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Biomonitoring of Heavy Metal(oid)s in the Residents of Abandoned Mining District in Northern Cyprus

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Abstract

Several heavy metal(oid)s are known mutagens and/or carcinogens. Exposure to these elements can lead to the development of malignancies. Gemikonagi, which is in the western part of Cyprus, was the hometown of mining operations. It is believed that the mining site is a significant heavy metal(oid) source for the environment and residents. In this biomonitoring study, a total of 60 blood samples from Gemikonagi region (n = 30) and from a control region located 40 km northeast from the mining site, Tepebasi (n = 30), and 5 soil samples from each region were collected to conduct heavy metal analysis using ICP-MS. To conduct genotoxicity analysis, alkaline comet assay and in vivo micronucleus assays were used. t test for independent samples and Mann-Whitney U tests were applied. Copper and iron were found to be enriched in Gemikonagi, while arsenic was found to be enriched in Tepebasi. Genotoxicity analyses demonstrated a statistically significant increase in parameters of micronuclei frequency (p value = 0.0001) and Comet Assay statistics upon exposure to some elements, such as arsenic (p value = 0.04) and copper (p value = 0.012). The results indicate that a general enrichment in heavy elements is not endemic to Gemikonagi, but a problem that might be generalized to the entirety of Cyprus.

Keywords Biomonitoring · Heavy metals · Environmental toxicology · Alkaline comet assay · In vivo micronucleus assay · Cyprus

Introduction

The term "heavy metal(oid)" refers to natural elements with a density greater than 5 g/cm^3 [1]. These ubiquitous elements persist in the environment for long periods of time. Regardless of their persistency, they are used in a wide variety of industrial applications due to the specific properties they have. Thus, several anthropogenic activities such as industry, agriculture, and pharmaceutics and as well as atmospheric sources release heavy metals to the environment continuously,

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causing a growing concern on both public and ecological health. Environmental pollution where mining or other metallurgical operations are carried is a significant and hazardous problem [2, 3].

During the past century, systemic biomedical research on the long-term effects of heavy metals has been started; however, even today, the precise extent of heavy metal contamination and its possible health effects is a major, underexamined hazard in Cyprus. Exposure to heavy metals not only affects humans but other organisms which show differences in their physico-chemical and biological properties [4, 5].

Induction of DNA damage and carcinogenesis via different pathways is one of the main characteristics of heavy metals [6, 7]. Heavy metals may act as genotoxins and they can cause single or double-strand DNA breaks (SSBs and DSBs, respectively) causing damage in DNA [8]. Heavy metal exposure may lead to genotoxicity via different modes of action. They cause oxidative stress by elevating the levels of production of reactive oxygen species (ROS), change cellular events that control repair mechanisms and alter the DSB repair mechanisms. They promote "false" DSB repairing and propagate DNA mutations, which in turn elevate the chance of carcinogenesis [9]. Induction of genetic damage driven by heavy metals occurs in several pathways. These include increased micronuclei formation, chromosomal aberrations, and anomalies at molecular DNA level. The type of DNA damage is subject to change depending on the chemical identity of the heavy metal exposed. The application of genotoxicity assays allows quantification of the effects of heavy metals. These assays include Alkaline Comet Assay (AC), Cytokinesis-blocked Micronucleus Assay (CBMN), Chromosomal Aberration Assay (CA), and Sister Chromatid Exchange Assay (SCE).

The island of Cyprus located in the Eastern Basin of the Mediterranean Sea. Cyprus is naturally rich in terms of heavy metals as evidenced by its name originating from copper ores. The island has been given several names through the time such as *Kryptos*, *Kypros*, and adjectives like *chalkoessa* (because of the copper veins)[10]. It is estimated that the history of mining started around 4000 Before Common Era (BCE) in Cyprus. In 1914, Cyprus Mines Corporation (CMC) was founded. CMC performed mining activities until the early 1970s. In 1974 following the conflict in Cyprus, mining operations in Gemikonagi region were abandoned completely [11]. Since then, the site has been left to its fate without adequate governmental oversight. The passage of time has deteriorated the site structures and resulted in the release of metals and ores into the environment. Gemikonagi was selected due to its proximity to the suspected heavy metal contamination, whereas Tepebasi was selected as a control area due to its relative isolation as well as a lack of mining operations (Fig. 1).

