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שם המוסד המחלקה והמוסד המגישים:

פקולטה למדעי הבריאות, אוניברסיטת בן-גוריון בנגב

כותרת המחקר בעברית:

ניטור ביולוגי אנושי במפרץ חיפה בהשוואה לאוכלוסייה הכללית ישראל: סיקור ארצי בקרב תורמי דם

כותרת המחקר באנגלית:
Human Biologic Monitoring in the Haifa Bay area compared to the general population: screening within blood donors

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מוגש למדען הראשי
המשרד להגנת הסביבה

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רקע ומטרות

במהלך העשור האחרון דווח על עודף בשיעורי תחלואה בקרב תושבי חיפה בהשוואה לשאר הארץ, כאשר הפער בתחלואה יוחס לזיהום אוויר מתעשייה מקומיות. מטרתו של המחקר הנוכחי הינו (1) להשוות ריכוזים של מתכות כבדות בקרב תורמי דם המתגוררים במפרץ חיפה עם תורמים מאזורים אחרים בישראל ו-(2) להעריך את הקשר בין ריכוזי המתכות לחשיפות הסביבה.

שיטות

המחקר תוכנן על בסיס שירותי הדם של מגן דוד אדום (מד״א), המספקים מנות דם לכלל אוכלוסיית ישראל. אוכלוסיית המחקר כללה מדגם אקראי של תורמי דם התורמים דם בכל המקומות בארץ, בשתי שכבות עיקריות: תורמים מאזור מפרץ חיפה ותורים מאזורים אחרים בארץ. הדגימה בוצעה במטה שירותי הדם של מד״א בתל-השומר. נאספו דגימות הדם המלא ונבדקו במעבדה הלאומית לבריאות הציבור. כתובות המגורים של התורמים ומיקומי אתרי התרומות אוחדו עם רמות המזהמים באוויר שאר נרשמו על-ידי תחנות הניטור הסמוכות. המזהמים כללו חנקן דו חמצני (NO2), דו תחמוצת הגופרית (SO2), אוזון (O3), חד תחמוצת הפחמן (CO) וחלקיקים בגודל פחות מ-10 ו-2.5 מ״מ בקוטר (PM10 ו-PM2.5).

את הקשרים חד משתניים בין נתוני מעבדה ונתוני זיהום אוויר אמדנו בעזרת מתאם ספירמן. ההשוואה החד משתנית של ריכוזי מתכת בדם של תורמים בין אזורים שונים בארץ נעשתה בעזרת ratio t-test. כמו כן, במטרה לתקנן את ההשוואה, השתמשנו ברגרסיה לוג-נורמלית, ובעזרתה תקננו לגיל, מין ועישון (עישון הוגדר על ידי קדמיום>1.0 ppb). השתמשנו בשיטה של רגרסיה לוג-נורמלית גם לזיהוי קשרים בין גורמים סביבתיים לבין רמות מתכות בדם תוך תקנון לגיל, מין ועישון. חשוב לציין, שעדות על קשר שנובע ממחקר הנוכחי, מצביע רק על קשר סטטיסטי ולא על קשר סיבתי.

תוצאות

במהלך מרס 2020 - פברואר 2022, אספנו 6230 דגימות ומתוך אלו, 911 דגימות נבדקו עבור 4 המתכות (ארסן (As), קדמיום (Cd), כרום (Cr) ועופרת (Pb)). ריכוזי המתכות היו דומים לאוכלוסייה הכללית באירופה, ובעלת ממוצעים גיאומטריים ו-95% CI כדלקמן:

As(μg/L): 0.53 (0.50; 0.57), Cd(μg/L): 0.22 (0.21; 0.24) , Cr(μg/L): 0.98 (0.95; 1.02), Pb(μg/dL): 6.68 .(7.00 ; 6.37)

ריכוזי רוב המתכות השתנו עם גיל, מגדר ועישון. תורמי ממפרץ חיפה היו יותר מבוגרים משאר התורמים בארץ.

לתורמים המתגוררים באזור מפרץ חיפה היו רמות נמוכות יותר של As (p-value<0.001) ו--Cd (p-value=0.029) בהשוואה לשאר התורמים בישראל, ורמות גבוהות יותר של Cr ו- Pb.

הריכוזים של המתכות האלו היו גבוהים פי 1.08-1.10 (תוספת סיכון של 8%-10%) בקרב תושבי מפרץ חיפה מאשר בשאר הארץ (עם מובהקות גבולית של 0.067 עבור Cr). Cr ו-Pb היו גבוהים פי 1.13 ו-1.15 (תוספת סיכון של 13%-15%) עבור אלו שתרמו דם באזור מפרץ חיפה, אך לא בהכרח התגוררו באזור (שניהם עם ערכי מובהקות שווים ל-0.004). כל הממצאים מהווים תוצאות הניתוחים המתוקננים לגיל, מגדר ועישון.

