluoride in kimberlite and fologophyte is an indication that it is an element of mantle origin.[6,7]

Rock forming minerals; F-1 only replaces OH-. Thus, mica, amphibole, apatite, clay and other water-containing minerals contain significant amounts of fluorine.[6,7]

Volcanic gases contain significant amounts of fluoride in the form of HF. In the meantime, it causes intense alteration of the rocks in the regions where volcanic gas outlets occur. Fluorine-containing hydrothermal solutions are acidic and react with limestone or calcium-containing minerals to raise the pH (basic) fluoride. The researchers found significant amounts of Na-Ca-Mg-Cl in the fluid inclusion studies in fluorites. Fluorites appear in nature as elevations because they are resistant to decomposition.[6,7]

**General Properties of Fluorine**

Chemical formula: $CaF_2$

Crystal system: Cubic

Specific gravity: 3,18 g/$cm^3$

Melting temperature: 1330°C

Hardness: 4 (Mohs)

Color Types: purple, green, blue, yellow, white, pink, brown and bluish black, transparent semi-transparent mineral. Colors vary according to the impurity element in the crystal lattice.

*Blue:* Yttrium and fluorine elements

*Pink:* Yttrium and oxygen elements

*Yellow:* Oxygen element

*Green:* Yttrium, cerium and fluorine elements

*Optical appearance differences:* Color change feature.

*Durability:* Weak and brittle

*Persistence:* Very sensitive to heat, generally resistant to light, soluble in sulfuric acid.

*Cutting types:* Generally faceted, cabochon, is cut in the shape and carving.

*Detection:* Can be detected by hot spot test (under microscope) and fluorescence property.

*Method:* Radiation is applied to obtain violet color from colorless.

*Care:* Ultrasonic washing and steam cleaning should never be performed. Keep away from heat. Can be cleaned with warm soapy water.

*Presence:* It is generally found as a gangue mineral in hydrothermal environments. It can also occur in all phases of limestone and dolomite and acidic magmatic rocks.

Some of the countries in which it was issued: England, Switzerland, the United States, Australia, Germany, Mexico, Norway and China.[8]

**Fig. 1:** Fluorine samples in the site.

## Formation of Fluorite Deposits

### *Pegmatite-Pneumatogenous fluoride deposits*

Pegmatites are magmatogen-pneumatogen mixture rocks. The average number of minerals found in pegmatites is 200 and these minerals are often rare. There are 30–40 kinds of minerals that can be produced economically in pegmatites and the first one is tin. Fluorite deposit formation occurs when magmatic or diagenetic hydrothermal fluids react with the side rock (eg.; South Africa). Some fluorite deposits have no relation with magmatic events. Examples of such fluorite deposits are Missipi type diagenetic hydrothermal mineralization. The fluorite they dissolve as a result of the cycle in the rocks is formed by precipitation in the appropriate faults and cavities. These are usually metasomatic formations. Whether their formation is syngenetic or epigenetic is still debated. Fluorite deposits formed as a result of diagenetic hydrothermal events are observed more frequently in nature.[6,7]

### *Hydrothermal Deposits*

There is another view that binds the formation of fluorite deposits to low-temperature hydrothermal solutions that refuse to bind pegmatite-forming alkaline granite. According to this view, fluorite deposits were formed in the last and outer phases of the hydrothermal phase. The structural features of the hydrothermal vein, the wall of the hydrothermal vein and the relationship between the breccias within the hydrothermal vein and the side rock vein give information about the development of the hydrothermal vein. The examination of this information helps to understand the physical and chemical development of the vessel and to analyze the tectonic mechanism that leads to the formation of the vessel. A series of deformation mineral tissues formed before and after mineralization in ores formed in hydrothermal vessels provide important information. These deformation tissues are shear lines, cleavage lines, deformation bands, twist bands, compositional band deformation mineral twinning, polygonization, powdering and fluidized structures (mylonitization). This deformation is the result of the geological universe that the fleet undergoes after its formation.[6,7]

### *Sedimentary Beds*

Fluorite is also found as sedimentary in the tricalcium carbophosphate, mainly in the vertebrate organism residues. Sometimes it is found in sedimentary apatite. Fluorine is found in human and animal bones. In the bones, the hydroxyl group can also be replaced by fluorine. The presence of fluorite as a sediment is known, but it is controversial at which depth granites are found as sedimentary.[6,7]

## Presence of Fluorine in Nature

Fluoride is very common in nature (Fig. 2). Fluoride is present in various minerals (Florspar, Fluorapatite, Cryolite, Mica, Topaz, Hornblende, Tourmaline), bones and teeth, waters and various biological materials. Aluminum facility, phosphorus fertilizer factories, brick, tile, and ceramic producing industrial zones, especially in the air, soil and water can be harmful to environmental health levels of fluoride.[9] The concentration of fluoride in surface waters is generally between 0.01 and 0.3 ppm. The concentrations of fluoride ions in groundwater depend on the geological, chemical and physical characteristics of the aquifer, the porosity and acidity of the soil and rocks, the


**Fig. 2:** Appearance of fluorites in the site.[7]

temperature, the movement of other chemical elements and the depth of the wells. Fluoride ion was found in groundwater at concentrations from less than 1 mg/L up to 48 mg/L. Fluoride is found in nature in the form of simple fluoride compounds and many complex ions. Its main compounds: NaF, $CaF_2$, $H_2F_2$, $Na_2SiF_6$, $H_2SiF_6$, $(NH_4)^2$, $Si_6$ and the like. Generally, fluoride is formed by hydrolysis of fluorosilicate ions.[10,11]

$$SiF_6^{2-} + 3H_2O \rightarrow 6F^- + 6H^+ + SiO_3^{2-}$$

Fluoride ion concentration values found in various media are listed below;