The aim of this study was to investigate heavy metal levels using soil, and human blood samples from the area of Gemikonagi, Lefke where the old mining area is located in immediate vicinity and compare these results with a control area, Tepebasi, Girne located 40 km away from the mining site and devoid of any mining activities, in addition to an investigation of any possible genotoxicity using human blood samples. During the study, inductively coupled plasma mass spectrometry (ICP-MS) was used to evaluate heavy metal levels and genotoxicity analysis was conducted by in vivo micronucleus (MN) and AC assays.

Materials and Methods

Ethical Statement

Prior to this study, the Ministry of Health of Turkish Republic of Northern Cyprus approved and accepted the study for ethical considerations. Ethics approval for the study was also obtained from The NEU Joint-Committee of the Research and Ethics Committee (YDU/2017/46-399). The study was conducted with respect to the "Guideline for Good Clinical Practice" published on 23 July 2015 by European Medicines Agency. After Informed Consent Forms were signed by the participants, they were asked to fill out study questionnaires. The questionnaire form was composed of 2 sections, a demographic section (12 questions) and a study-oriented section (16 questions).

The exclusion criteria were as follows: being under 18 or above 65 years old, living outside of the selected regions, living in the selected regions for less than a year, being diagnosed with any type of cancer or having received any type of cancer treatment.

Sample Collection, Storage, and Pre-treatment

Soil Samples

A total of 10 soil samples (from 15 cm depth, as per the method by Baycu et al. and Lee et al. [12, 13]) were collected in November 2017, following the first seasonal rainfalls of Cyprus and subsequent cultivation of agricultural plants in the region, simultaneous with the collection of blood samples from human participants [14]. The coordinates of the sampling sites are given in Table 1. The collection was held by a pre-sanitized, PTFE-coated large spoon and collected samples were transferred to a sample collection bag (500 g). After the evacuation of air, the bag was sealed. The samples were immediately transferred to the Near East University Toxicology Laboratory, sieved with a fine sieve to exclude stones, pebbles, and large chunks of soil and refrigerated at 4 °C to minimize microbiological contamination and environmental exposure until analysis.

Fig. 1 Locations of Gemikonagi and Tepebasi (left) and a view of the mining site (right)

Immediately before the analysis, the soil samples were dried in a dust-free environment at room temperature for 72 h and ground to ensure fine particle size. A mass of 0.25 g of each sample was weighed and 5 mL of 65% nitric acid was added. The samples were heated to 180 °C to near dryness in a fume cupboard. Acid addition and heating processes were repeated two more times [15]. Deionized water was added to the residue. The acid-insoluble suspended material was filtered (Whatman Filter Merck, 0.45 μm) and deionized water was added up to a final volume 50 mL. The analysis was carried out using ICP-MS 7500ce (Tokyo, Japan) housed within the Advanced Technology Education Research and Application Centre Laboratory of Mersin University, Turkey [1].

Blood Samples

Blood samples were obtained from the volunteers residing in Gemikonagi (35° 08′ 28.8″ N 32° 49′ 59.8″ E) and Tepebasi (35° 18′ 22.2″ N 33° 03′ 17.6″ E) located in Northern Cyprus. A volume of 5.0 mL of human peripheral blood sample was collected using heparinized vacuum tubes from 60 individuals aged between 18 and 65. Blood was collected only from the participants that signed the informed consent form and filled the questionnaire.

Heavy Metal Analysis

The method established by Alkas et al. [1] was utilized with minor modifications [16] (Table 2). The ICP-MS instrument used was an Agilent 7500ce Octupole Reaction System with 99.99% helium from Agilent Technologies (Tokyo, Japan). The operating conditions were as follows: nebulizer type, concentric nebulizer; nebulizer gas (argon) flow rate, 0.9 L/min; auxiliary gas (argon) flow rate, 0.14 L/min; plasma gas (argon) flow rate, 15 L/min; reaction gas (helium) flow rate, 0.14 L/min; spray chamber (S/C) temperature, 2 °C; and ICP RF power, 1500 W. The argon gas utilized was of spectral purity (99.998%).