במטרה לבודד מקורות אפשריים לריכוזי מתכת בדם, השתמשנו ברגרסיה שבעזרתה ניבינו את ריכוזי המתכת (משתנה תלוי) בעזרת רמות מזהמי אוויר בסיבת מגורים או אתר התרמה של תורמי דם (כגורמים בלתי תלויים) ותוף תקנון לגיל, מין ועישון. תוצאות הניתוח הצביעו על קשרים סטטיסטיים בלבד ולא קשרים סיבתיים. להלן התוצאות של הניתוח הזה:

- ריכוזי As היו במתאם חיובי עם NO_2, (p-value<0.001) Pb, היה קשור עם PM_{10}, CO ו-SO2 (ערכי מובהקות 0.001>, 0.013 ו-0.001>, בהתאמה).
- קרבה למחצבות נמצאה קשורה לריכוזי Pb בדם (p-value=0.014).

מסקנות

תורמי דם מאזור מפרץ חיפה מאופיינים ברמות נמוכות של As ו-Cd, וברמות גבוהות של Cr ו- Pb, בהשוואה לתורמים משאר הארץ. תורמים עם ריכוזי Pb גבוהים נוטים להתגורר קרוב יותר למחצבות ולהיות חשופים לרמות גבוהות יותר של PM_{10}, CO ו-SO_2. באופן כללי, רמות הזיהום אוויר נמצאו קשורות לריכוזי המתכות בדם.

המלצות להמשך המחקר

לאור ממצאי המחקר הנוכחי יש חשיבות רבה בחקירה מקיפה של מקורות חשיפה אפשריות כמו תעשיות ומחצבות במפרץ חיפה ובאזורים נוספים בארץ המתאפיינים ברמות גבוהות של מתכות. הניתוח הזה מתוכנן על ידי החוקרים בהמשך העבודה.

בנוסף, נראה כי יש צורך בבדיקת יתר הדגימות שרק נאספו אך לא נבדקו בגלל מגבלות התקציב. בדיקות נוספות יוסיפו עוצמה סטטיסטית ופירוט לממצאים אשר התקבלו עד כה ויעזרו לאתר מקורות אפשריים לחשיפה.

כמו כן, יש לבחון קשר עם מדדי תחלואה באזור מפרץ חיפה , במיוחד הקשורים לחשיפה ל-Cr ו- Pb.

Abstract in English

Background and objectives. During the last decade, higher morbidity rates have been reported among Haifa residents as compared to the rest of the country, possibly contributed by air pollution from local industries. This study was aimed (1) to compare concentrations of heavy metals in blood among blood donors residing in Haifa Bay with donors from other regions in Israel and (2) to estimate the association between the metals' concentrations and ambient exposures.

Methods. The study design was developed on the platform of the Magen David Adom (MDA) Blood Services, providing a supply of blood units to the entire population of Israel. The study population comprised a random sample of blood donors donating blood at all locations in the country, stratified by their location, i.e., from the Haifa Bay or non-Haifa Bay area. The sampling was performed at the headquarters of the MDA Blood Services. The samples of whole blood were tested at the National Laboratory of Public Health. The donors' residential addresses and donations sites' locations were geocoded and merged with the levels of pollutants recorded by the nearby monitoring stations. Pollutants included nitrogen dioxide (NO_2), sulfate dioxide (SO_2), ozone (O_3), carbon monoxide (CO) and particulate matter of size <10 and 2.5mm in diameter (PM_{10} & $PM_{2.5}$).

We used Spearman correlation coefficient to estimate the correlation between metals' concentration and ambient pollutants. Metals' concentrations were statistically compared between geographical areas in Israel using a ratio t-test. We used a log-normal regression, to adjust to age, gender and smoking (defined by Cadmium>1.0µg/L). The same approach of log-normal regression was used for estimating the independent contribution of environmental pollution to the variation of metals' concentrations, after adjustment to age, gender and smoking. Important to note that all associations established in the current analyses are statistical, and do not indicate a causality between the analyzed factors.

Results. During Mar 2020 - Feb 2022, we collected 6230 samples and out of these, 911 samples have been tested for the 4 metals (arsenic (As), cadmium (Cd), chromium (Cr) and lead (Pb)). The metals' concentrations were comparable to the general population in Europe, with geometric means and 95%CI as follows: As(µg/L): 0.53 (0.50; 0.57), Cd(µg/L): 0.22 (0.21; 0.24), Cr(µg/L): 0.98 (0.95; 1.02), Pb(µg/dL): 6.68 (6.37; 7.00). Age, gender and smoking modified concentrations of most of the metals.