- In sea water: Average concentration 1.4 mg/L.[12]
- In river water: Average concentration 0.2 mg/L
- In groundwater in limestone and dolomite environments: Average concentration 8.7 mg/L
- Groundwater with granitic rocks: average concentration 9.2 mg/L.[11]

## Usage Areas of Fluoride

Fluoride is used in different fields according to its purity.[6,7]

### *Use of Fluorite in Metallurgy*

Low-grade fluoride (70–85% $CaF_2$) is sufficient for use. Slag is used as a fluidizing medium in steel furnaces while producing steel. When fluoride is used in metallurgy, its reaction with phosphorus, sulfur and silica ensures good steel construction. But if these impurities are found in the ore, the situation changes slightly. Therefore, the proportion of these impurities in the ore is limited. The use of fluoride in metallurgy can be summarized as follows. It drains the phosphorus together with the slag and prevents the abrasion of the bricks in the furnaces covered with refractory bricks.[6,7]

### *Use of Fluoride in Chemical Industry*

In principle, the fluoride is used as 44% fluorite, 18% cryolite, 35% hydrofluoric acid and 3% fluoslicate. Fluoride is the major raw material of hydrofluoric acid.[6] Fluorine and its compounds are used in the production of a large number of commercial chemicals, especially uranium.[7]

Toothpastes, as it is an essential ingredient for dental health. Single atom fluorides, in the production of semiconductor products. The fluorochlorohydrocarbon compound is used in refrigerators, air conditioners and deodorants. However, the fluorochlorohydrocarbon compound is a substance that is harmful to the ozone layer.[11] Teflon contains fluorine. Elemental fluorine is used to provide propulsion in rockets due to its high specific propulsion.[11]

### *Usage of Fluorite in Ceramic and Glass Industry*

It is used for making fine-grained fluorite opal and flint-containing glasses. Fluorite of the same composition is used for enamel coating on steel and iron in the ceramic industry. Ceramic grade fluoride is also used in the preparation of fiber glass, the magnesium industry and clay briquettes. Hydrofluoric acid (HF) is the only compound that acts on the glass surfaces.[6,7]

### *Use of Fluorite as Ornaments*

Ornaments such as ashtrays, bowls and plates hanging on the wall are made of fluorites in dark pink and yellow colors (Fig. 3).[6,7]

### *Use of Fluorite in the Optical Industry*

Especially used as microscope lenses and glasses.[6,7]

### *Usage of Fluorite in Cement Industry*

Fluorite was used to ensure low sintering in cement construction. However, it has been abandoned in many places since it has corrosive activity in the furnace.[6,7]

**Fig. 3:** Use of fluorite as ornaments

## Fluoride and Toxic Effects in Waters

As one of the trace elements necessary for the human body, fluorine has an important role especially in bone and tooth development, prevention of mineral loss on tooth surfaces, and cellular activation and reduction of bacterial enzyme activity.[13–16] Fluorine is an element of biological importance in addition to its industrial use. There is 0.5 ppm fluoride in human blood, 2000–12000 ppm in bone, 0.22–7 ppm in lungs and 0.005 ppm fluoride in muscle tissue, 950 ppm in crust and 1.3 ppm in sea water.[15,17]

It may be beneficial or harmful depending on the amount of fluoride present in the waters. The permissible concentration of fluoride ion in water is 1.0 mg/L and the lethal dose for adults is 0.20–0.35 g/kg body weight. Several methods have been developed for the determination of fluoride in aqueous solutions and waters, such as chromatographic, spectrophotometric and potentiometric methods.[18]

Removal of excess fluoride ions from water has been one of the major environmental problems investigated in the world due to the necessity of a certain concentration range in terms of human health in drinking water. The desired fluoride concentration in drinking water is in the range of 0.8–1.7 ppm.[15] The presence of fluoride at these levels shows protective properties against tooth decay. Prolonged consumption of a drinking water containing fluoride ions of more than 1.5 mg/L causes fluorosis in the teeth and skeletal system.[19]

Fluorine intoxications occur in two forms: acute and chronic. Acute intoxications are rare, but chronic fluoride intoxication may cause loss of appetite, long bones, tooth loss, chalky to brown speckles.[17,20,21]

Nutritional level, age and climatic conditions are significantly effective in the occurrence of fluoride deficiency or toxicities.[9] The structure of the soil, volcanic formations phosphate rocks, superphosphate fertilizers used in agriculture, preparations used in veterinary fields, aluminum, glass, iron, brick and cement factories are factors that cause fluorosis risk in the environment.[20,22, 23]

Since fluoride is a very electronegative ion, it combines with calcium in the bones and teeth, causing high concentrations of fluorosis in the teeth and skeleton. Very high concentrations can be the source of cancer cases. Fluoride intoxication caused by high intake of fluorine is called fluorosis. In the formation of fluorosis, the fluorine concentrations of soil, water and plants are important in relation to the background value of fluorine in the region.[24]

On the other hand, fluorine, which is widely used in the industry, is given to the atmosphere and thus, a high proportion of fluorine can be transferred from anthropogenic sources to human and other living bodies.[25] It is not an essential element for plants. However, it was observed that the seeds germinate or the growth of plants was not normal in environments with high fluoride content. In waters with fluorine, this amount taken with tea daily can increase to 8–10 mg.[17,26,27]

## Fluoride Toxicology

### *Acute Fluoride Poisoning*

In healthy individuals, the acute toxic dose for the fluorine element is between 1 and 5 mg/kg, and fluoride intoxication with doses of 15–30 mg/kg can result in death.[28–30]

In acute fluoride poisoning, these symptoms appear minutes after excessive intake of fluoride, nausea-vomiting-diarrhea, dizziness, hypersalivation, and abdominal pain. In the case of mild intoxication, these symptoms usually disappear within 24 hours. In severe cases, these symptoms are accompanied by cardiac arrhythmia and coma. Prognosis is generally good in patients who survive 24 hours after poisoning.[29,30,32,33]