Genotoxicity Analyses

In order to isolate blood lymphocytes, 1 mL of the blood was resuspended in a 15 mL centrifuge tube with 5 mL of phosphate buffered saline (PBS) solution, followed by the application of 1 mL Histopaque 1077 and centrifugation at 1640 rpm, 250*g* for 5 min at 4 °C. A total of 1 mL of the peripheral blood lymphocytes was then collected and kept at 4 °C for 30 min.

Table 1 Locations of the soil sampling sites in Cyprus

	Gemikonagi (coordinates)	Tepebasi (coordinates)
Sampling site 1	35° 13′ 66.8″ N 32° 83′ 49.13″ E	35° 30′ 33.26″ N 33° 05′ 74.08″ E
Sampling site 2	35° 13′ 89.27″ N 32° 83′ 12.63″ E	35° 30′ 42.73″ N 33° 06′ 54.84″ E
Sampling site 3	35° 14′ 30.49″ N 32° 81′ 29.42″ E	35° 30′ 93.65″ N 33° 06′ 15.54″ E
Sampling site 4	35° 13′ 52.06″ N 32° 83′ 25.25″ E	35° 31′ 05.31″ N 33° 04′ 98.86″ E
Sampling site 5	35° 14′ 35.75″ N 32° 84′ 04.65″ E	35° 29′ 89.40″ N 33° 04′ 56.95″ E

Table 2 Validation parameters of the inductively coupled plasma mass spectrometry method

	Cr	Mn	Ni	Cu	Cd	Pb	As
Calibration range (ng/mL)	0–50	0–50	0–50	0–50	0–50	0–50	0–50
Determination coefficient (R^2)	0.9998	0.9997	0.9999	1	1	0.9999	1
Recovery (%)	91	99	89	95	110	93	94
RSD (%) (n = 10)	6.9	7.8	10.2	9.9	10.1	11.9	12.4
LOD (μg/kg)	0.08	0.11	0.06	0.03	0.15	0.12	0.06
LOQ (μg/kg)	0.005	0.005	0.007	0.001	0.008	0.006	0.004

RSD relative standard deviation, *LOD* limit of detection, *LOQ* limit of quantitation

In Vivo Micronucleus Assay

In order to perform micronucleus test, 100 μL of isolated lymphocytes was transferred to slides and allowed to air-dry for 30 min, then fixed for 5 min with absolute ethanol. Cells were stained with Giemsa (5%) (Merck, Germany) for 5 min [17]. After staining, slides were analyzed in trans illuminating stereomicroscope (Olympus CX51), observing the frequency of micronucleated lymphocytes in a total of 1200 cells per patient.

In Vivo Alkaline Comet Assay

Isolated lymphocytes were mixed with equal volume of low-melting agar (LMA) (Sigma-Aldrich, USA) and 100 μL of LMA-lymphocyte suspension each was transferred and spread onto 3 slides. The slides were then refrigerated at 4 °C for 30 min for the agar to solidify. Then, a second coating of 100 μL LMA was added and spread to space the lymphocytes away from the surface of the slide. Following solidification, the sample lymphocytes were lysed with 10% DMSO and 1% Triton X-100 in stock lysis (14.6% NaCl, 3.7% Na_2-EDTA and 0.1% Tris-HCl, pH 10 with HCl). The samples were then alkalinized for 40 min and electrophoresed for 30 min in electrophoresis buffer (30 mL 10 M NaOH and 5.4 mL 0.2 M Na_2-EDTA in 1.0 L of distilled water). Following electrophoresis, the samples were neutralized with 0.4 M Tris-HCl solution (pH 7.5) for 15 min (5 min, thrice), fixed with 50%, 75% and absolute ethanol successively and stained with 50 μL of 0.1 mg/mL ethidium bromide solution and observed under epifluorescence.