Donors residing in the Haifa Bay area had lower levels of As (p-value<0.001) and Cd (p-value=0.029) as compared to the rest of the donors in Israel, and higher levels of Cr and Pb. These metals appeared to be 1.08-1.10 times higher among Haifa Bay residents than in the rest of the country (with borderline significance of 0.067 for Cr). Cr and Pb were 1.13 and 1.15 times higher for those who donated blood in the Haifa Bay region, but not necessarily resided in the area (both p-values equal 0.004). All analyses were adjusted to age, sex and smoking.

To estimate the possible contribution of environmental pollution to metals' concentrations in blood, we regressed the metal concentrations in blood (dependent variable) over each of the environmental factors (independent variable) while adjusting all associations to age, gender and smoking. The findings indicated only the statistical associations, and cannot be interpreted as causal. The following are the findings from this analysis:

- As concentrations were positively associated with NO_2 (p-value<0.001) and Pb - with PM_{10}, CO and SO_2 (p-values, <0.001, 0.013 and <0.001, respectively).
- Proximity to quarries was associated with Pb concentrations in blood (p-value= 0.014).

Conclusions. Blood donors from Haifa Bay area are featured by low levels of As and Cd, and by high levels of Cr and Pb, as compared to the rest of the country. Donors with high Pb concentrations are likely to live close to quarries and be exposed to higher levels of PM_{10}, CO and SO_2. An association between ambient levels of pollution and internal metals' concentrations, reaffirms the link between the two in the pathological pathway from air pollution to morbidity.

Recommended research in future. An extensive investigation of industries in Haifa Bay and other areas in the country featured by high levels of metals is warranted and will be conducted by the study researchers. Additionally, testing of the remaining samples collected in the study seems to be of highest importance and potential to reveal possible sources of exposure and prompt their elimination in future. Association of morbidity rates with Cr and Pb exposure in Haifa Bay area, should be explored.

Scientific Background

During the last decade, investigators have reported increased rates of cancer, respiratory illnesses and adverse birth outcome among Haifa residents [1-3]. In 2015, following multiple reports on the excessive morbidity levels in Haifa Bay area, the Ministry of Health issued a position paper warranting mitigation of air pollution levels in the region and investigation of the impact of environment on health at individual level of residents[4]. The latter was recommended to perform with individually verified exposures, as opposed to the ecologic nature of the initial investigations in the area.

The most widely used approach in assessing human exposure is based on the semi-ecological studies where the exposures measured in the certain geographical areas are assigned to the subjects' addresses as an estimate of the individual exposure. The underlying assumption in these studies, is that ambient pollution is a valid proxy of the true internal levels of exposure. Nevertheless, the ambient exposure is frequently confounded by socio-economic status, occupation, smoking and other factors, that are hard to account for in a standard analysis, resulting in residual confounding and spurious or biased associations.

Alternatively, human biomonitoring (HBM) provides a direct personal assessment of external exposures at the time of sampling and has become the ‘‘gold standard’’ for the characterization of chemical exposure [5, 6]. Metals concentrations in human fluids are expected to reliably reveal the unwanted environmental exposures, and therefore are frequently tested by HBM. The main drawback of HBM is clearly its laboratory cost and complicated logistics required for recruitment and sampling. Some tests are invasive adding to HBM limitations [6]. As a result, HBM cycles are frequently limited to annual or bi-annual cycles.

European countries have initiated similar programs separately in each country and lately, joined their efforts in projects like HBM4EU [7] and Partnership for the Assessment of Risks from Chemicals (PARC)[8] bringing together nearly 200 partners from up to 30 countries in attempt to generate knowledge on population exposures to chemicals and their safe management in Europe.

In Israel, the Ministry of Health conducted a biomonitoring study (HBS) in 2011 involving 250 adults [9-13] and assessed exposure to bisphenol A, organophosphate pesticides, phthalates, cotinine, polycyclic aromatic hydrocarbons, and the phytoestrogenic compounds, genistein, and daidzein, based on analysis of spot urine samples. A National Health and Nutrition Study (MABAT) was another attempt of ongoing surveillance in Israel [14].

The HBM projects are usually expensive, labor intensive and therefore, result in spatially and temporally low-density sampling of the population. To meet the study objective of comparing Haifa Bay residents to the general population, we proposed establishing a framework for a national HBM survey, based on blood donations collected and processed on a daily basis by the Magen David Adom (MDA) National Blood Services located in Tel-Hashomer, Israel. The MDA is responsible for collection, processing, testing and distribution of blood products throughout the country (https://www.mdais.org/en/n-b-s/mda-national-blood-services). The MDA donors' population comprises non-paid volunteers, 78% being Israeli natives, 75% are 17-40 years old males. Ninety (90) percent of blood donors are recruited by MDA Mobil Units in schools, factories, community centers and army camps and the other 10% - in fixed-sites donor rooms at MDA first-aid stations all over Israel. The daily count of blood donations handled by MDA is close to 1000 samples.