The effects of fluoride on nerve tissues can be defined as headache, convulsions, visual impairment, paresthesia, optic neuritis, and change of consciousness.[29,30,34]

The most serious symptoms of acute fluoride poisoning are related to the respiratory system. Respiration is initially stimulated and then depression develops. Laryngeal or pulmonary edema may occur. Depending on the dose of fluoride taken, respiratory-related symptoms can occur even within 30 minutes, under which conditions death can generally occur within 2–4 hours.[29,30,32,33]

Pathophysiological changes in individuals during acute fluoride poisoning can be listed as follows.[29,30,32]

1. Disorders of cardiac and muscle functions and coagulation mechanisms due to sudden hypocalcemia.
2. Gastrointestinal system side effects due to hydrogen fluoride caused by fluoride and gastric content.

3. Neurological symptoms with direct effects on muscle and nerve tissue.
4. Anaerobic glycolysis enzymes, cholinesterase function. impaired tissue respiration by affecting enzymes containing magnesium and zinc.
5. Vasomotor disorders caused by the involvement of smooth muscles in the vessels.

### *Chronic Fluoride Poisoning*

*Dental Fluorosis*

For the first time Morichini reported that teeth contain fluoride, and in the following ways, many researchers have argued that the amount of fluoride in the teeth affects dental health. In 1902, dentist Mc Kay found that most of his patients had brown and permanent stains on his teeth, and he thought these teeth could occur for local reasons and called them stained enamel (fluorosis).[11]

In the formation stages of the teeth, the excessive amount of flora in the environment is affected by enamel and then dentin formation and dental fluorosis occurs. Changes in the enamel structure of dental fluorosis starts from fine-white lines and changes as enamel pits.[30,35–42]

When taken systemically during the enamel formation step, fluoride enters the enamel structure and replaces the hydroxyl ion in the hydroxyapatite to form the fluorapatite form. Since the electrostatic bonds in the fluorapatite structure are stronger, this structure is more resistant than hydroxyapatite. In this new structure, fluoride stabilizes the hydroxyapatite structure of the enamel by establishing additional hydrogen bridges.[28,35,36,39,43,44] However, an increase in porosity and intercrystalline cavities also occur in the enamel structure. This is responsible for the weakening of the enamel structure.[30,39,42,45,46]

*Skeletal Fluorosis* (Fig. 4)

Chronic administration of fluoride by oral route over long periods of time, particularly during growth and development, leads to fluoride uptake in skeletal tissues and pathological bone formations.[1,3] In the case of skeletal fluorosis, excessive intake of fluoride is usually involved by the oral route during the bone growth stage or during various periods of the restructuring stage. It is thought that at least 20,000,000 people in the world are affected by skeletal fluorosis of varying degrees.[30,35,42,44,47–49]

Bone tissue functions as the largest fluoride store in our body. Bone tissue fluoride concentrations are a good indicator for long-term fluoride use. Therefore, bone tissue fluoride concentrations are a recommended biomarker for the total amount of fluoride in the body.[30,44,50,51]

Almost all of the fluoride uptake in the body depends on the ability of the fluoride ion to integrate into the apatite crystal structure. Fluoride selects the hydroxyapatite crystal form in the skeletal system due to the similar electrical charge and size and replaces the hydroxyl ions and fluoride ions. By replacing the hydroxyl ions in the bone apatite structure with fluoride, crystal stabilization is achieved in the bone structure while increasing the average crystal structure size. Fluorapatite crystal structure has less solubility and larger crystal volume than hydroxyapatite.[30,35,38,40,48,52–55]

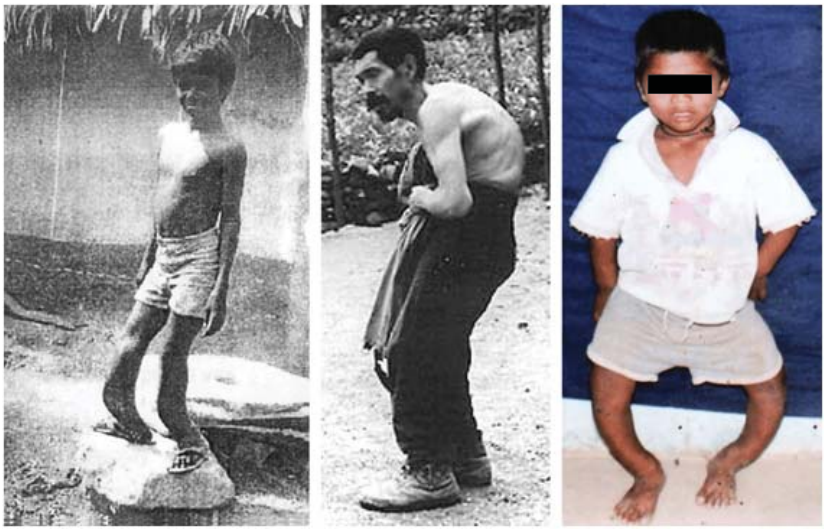

**Fig. 4:** Skeletal fluorosis.

**Effects of Fluorine on Organism**

Acute or chronic exposure to fluoride compounds results in long-term exposure to many systems of the organism. Fluoride compounds have major effects on respiratory, digestive, hematologic, cardiovascular, renal and endocrine systems and musculoskeletal system.[30]

**Daily Fluorite Ingested**

Fluoride has been added to drinking water since 1945 in various countries around the world. An insufficient amount of fluoride in the process of adding fluoride to the water, which is accepted in modern water engineering and although it is quite a common procedure is not yet widely used in Turkey. However, it is known that fluoride is added to some spring waters.[30]

The purpose of adding fluoride to water with insufficient fluoride content is to protect the dental health of consumers. Fluoride is added to the water using one of the following eight compounds.[56–58]