Statistical Analyses

Statistical analysis was carried out using IBM SPSS Statistics (IBM SPSS Statistics 21. SPSS Inc., an IBM Ca. Somers, NY). During the analysis of the data, Levene's test is used to calculate normal distribution. For normally distributed data, t test for independent samples was applied. For the non-parametric data, Mann-Whitney U test was applied. Statistical significance was accepted at p value < 0.05 level.

Results

The demographical characteristics of the sample population are given in Table 3. These results indicate no significant differences in the age and gender distribution of the participants. Similarly, hormone therapy and/or radiography do not seem to produce statistically significant differences in distribution among the groups.

Heavy Metal Analysis of Soil Samples

The results obtained for soil heavy metal levels in the sampled regions exhibited excessive metal concentrations, above established limits, in Gemikonagi soils, except iron, manganese and nickel. In contrast, in Tepebasi soils, iron, nickel, and manganese were present below their limits, with all other elements concentrated above their respective limits. The copper concentration in Gemikonagi of 3349 mg/kg is greatly more than that of Tepebasi with 221.6 mg/kg (Table 4).

Heavy Metal Analysis of Blood Samples

As demonstrated in Table 5, iron and copper levels were within acceptable limits in either region, whereas the other elements were above acceptable limits in either region. The levels of arsenic, copper, iron, nickel, and manganese were found to be in higher concentrations in the residents of Gemikonagi with statistical significance, compared to the control region of Tepebasi (p value < 0.05). The results indicate that the arsenic levels in the populations sampled are significantly higher than the reference utilized in this study, 0.012 mg/mL as established by Mayo Clinic in 2017[31]. There also exists a significant difference (p value = 0.021), with Gemikonagi being higher. There exists a higher blood copper concentration in Gemikonagi than Tepebasi with a significant statistical difference (p value = 0.016). Similarly, the iron

Table 3 Demographical characteristics of the sample populations

Category		Gemikonagi (n = 30)	Tepebasi (n = 30)
Mean age ± SD		41.67 ± 13.62	42.03 ± 14.13
Gender	Female	13	19
	Male	17	11
	Total	30	30
Smoking status	Smoker	9	7
	Non-smoker	15	19
	Quit smoking	6	4
Radiography	Yes	6	12
	No	24	18
Diabetes (types I and II)	Yes	4	7
	No	26	23
Familial cancer	Yes	4	16
	No	26	14
Hormone therapy	Yes	2	4
	No	28	26

levels of Gemikonagi population are significantly higher (p value = 0.011) than Tepebasi population. In Tepebasi, the average concentration of nickel obtained was 0.37 μg/L, but the levels in Gemikonagi were much greater, at 132.15 μg/L.

Alkaline Comet Assay

The data obtained from the in vivo comet assay were evaluated using the parameters of the tail intensity, tail length and tail moment of the ethidium bromide-stained cells investigated under fluorescence microscope (Fig. 2). Upon the evaluation of the DNA damage in the chronically heavy metal-exposed individuals with in vivo comet assay in terms of tail length, even it was observed that the median tail length was higher in Tepebasi residents than that of Gemikonagi residents, there was no statistically significant difference (p value = 0.448). When DNA damage was compared from the point of tail intensity, it was observed that tail intensities' median values did not show a significant difference (p value = 0.258). Nevertheless, Gemikonagi residents' maximum tail intensity value was significantly higher than Tepebasi residents' (p value = 0.024). According to the statistical evaluation conducted on tail moment, no significant difference was found between two groups (p value = 0.602) (Table 6).

In Vivo Micronucleus Assay

Individuals who are chronically exposed to heavy metals showed an increase in MN frequency compared to control group. MN frequency of Gemikonagi residents was significantly higher that the MN frequency of Tepebasi residents (Table 6).