1. NaF (sodium fluoride)
2. KF (potassium fluoride)
3. $CaF_2$ (calcium fluoride)
4. HF (hydrofluoric acid)
5. $Na_2SiF_6$ (sodium silicofluoride)
6. $MgSiF_6$ (magnesium silicofluoride)
7. $H_2SiF_6$ (silicofluoric acid)
8. $(NH_4)_2\ SiF_6$ (ammonium silicofluoride)

The most common of these is sodium fluoride. All these compounds ionize in water to release fluoride ($F^-$) ion. When alum is used for turbidity and/or organic matter removal, the fluoride addition process should be added at the outlet of the treatment plant (at least after flocculation and settling) as the fluoride will also be partially removed. Otherwise some of the added fluoride will be wasted. During manufacture, trace amounts of arsenic, lead or zinc may be mixed into the fluoride compounds. Normally, their concentrations are so low that they are not harmful.[58]

The World Health Organization study on the relationship between fluoride and drinking water permits 1.0 mg/L fluoride in drinking water and considers it the optimum dose. The optimum amount of fluoride required in drinking water is controversial for the regions. People living in warm climates consume more water because they need more water, and although they have drunk optimum fluorinated water, they will have more fluoride in their bodies than the recommended dose.[35] For this reason, it is not correct to determine the optimum fluoride values for the population through studies based only on drinking water. All possible resources in the region should be taken into account.[30,31]

Depending on the average regional temperature, 0.7–1.2 ppm fluoride in drinking water are the concentrations recommended by the World Health Organization.[28,30,35,36,59]

The fluoride values in Table 1 are the best fluoride concentrations recommended by the CDC (Center for Disease Control) in the USA for drinking water.[56,58]

**Table 1:** Recommended amount of fluoride in drinking water[56]

| Average annual temperature per day (°C) | Recommended amount of fluoride (mg/L) |
|---|---|
| 10.0 – 12.0 | 1.2 |
| 12.1 – 14.6 | 1.1 |
| 14.7 – 17.6 | 1.0 |
| 17.7 – 21.4 | 0.9 |
| 21.5 – 26.2 | 0.8 |
| 26.3 – 32.5 | 0.7 |

In some studies, the recommended daily safe fluoride dose for children was 0.1 mg/kg/day.[30,37] Growth and development periods and the amount of fluoride needed vary. Therefore, daily fluorine needs are calculated separately for children and adults. However, it is generally agreed that the daily fluoride dose should not exceed 0.1 mg/kg for all individuals. It is thought that with the recommended doses in this way, healthy individuals can be protected from dental and skeletal fluorosis.[30,31,35,47,60,59]

In accordance with the new recommendations, the dietary reference amounts of regulated fluoride are regulated by the American Dietetic Association as shown in Table 2.[30,61]

**Table 2.** Fluoridine reference amounts in the diet.

| Age Groups | Reference weıghts (kg) | Optımum doses (mg/day) | Tolerable upper limit (mg/day) |
|---|---|---|---|
| Infant 0–6 months | 7 | 0.01 | 0.7 |
| Infant 6–12 months | 9 | 0.5 | 0.9 |
| Child 1–3 years | 13 | 0.7 | 1.3 |
| Child 4–8 years | 22 | 1.0 | 2.0 |
| Child 9–13 years | 40 | 2.0 | 10.0 |
| Boy 14–18 years old | 64 | 3.0 | 10.0 |
| Girl 14–18 years old | 57 | 3.0 | 10.0 |
| Male 19 years and over | 76 | 4.0 | 10.0 |
| Female 19 years and over | 61 | 3.0 | 10.0 |

Daily optimum fluoride amounts were calculated according to body weight and optimum daily doses are presented in Table 3.[30,35]

The dental fluorosis index developed by Dean in 1934 and the modified version of this system by Moller in 1982 have been reported in litreature.[35] Accordingly, scores of Dean dental fluorosis classification are shown in Table 4.[30]

**Table 3.** Fluoride intake by body weight[35]

| Body weight (kg) | Optimal daily fluoride intake (mg F) | Potentially harmful daily fluoride intake (mg F) |
|---|---|---|
| 10 | 0.50 – 0.70 | 1.00 |
| 20 | 1.00 – 1.40 | 2.00 |
| 30 | 1.50 – 2.10 | 3.00 |
| 40 | 2.00 – 2.80 | 4.00 |
| 45 | 2.25 – 3.15 | 4.50 |
| 50 | 2.50 – 3.50 | 5.00 |
| 55 | 2.75 – 3.85 | 5.50 |
| 60 | 3.00 – 4.00 | 6.00 |
| 65 | 3.25 – 4.00 | 6.50 |
| 70 | 3.50 – 4.00 | 7.00 |
| 75 | 3.75 – 4.00 | 7.50 |

**Table 4:** Dean dental fluorosis index[35]

| Scores | Classifications | Properties |
|---|---|---|
| 0.0 | Normal | Flat, glassy, cream-white, translucent tooth surface. |
| 0.5 | Suspicious | Several white spots or blemishes. |
| 1.0 | So light | Small opaque, paper-white areas that are observed in less than 25% of the tooth surface. |
| 2.0 | Light | Small opaque, paper-white areas that are observed in less than 50% of the tooth surface. |
| 3.0 | Middle | Significant wear that can be observed on incisal and occlusal surfaces followed by brown stains affecting all tooth surfaces. |
| 4.0 | Severe | Combined pit formations with brown staining where all tooth surfaces are significantly affected |

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*Review Article*

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# Industrial and Environmental Toxic Exposures and Osteoporosis