Table 4 Heavy metal levels by region in soil

Descriptive statistics	Gemikonagi (n = 5)		Tepebasi (n = 5)		Accepted limits	
	Mean (mg/kg)*	Std. deviation	Mean (mg/kg)*	Std. deviation	Accepted limits (mg/kg)	Reference
Arsenic levels	138	49.8	273	52.4	0.39	[18]
Chromium levels	749	150.2	1342	99.6	37	[19]
Copper levels	3349	1559.4	222	41.8	50	[20]
Iron levels	557,219	330,830.4	213,994	13,935.6	5.5×10^5	[21]
Nickel levels	158	102.2	684	260.1	1000	[22]
Vanadium levels	1067	278.1	832	145.2	310	[23]
Manganese levels	4074	2378.0	4891	1846.3	330	[24]

*Rounded to the nearest integer

Table 5 Heavy metal levels by region in human blood

Descriptive statistics	Gemikonagi (n = 30)		Tepebasi (n = 30)		Significance (p value)	Accepted Limits (mg/mL)	Reference
	Mean (mg/mL)[c]	Std. deviation[c]	Mean (mg/mL)[c]	Std. deviation [c]			
Arsenic levels[a]	0.2	0.1	0.2	0.2	0.021[b]	0.012	[25]
Chromium levels	0.004	0.004	0.003	0.001	0.257	1.4	[26]
Copper levels[a]	0.8	0.3	0.6	0.2	0.016[b]	1.4	[27]
Iron levels[a]	521.9	238.7	423.6	82.5	0.011 [b]	600	[28]
Nickel levels[a]	0.1	0.6	0.001	0.003	0.017 [b]	2×10^{-4}	[29]
Vanadium levels	0	0.3	0.7	0.3	0.058	5×10^{-5}	[30]
Manganese levels	0.04	0.01	0.02	0.02	0.0001 [b]	1.5×10^{-2}	[30]

[a] Due to non-normal distribution, Mann-Whitney U test was applied instead of t test for independent samples

[b] Statistically significant difference between groups

[c] Rounded to 1 s.f.

Correlation Analyses

In Gemikonagi, correlation analysis suggests that there exists a significant positive correlation between participant arsenic levels and the tail intensity max parameter (p value = 0.04). Similarly, copper levels in blood were associated with increases in tail length min, tail length max, tail moment max, and tail moment median parameters, with significance at p value = 0.012, p value = 0.0001, p value = 0.01, and p value = 0.0001, respectively. Iron levels produced a complex relationship with genotoxicity profiles, with significant increase in micronuclei frequency (p value = 0.045) but produced a significant reduction in tail length min (p value = 0.017). In addition, nickel levels also produced a significant (p value = 0.037) increase in the tail length median parameter. In Tepebasi, the abovementioned significances in Gemikonagi failed to produce p values less than 0.05, therefore were deemed insignificant (Table 7).

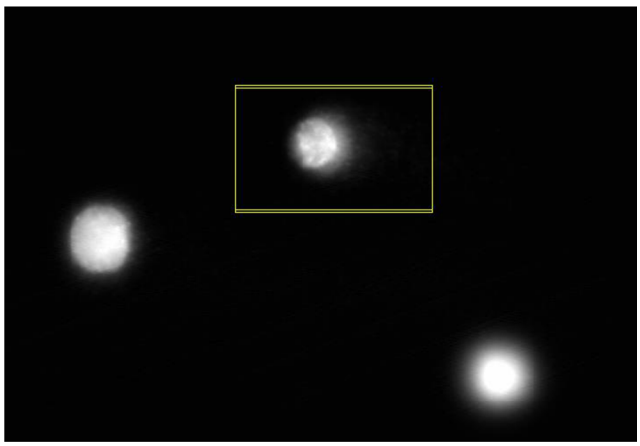

Fig. 2 Comet images from cells obtained from the residents of Gemikonagi using Comet Assay III image analysis system (Perceptive Instruments, Steeple Bumpstead, UK)

There is no statistical difference of heavy metal accumulation between genders (p value > 0.005 for each metal). However, in Gemikonagi region, tail intensity maximum parameter is significantly higher in women (p value = 0.044) and tail intensity minimum parameter is higher in men in Tepebasi (p value = 0.031). There is no statistically significant difference in other genotoxicity parameters between genders.

To conduct multiple regression analysis, As, Ni, V, and Mn were considered to be independent variables and they were compared with dependent variables tail intensity maximum and micronucleus frequency for each group separately. Only As levels of Gemikonagi region was found to be correlated with the tail intensity maximum.

Discussion

Environmental exposure to carcinogenic metals and metalloids has long been a major concern in the collective consciousness of the healthcare system in Northern Cyprus. The abandonment of the mine, and its subsequent dereliction, has allowed a massive amount of these elements to penetrate deep and wide into the surrounding environment, consorted by potential exposure to these dangerous chemical species and consequent severe health problems.

In the light of previous studies, it is known that heavy metal exposure induces DNA damage and carcinogenesis via several modes of actions [9]. Heavy metal exposure may elevate the production of ROS causing oxidative stress, negatively affect the DNA repair mechanism and favor “false” repairing of DSBs [32–34]. With respect to this expertise, it is important to investigate blood and soil heavy metal levels and correlate this data with genotoxicity assessments in mining areas. There are vast numbers of techniques used to detect metal levels and

Table 6 In vivo micronucleus assay and in vivo comet assay results for the populations sampled

Genotoxicity analysis	Parameter measured	Gemikonagi (n = 30)		Tepebasi (control) (n = 30)		Significance (p value)
		Mean	SD	Mean	SD	
Micronucleus assay	MN frequency	279.5	52.4	164.1	51.9	0.0001*
Alkaline comet assay	TL min.	17.3	4.5	16.8	6.5	0.859
	TL max.	73.1	17.7	87.0	33.6	0.209
	TL median	32.5	5.2	34.1	9.7	0.448
	TI min.	0.0	0.0	0.0	0.0	1.000
	TI max.	47.4	10.7	39.6	15.0	0.024*
	TI median	3.2	9.1	1.3	1.4	0.258
	TM min.	0.1	0.7	0.0	0.0	0.317
	TM max.	12.1	3.0	11.9	7.1	0.132
	TM median	0.3	0.2	0.3	0.4	0.602

TL tail length, *TI* tail intensity, *TM* tail moment

*Statistically significant difference between groups by t test for independent samples

assess genotoxicity. In this study, heavy metal analyses were conducted using ICP-MS, which is one of the most technologically advanced and sensitive devices. On the other hand, alkaline comet assay and MN were used to assess genotoxicity. The comet assay was preferred due to its capability of detecting overall damage and as well as primary molecular DNA damage. Furthermore, in vivo micronucleus assay is one of the pioneers of clastogen and/or aneugen screening testing. An increased micronuclei frequency indicates the induction of chromosomal damage [19].

In the present study, 5 soil samples from each region were collected to conduct heavy metal analysis. To assess heavy metal induced genotoxicity and the heavy metal levels, a total of 60 blood samples were collected from Gemikonagi (n = 30) and Tepebasi, control (n = 30).

The soil samples obtained from each region represent a stark contrast to each other in pertinence to each element sampled. The most striking result was that there existed significantly higher soil arsenic and chromium concentrations in Tepebasi than in Gemikonagi. While exact explanations for the observed results will likely prove to be multifactorial, the

Table 7 Correlation analysis between blood heavy metal levels and genotoxicity parameters