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**Abstract**

Exposure of industrial and environmental toxic substances to air, drinking water, soil, plants, animals and food chains and their continuous release to the human environment have a negative impact on human health. When exposed to heavy metals due to these toxic exposures, many effects on body functions can occur. Generally, due to the long half-life of the metal in the body, it can accumulate biologically in soft and hard tissues/organs. Bone tissue undergoes a continuous remodeling throughout life. This involves the simultaneous action of resolution, synthesis and mineralization of the bone matrix. In general, metals have two effects on bone tissue: the first is their direct toxicity to bone cells and the second is their accumulation in the bone matrix. Their direct toxicity mainly affects osteoblasts, inhibits osteoblast differentiation, synthesis activity and mineralization of the extracellular matrix. Their effect on osteoclasts differs from that of metal, increases or decreases the activity of tartarate resistant acid phosphatase enzyme and prevents the maturation of the precursors. As a result, it causes imbalance in the bone remodeling process, reduces bone formation and contributes to the formation of bone diseases such as osteopenia and osteoporosis. Despite our knowledge of the effect of metals on bone tissue, many things are still unclear. Understanding the mechanisms of action will ensure that appropriate therapies are available to address their adverse effects on bone tissue.

**Keywords:** Heavy metal; Bone; Osteoporosis.

## Industrial and Environmental Toxic Exposures and Osteoporosis

Contamination by air, drinking water, soil, plants, animals and food chains with metals has a negative impact on human health. Their toxicity and prolonged biological half-life is a serious problem due to their accumulation in the environment and living organisms. Metal exposure affects many systems in our body and has a negative impact on the skeletal system. Depending on the concentration and duration of the metal species, it may result in increased risk of osteoporosis and fracture.[1–4]

The aim of this review is to focus on the effects of toxic metals on bone tissue. It was to taking into account of the mechanisms of these metals with long/short term non-physiological deposition on the skeletal system.

## Normal Bone Tissue and Osteoporosis

In addition to providing normal posture and bodily movements, bone tissue serves as a reservoir for many minerals, including calcium and phosphorus, to preserve important structures. Bone remodeling, which is a dynamic process, consists of bone resorption and construction processes.[3,4]

Osteoblasts are cells that originate from bone marrow (Mesenchymal), responsible for bone

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formation and mineralization. Active osteoblasts perform bone formation while inactive osteoblasts form cells that cover the bone surface. Osteoblasts synthesize the bone matrix, 90% of which consists of collagen. These matrix elements; Type 1 consists of collagen, osteocalcin, bone sialoprotein, osteopontin, proteoglycans, cytokines, growth factors and alkaline phosphatase. Alkaline phosphatases are responsible for the mineralization phase. During the mineralization phase, phosphate and calcium precipitate on the bone matrix. For intestinal absorption of calcium, 1,25 dihydroxy-vitamin $D_3$ is required.[5,6]

Osteoclasts are cells that originate from monocyte/macrophage precursors of the hematopoietic system. Osteoclasts are rich in lysosomal enzymes including tartrate-resistant acid phosphatase, collagenase, and cathepsins, and through these enzymes, they resorb the bone matrix.[6] Osteoprotegerin (OPG), which inhibits bone destruction, is the receptor activator nuclear kappa B (RANK), which controls the physiological and pathological bone resorption, and is the receptor in osteoclasts that causes bone destruction by stimulation with RANK ligand (RANKL).[7-9] The RANK, RANKL axis adjust osteoclast activity. Osteoprotegerin (OPG) binds to RANKL, inhibiting RANK activation and inhibits osteoclast formation.[10] Increased osteoclast activity leads to osteoporosis and osteopenia. Therefore, osteoclasts play an important role in bone hemostasis.[11]

## Metal Toxicity in Bone Tissue

### *Chromium*

Chromium (Cr) occurs in different states in natüre. It is commonly found as Cr (III) and Cr (VI). Chromium rock, soil, water and dust are also available. Especially chromium (III) is naturally found in some foods such as meat, fish, nuts, eggs, fruits and vegetables and is required in small doses for the human body due to its participation in carbohydrate metabolism. Chromium has been shown to reduce ALP activity and mineralization of osteoblasts.[4,12,13] The study in rats showed[14] that Cr (VI) accumulates in the femur. Some studies have shown that long-term supplementation with Cr (III) may have adverse effects on Fe metabolism, since these metals bind to transferrin.[15,16] Such interaction may also be observed between Cr (III), Zn and Cu due to similar mechanisms of absorption.[17,18] Therefore, over-administration of Cr may cause osteoporosis due to Fe, Cu and Zn deficiency.[4]

### *Lead*

Lead (Pb) is found in various forms in nature including Pb salts (ionic Pb) and organic Pb tetraethyl lead compounds.[19] The main sources of environmental Pb contamination include the steel, metal and mining industries. When Pb content increases, physiological mineralization decreases.[20] The main target of Pb is the bone matrix, capable of replacing other divalent cations in the body such as $Ca^{2+}$, $Mg^{2+}$ and $Fe^{2+}$, to a lower degree of monovalent cations such as $Na^{+}$. It may alter osteoblast function and thereby impair the hormonal regulation of calcification.[21] It inhibits the enzyme 1-hidroks-hydroxylase in the kidney. Affects vitamin D production. It results in hypocalcemia and hypophosphatemia.[22] Even subtoxic Pb doses may alter bone biomarker values such as alkaline phosphatase, collagen Type 1, osteocalcin and transcription factor 2, thereby preventing bone formation by reducing the differentiation of osteoblasts.[23]

### *Aluminum*

Aluminum (Al) is a metal found everywhere. Aluminum is known to cause nervous system, hematopoietic system, renal osteodystrophy, hemodialysis encephalopathy, osteomalacia, osteoporosis and anemia.[24–28] Aluminum is accumulates on trabecular bone surfaces and on the surfaces of vascular ducts that penetrate the compact bone.[24,29] *In vitro* studies, there is evidence that aluminum prevents osteoblast differentiation. It inhibits type I collagen. Al, reduces expression of osteocalcin, bone sialoprotein, osteopontin.[30] Deposition of Al in the bone can reduce Mg, Ca and P levels and lead to inhibition of the bone mineralization process.[31]