	As		Cr		Cu		Fe		Ni		Mn		V	
	p value[1]	p value[2]	p value[1]	p value[2]	p value[1]	p value[2]	p value[1]	p value[2]	p value[1]	p value[2]	p value[1]	p value[2]	p value[1]	p value[2]
MN frequency	0.751	0.352	0.268	0.310	0.643	0.542	0.045*	0.608	0.235	0.070	0.036*	0.751	0.108	0.820
TL min.	0.337	0.331	0.0001*	0.546	0.012*	0.794	0.017*	0.075	0.081	0.551	0.337	0.878	0.004*	0.324
TL max.	0.093	0.616	0.495	0.909	0.0001*	0.827	0.765	0.743	0.114	0.700	0.819	0.093	0.558	0.675
TL median	0.208	0.751	0.012	0.853	N/A	0.092	0.674	0.541	0.037*	0.787	0.558	0.208	0.014*	0.302
TI min.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
TI max.	0.934	0.761	0.865	0.394	0.193	0.486	0.229	0.232	0.908	0.172	0.519	0.934	0.816	0.764
TI median	0.035*	0.332	0.335	0.275	0.895	0.154	0.470	0.894	0.858	0.438	0.346	0.035*	0.425	0.346
TM min.	N/A	N/A	0.459	N/A	0.732	N/A	0.781	N/A	0.825	N/A	0.828	N/A	0.388	N/A
TM max.	0.181	0.966	0.654	0.591	0.010*	0.659	0.457	0.363	0.534	0.453	0.615	0.181	0.628	0.591
TM median	0.318	0.977	0.187	0.646	0.0001*	0.153	0.511	0.966	0.122	0.557	0.403	0.318	0.085	0.591

[1] Gemikonagi p values

[2] Tepebasi p values

*Statistically significant (p value < 0.05)

N/A statistical analysis is not applicable

geomorphology of the region is likely to account for at least a significant role in the development of such discrepancies. As expected, the copper concentration in Gemikonagi is greatly more than that of Tepebasi. This discrepancy is expected due to the presence of a defunct copper mine in close vicinity to Gemikonagi, as well as the presence of unused copper ore pools in close vicinity to the town, some of which were known to have leaked or are currently leaking. However, it stands to interest that both regions yielded values greater than the soil copper limits of 50 mg/kg as established by the ATSDR[35]. Besides the obtained results, a study conducted by Barkett and Akun in 2018, showed 56% of considerable potential ecological risk in a town called Yedidalga, which is located immediate vicinity to Gemikonagi [17]. All tested elements in soil except iron and nickel exceeded the guideline limits established by reputable organizations. This result indicates that, working under the assumption that Tepebasi is an uncontaminated region, vast stretches of North Cyprus bedrocks and/or soils may be naturally enriched in chalcophile and siderophile elements and these large degrees of enrichment may not be isolated to solely the Gemikonagi region.

The results indicate that the arsenic levels in the populations sampled are significantly higher than the reference utilized in this study, 0.012 mg/mL as established by Mayo Clinic in 2017 [36]. Similarly, there exists a higher blood copper concentration in Gemikonagi than Tepebasi with a significant statistical difference. It must be noted, however, that neither result is higher than the limit of 1.4 mg/mL established by URMC Rochester in 2017 [37]. Similarly, the iron levels of Gemikonagi population are significantly higher than Tepebasi population. Iron occupies a special position in blood analysis, as the natural iron content of human blood is extremely high due to the high prevalence in blood of hemoglobin, each molecule of which bears 4 Fe^{+2} ions. Therefore, the iron levels in blood are below limit for either sample population. Blood nickel limit is established by ATSDR as 0.2 μg/L [38]. As a result, the blood nickel levels exceed the allowed limits in either population. The results obtained in this study indicate that there exists a significant enrichment of several elements in the Gemikonagi population. Such elements include arsenic, copper, iron, and nickel. Gemikonagi and its associated mines have been important loci of mining on the Island of Cyprus since prehistory. The extracted ores in these mines have primarily been chalcopyrite, the chief copper ore, and an important iron ore. The results thus obtained are therefore expected, as nickel is a siderophile element, thus has an affinity to copper. Similarly, arsenic is a chalcophile element, and also has a high affinity to copper. This provides a natural explanation for the detection of these elements together, although only arsenic is truly above the limits established by a credible health regulatory agency.

In genotoxicity, there was observed a significant (p value = 0.0001) increase in micronuclei frequency in Gemikonagi as opposed to Tepebasi, as well as a significant increase in Maximal Tail Intensities between the two populations. This suggests that more aneugenic damage is inflicted upon the peripheral blood lymphocytes of the Gemikonagi population, as well as to suggest that the individuals expressing the greatest amount of direct DNA damage (as expressed in the tail intensity max value) develop more extensive DNA damage compared to Tepebasi population.