### *Cadmium*

Cadmium (Cd) is an environmentally and occupationally important contaminant. Cadmium compounds can be in powder and aerosol form. Toxic effects may result from chronic inhalation. Adverse effects on vitamin D levels and bone mineralization have been reported, particularly in the lung and kidney (renal tubular dysfunction, renal stone and hypercalciuria). Many studies have shown that increased risk of renal failure, osteoporosis and fracture can be triggered by exposure to Cd.[29,32–34]

Two mechanisms of action are mentioned: the direct effect of metal on bone cells. It has been reported that it directly affects osteoclasts

and causes the destruction of matrix tissue.[29,35–37] Cadmium causes renal failure first, increases excretion of calcium and phosphorus, decreases vitamin D synthesis and consequently decreases calcium absorption in the digestive tract and affects bone mineralization.[38–40] Studies have shown that chronic exposure to Cd reduces the mineralization of vertebral bodies and makes them more susceptible to fracture.[41] Youness E. et al. They found that a decrease in vitamin $D_3$, osteocalcin and bone-specific alkaline phosphatase activity and an increase in serum Ca, P and parathyroid hormone levels.[42] Other studies have shown that chronic exposure to Cd reduces bone volume and increases TRAP positive cells in the subchondral tibial bone.[43,44]

### *Mercury*

Inorganic mercury (Hg) is absorbed by the lungs and accumulates in the brain, while methyl mercury is absorbed in the intestine and accumulates in the soft organ. Methyl mercury has been shown to reduce calcemia and directly affect the metabolism of bone cells.[4,29,45] Mercury inhibits the activity of both osteoclasts and osteoblasts. Prenatal poisoning of experimental animals with methyl mercury has shown that the rat fetus retards ossification and reduces long bone length.[46]

### *Iron*

Iron (Fe) is one of the most abundant metals in nature and is required for many biological processes.[47,48] It catalyze the formation of free radicals in different cells. The RANK/RANKL/OPG system also showed a change, an increase in osteoclastactivity and osteoblastic dysfunction.[49] Iron overload also reduces the formation of mineralization and inhibits the growth of hydroxyapatite crystals by changing their crystallization.[29,50,51]

However, the exact mechanism by which Fe deficiency can affect bone health has not yet been fully understood. Some experimental studies have shown that Fe-deficient animals have reduced trabecular thickness and total bone volume compared to non-deficient animals.[52] Changes in iron metabolism inhibit osteoblast proliferation and osteoclast differentiation.[53] It has been suggested that there is a relationship between adequate Fe intake and bone health.[54–56]

### *Arsenic*

Arsenic (As) is not a metal, it is a metalloid that occurs as inorganic As (III) and As (V) and organic arsenic species. Arsenic and Paget's disease have shown a relationship between them.[57] There are studies reporting that osteomyelitis and osteonecrosis of the alveolar bone develop as a result of the use of dental paste containing arsenic trioxide.[58] Chronic exposure to low-level arsenic can cause bone resorption by promoting osteoclast differentiation.[59] *In vivo* arsenic trioxide poisoning has been shown to alter bone resorption by decreasing the maturation of osteoclast precursors and decreasing osteoclastic activity. Non-cytotoxic doses of Arsenic have been reported to cause dysfunction of some signal transduction including steroid receptors due to osteoclast and osteoblast differentiation.[4,29] In addition, it has been reported that *in vitro* exposure of Arsenic may reduce osteoblastic activity and osteoblastogenesis, thereby affecting bone formation process.[60]

### *Nickel*

*In vitro* studies have shown that high Ni concentrations inhibit alkaline phosphatase activity and bone mineralization.[61]

### *Titanium*

Titanium (Ti) is used the production of orthopedic prostheses and dental implants. There are studies showing that titanium inhibits osteoblastic differentiation.[62] It was found to stimulate osteoclastogenesis and osteoclast activity in the presence of RANKL.[63]

## Results

Various remains unclear as to the effect of metals on bone tissue. Understanding the mechanisms of effect will ensure that adequate therapies are available to address their adverse effects on the bone.

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*Case Report*

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# Methyl Alcohol Intoxication with Extensive Involvement of the Central Nervous System Shown By Magnetic Resonance Imaging

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**Abstract**

The focus of this study was to present methyl alcohol intoxication by citing clinical and laboratory, as well as radiological findings. A case of a 47-year-old man with a 20-year history of alcohol use disorder, brought to the emergency department in a coma. He had headache and blurred vision symptoms that started after drinking cologne and spirits. He had a Glasgow Coma Scale score of four. Increased anion gap metabolic acidosis was detected. Diffusion weighted imaging showed diffuse restriction regions in the subcortical white matter, cerebellar hemispheres, brain stem, bilateral optic nerve and putamen. He treated with hemodialyze, sodium bicarbonate, dextrose and ethanol infusion. However, the patient died on the seventh day. Bilateral putaminal necrosis is the most characteristic radiological finding, but white matter necrosis rarely reported in the literature. As in our case, such extensive involvement of the brain may be correlated with mortality in the acute period.

**Keywords:** Methyl alcohol; Methanol; Intoxication; Magnetic resonance imaging.

## Introduction

A Acute methanol poisoning is frequently seen in suicidal or accidental. Drinking cologne and spirits to get drunk is not a usual practice, however, it can be seen in rare cases of alcohol use disorder when ethyl alcohol is not available.

The aim of this report is to emphasize the recognition of methanol intoxication in respect to clinical and radiological features, and the importance of early diagnosis and timely treatment. In addition, it is thought that the diffuse involvement of the Central Nervous System (CNS) in neuroradiological imaging may be correlated with the risk of death in the acute phase.

## Case Report

A 47-year-old male was intubated due to respiratory arrest, referred to the emergency department on admission. According to the medical history taken from his family, he had a history of alcohol abuse for 20 years. Thirty hours before admission he had ingested intentionally an unknown amount of methanol to get drunk. It was learned that he had headache and blurred vision symptoms started 24 hours after ingestion, gradually became unconscious. Although the patient did not receive medication for sedation, he had a Glasgow Coma Scale (GCS) score of 4/15 (E1V1M2).

Biochemical investigation revealed wide anion gap metabolic acidosis. Methyl alcohol levels were not measured due to technical inability. The laboratory findings are shown in Tables 1 and 2.

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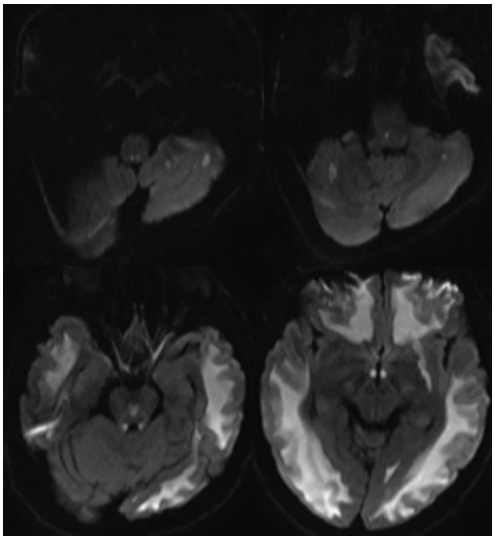
**Table 1:** Investigated laboratory findings related to acute methanol intoxication

| Detected hematological and biochemical abnormalities | |
|---|---|
| Serum osmolality | 286.4 $\mu$mol/kg |
| Blood ethanol level | Below 0.1 g/L |
| Arterial blood gas | |
| pH | 6.7 |
| $pO_2$ | 406.9 mm Hg |
| $pCO_2$ | 20.0 mm Hg |
| $HCO_3$ | 3.0 $\mu$mol/L |
| Base deficit | -38.2 $\mu$mol/L |

**Table 2:** Other laboratory findings examined in blood

| Detected hematological and biochemical abnormalities | |
|---|---|
| Glucose | 44 mg/dl |
| Urea | 60 mg/dl |
| Creatinine | 2.0 mg/dl |
| Aspartate Aminotransferase (AST) | 73 u/L |
| Gamma glutamyl transferase (GGT) | 80 u/L |
| Lactate dehydrogenase (LDH) | 436 u/L |
| Amylase | 667 u/L |
| Potassium | 2.9 $\mu$mol/L |
| White blood cell (WBC) | 18.5 $10^9$/L |
| Hematocrit (HCT) | 55% |
| Mean corpuscular volume (MCV) | 102.3 fl |
| International normalized ratio (INR) | 1.3 |
| Prothrombin time (PT) | 15.5 s |
| Activated partial thromboplastin time (aPTT) | 66.6 s |

Brain Computed Tomography (CT) and Diffusion weighted imaging (DWI) and Apparent Diffusion Coefficient (ADC) map were performed on admission. CT showed bilateral putaminal low attenuation, DWI demonstrated bilaterally putaminal restricted diffusion, ADC maps demonstrated low values suggesting necrosis in the same areas. Conventional Magnetic Resonance Imaging (MRI) was performed while DWI and CT were repeated on the third day of exposure and showed new lesions (CT performed after MRI) (Figs. 1A-E).



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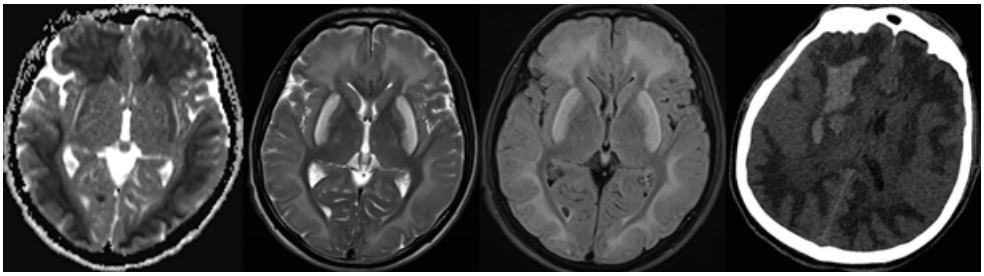

B C D E

**Fig. 1:** Methanol-induced brain lesions (A) – DWI, (B) – ADC maps demonstrated restricted diffusion in the cerebellum, brain stem, putamen, subcortical white matter; (C) – T2-weighted, (D) – T2 flair MRI showed hyperintensity in the same regions and (E) – CT also showed right putaminal and frontal hemorrhage.

Hemodialyze, intravenous (i.v.) sodium bicarbonate and dextrose infusions, 40% ethanol from the nasogastric until i.v. form was provided, followed by i.v. ethanol infusion was performed. Blood ethyl alcohol levels were monitored frequently. Fornepizol could not be given because it was not found in the country. The patient did not respond to treatment and died on post-admission day seven.

## Discussion

A 47-year-old male patient who was brought to the emergency department in a coma, who had a history of alcohol use disorder, who had been drinking cologne and spirits to get drunk, with diffuse subcortical white matter, brain stem, optic nerve, cerebellum, basal ganglia involvement on brain imaging, whose follow-up resulted in death, is presented.