The results obtained from arsenic levels and genotoxicity results suggest that arsenic does not affect every participant in the same magnitude. This suggests that there exist important personal determinants that can impact the manifestation of arsenic-induced genotoxicity. Those that have the greatest capability to mitigate arsenic-induced genotoxicity do not develop appreciable levels of DNA damage, thus representing the constant tail intensity min, but the more susceptible members develop increased genotoxicity, thus producing a higher tail intensity max. Copper, on the other hand, produced marked increases in 4 different metrics of genotoxicity, including minima and maxima, suggesting that all participants in the Gemikonagi group were affected roughly similarly by copper exposure, despite the copper level being less than the limits established by reputable organizations. However, a lower level of copper does not produce any genotoxic symptoms in Tepebasi. The authors suggest that copper is biopotentiated by the presence of another element, specifically nickel. There exists previous literature to suggest toxic synergism between Cu and Ni [39] and the nickel levels are quite different between the two sampled regions, lending further support to the suggestion. Nickel is a known genotoxin with multiple pathways on its own [40].

The metals and metalloids analyzed in this study are known to have detrimental health effects in high concentrations [41], where such effects may include genotoxicity secondary to what is usually increased oxidative stress. Hydroxyl radicals have been touted as the primary effectors of molecular-level damage to both naked and intracellular DNA. It was observed that metals such as iron and copper are indispensable in the translation of oxidative stress exemplified by superoxide (O_2^-) into actual DNA damage through molecular damage mechanisms associated with hydroxyl radical (OH^-). Thus, metals such as iron and copper can be said to induce an amplification of molecular insults inflicted upon DNA by oxidative stresses, yielding major increases in the extent of damage to DNA. To further this point, iron has been found to be strongly associated with the development of multiple cancers, most profoundly colorectal cancer [41]. In addition, iron and copper, in particular, are thought to have synergistic and amplificative

effects on the oxidative stress generated by each other [42]. Iron or copper overload is a known inducer of mitochondrial metabolic disfunction. Copper, for example, is known to reduce mRNA and protein expression in cells [13, 43]. In addition, reduced mitochondrial function was observed upon exposure to copper as well as a reduction in metabolism in the affected cells.

Conclusion

In short, in this study, a causal relationship between heavy metal exposure and resultant genotoxic effects have been evaluated in Cyprus within a unified framework in the light of the previous studies investigating heavy metal accumulation in the afflicted areas [44].

The results obtained in this study revealed a generalized enrichment of heavy metals in the soils of both sampled regions. This indicates that, in addition to the release of heavy metal content by the mine, there exists a relatively high level of heavy metals in North Cyprus, even in areas considered to be industrially pristine. This in turn indicates that there also exist mechanisms independent of industry that generate and preserve an elevated heavy metal concentration in the soil, such as the geochemical makeup of the bedrocks that constitute the landmass of North Cyprus.

If the geology of the Island is indeed responsible, at least partially, for the elevated heavy metal levels, then this suggests that the issue of heavy element contamination of the soil is not a problem isolated to the region of Gemikonagi, but may instead be affecting the entirety, or at least a significant proportion of the Island. The geology of the Island being a significant contributor to the soil heavy metal inventory is corroborated by a number of previously conducted studies, which have uncovered an enrichment in the soils in locations all over the Island [1, 13, 35, 45, 46]. The results indicate that a general enrichment in heavy elements is not endemic to Gemikonagi, but a problem that might be generalized to the entirety of Cyprus.

Nevertheless, the study had some limitations. The study was designed as a pilot study to enlighten future studies. Thus, the number of the participants should be increased in future studies. Furthermore, the number of the soil samples and sampling sites should be increased in the future. Consequently, this study will be a framework for future studies that will concentrate on a causal relationship between heavy metals and their possible genotoxic, therefore potentially carcinogenic risk.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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