The main feature of methanol intoxication is the latent period between ingestion and onset of symptoms. Symptoms and signs are nausea, vomiting, impaired visual function, ranging from blurred vision to complete blindness, change of consciousness, ranging from confusion to coma, respiratory arrest and death.[1]

The CNS effects of acute methyl alcohol intoxication are caused by its toxic metabolite, formic acid. Formic acid inhibits cytochrome oxidase, a mitochondrial enzyme that is functionary for oxidative phosphorylation; this inhibition leads to anoxia, edema and ultimately cell death.[2]

Especially if medical history is insufficient, diagnosis of methanol poisoning is difficult. Therefore neuroimaging findings and clinical manifestations of methanol intoxication should be better understood and recognized. It was aimed to recognize methyl alcohol intoxication by presenting patient's clinical features, blood biochemical examinations and radiological findings, and interrogates the history of alcohol use disorder and to suggest methanol intoxication in the differential diagnosis. In addition, it was aimed to emphasize the importance of the early treatment of this intoxication which could result in death.

MRI and CT as neuroradiological imaging techniques are able to show toxic effects of methanol intoxication at the CNS but the neuroimaging finding can be normal in acute phase.[3] Putaminal necrosis is the most common and characteristic,[4] but not a specific radiological finding of methanol intoxication. Subcortical white and grey matter necrosis and edema, cortical cerebellar and optic nerve lesions, intracerebral hemorrhage, tegmental necrosis have all been described as well as other brain lesions, in previous reports.[5,3]

Here, a case with a very diffuse involvement of CNS, which is rarely detected, is presented. Conventional MRI and DWI showed diffuse subcortical white matter lesions accompanied by bilateral basal ganglia, brainstem, optic nerve and cerebellum. There was no case that showed the involvement of all these areas together in the literature. This is a unique case that showed all MRI finding seen in the methanol poising.

Target areas of methanol poisoning are basal ganglia especially putamen. It is not precisely known why especially putamen is affected—possibly because of its high metabolic demand, its cerebral microvascular anatomy or direct toxic effects of methanol metabolites.[4] Probably it is multifactorial.

Also, methanol intoxication has a characteristic necrosis structure in relation to parenchymal involvement and it is remarkable that subcortical arcuate fibers are preserved. The difference in the involvement of gray and white matter is due to the fact that the vascular networks where these

regions provide blood and venous drainage are different. Although there are rich vascular networks for arterial circulation of gray matter and subcortical arcuate fibers, arterial circulation of deeper white matter is provided by longer and larger caliber vessels that reach through the cortex without branching. Thus, while diffuse cerebral edema disrupted arterial and venous drainage of the deep white matter, subcortical arcuate fibers continue to be perfused due to rich anastomosis vascular networks. In addition, penetrating vessels that deliver the blood supply to the deep white matter are more susceptible to hypoxia-induced vasospasm.[6]

Treatment of acidosis and removal of toxic substances are intended. Therapeutic procedures are gastric lavage when the patient arrives early, ethanol or fomepizole, sodium bicarbonate, folinic acid and hemodialysis. Fomepizole and folinic acid could not be given to the patient due to technical insufficiency, but all other treatment options were applied quickly. Early diagnosis and timely treatment can be life-saving. While one-third of untreated cases result in death, sequelae such as blindness, dementia and parkinsonism.[2] Severe metabolic acidosis, coma at the presentation, brain edema, infarction and increased $pCO_2$ are associated with poor prognosis. There is no statistically significant association between prognosis and radiological abnormalities.[7,5] However, comatose patients showed more diffuse involvement of CNS.[5]

Considering the poor prognosis and comatose status of this presented case it is avowable that it was a serious intoxication in this case and it can be suggested that increasing degree of poisoning damage started from the optic nerve and the central gray matter and may cause spread to areas other than peripheral gray matter like subcortical white matter, cerebellum and brainstem. MRI and especially DWI should be performed as soon as possible to understand the degree of methanol intoxication. This case was found to be worth presenting due to the detection of such diffuse CNS lesions. There may be a correlation between the clinical outcome and the diffusiveness of radiological abnormalities. It may be suggested that the more extensive CNS involvement is indicative of poor prognosis and worse clinical outcomes. In addition, it may also be correlated with the degree of intoxication and the risk of death in the acute phase. This case is also important due to the progression of MRI findings on the 2nd and 4th days of methanol exposure.

Some of the previous studies have used diffusion weighted imaging in methanol poisoning.[5] In this case, DWI demonstrated bilaterally putaminal hyperintensity, ADC maps demonstrated low values corresponding to the lesions at DWI with restricted diffusion at the presentation. This case developed new lesions in the subcortical white matter, brainstem, optic nerve, cerebellum and CT demonstrated right basal ganglia hemorrhage on the third day after admission.

## Conclusion

Especially in unconscious patients, both clinicians and radiologists should be familiar with the symptoms and recognize the finding of methanol poisoning and begin treatment early.

Prognosis studies involving a large number of patients associated with methanol intoxication may reveal the relationship between the degree of mortality and the extent of brain parenchyma involved in neuroimaging.

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7. Hassanian-Moghaddam H, Pajoumand A, Dadgar SM, Shadnia S. Prognostic factors in methanol poisoning. Hum Exp Toxicol. 2007;26(7):583–86.

# Guidelines for Authors

Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journal" developed by international committee of medical Journal Editors

**Types of Manuscripts and Limits**

Original articles: Up to 3000 words excluding references and abstract and up to 10 references.

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1) Type of manuscript (e.g. Original article, Review article, Case Report)
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**Standard journal article**

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540–7.

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**Article in supplement or special issue**

[3] Fleischer W, Reimer K. Povidone-iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3–9.

**Corporate (collective) author**

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792–801.

**Unpublished article**

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

**Personal author(s)**

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

**Chapter in book**

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O,

Kidd EAM, editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. pp 7–27.

**No author given**

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

**Reference from electronic media**

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979–2001. www.statistics.gov.uk/downloads/theme_health/HSQ20.pdf (accessed Jan 24, 2005): 7–18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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