Study objectives

In the current study, *we aimed to* compare concentrations of heavy metals in blood of Haifa Bay residents to residents in other regions in Israel. Specifically, we sought to develop a geographically representative sampling of the blood donations, on which this comparison could be performed. Furthermore, we aimed to estimate the association between the biomarkers and ambient environmental exposures, relying on the location of the blood donation collection as a proxy for a short-term exposure and permanent address of the donor – for a long-term exposure.

Methods

The methodology of the current study has been described elsewhere [15]. Briefly, the study population comprised a random sample of blood donors donating blood at all locations in Israel during 5 working days of a week, and stratified only by their location, i.e., from the Haifa Bay or non-Haifa Bay area. Donors not agreeing to their donation being used for research, as well as donors at military bases, were not included in the sample.
The sampling procedure was performed at the headquarters of the MDA Blood Services at the Tel-Hashomer once in 2-3 days during the span of 25 months, Feb 2020-Feb 2022. A test tube of whole blood of each chosen sample left over from the routine testing procedure was frozen in -80^{0}C freezer until testing. In all, we planned to collect up to 4800 samples and test 20% of them. The testing procedure was performed at the National Laboratory of Public Health.
To account for smoking habit, we tested a sample of 45 blood donors for Cotinine. When further compared the results to Cd readings, in attempt of classifying smoking based on Cd testing alone.

Environmental Exposures
To link blood collection locations (for short-term exposures and working places) and donors addresses of residence (for long-term exposures) with ambient exposures, the geographical data on locations and addresses was geocoded using ESRI ArcMap. Data on pollutants, i.e. nitrogen dioxide (NO_2), sulfate dioxide (SO_2), ozone (O_3), carbon monoxide (CO), $PM_{2.5}$, PM_{10}, and relative humidity (%) and air temperature were obtained from all the monitoring station managed by the Ministry of Environmental Protection spread throughout Israel. Throughout Israel we used 122 stations for NO_2, 92 stations for SO_2, 14 for CO, 44 for PM_{10}, 75 for $PM_{2.5}$, 57 for relative humidity and 64 for temperature. For each donor we chose 5 closest stations recording a relevant environmental factor within 20km of his/her donating site or residence town location. The ambient pollution values were further averaged for the date of donation.

At a later stage, exposure to $PM_{2.5}$, PM_{10} and air temperature (°C) will be defined using hybrid satellite-based exposure models[16]. Their estimates normally take two years to obtain (since our last enrollment in Mar 2022) and depend on the data release schedule of NASA database. This approach provides with accurate estimates of exposure at a grid of 1x1 km^2 and has a potential to prevent a selection bias, when assigning exposure to locations without monitoring stations in vicinity.

Data processing

We compared the Haifa Bay area donors with the general population of non-Haifa Bay area donors, in terms of their main demographic characteristics, using standard statistical methods, i.e., mean ± sd, median, 95% confidence intervals, Chi-square, t and non-parametric tests. The biomonitoring data was further described by geometric mean, minimum and maximum. The description was provided separately for main geographical locations and the main municipalities in Israel. Biomarker readings by regions were compared by ratio t-test. The association between ambient environmental exposures and biomarker readings was analyzed using a log-normal regression, whereas the biomarker was the predicted outcome in the regression and ambient exposure - as the main independent variable. The analysis was adjusted to all possible confounders recorded in the database. Prevalence ratio (PR) represented the main point estimate of association at study, showing a multiplicative effect of risk factor on metals concentrations. The levels of metals adjusted to age, gender and smoking were displayed on a map, in a form of a ratio of observed concentrations over expected by a model.

Environmental chemicals for testing

Following the guidelines issued by the Ministry of Environmental Protection in Israel ("Regulations on Clean Air", 2008), [17] there is a public urgency for testing Cadmium (Cd), Lead (Pb) and Arsenic (As), as a part of human biomonitoring; whereas Cd and Pb, might indicate exposure to transport, and As – to industry. Additionally, Chromium (Cr) has been indicated as a possible hazardous exposure featuring the Haifa Bay area. All the four chemicals have been defined as carcinogenic to human by International Agency for Research on Cancer (IARC)[18]. The half-life of metals' concentrations in blood is often measured in months. Specifically, the half-life for Pb with half-life of 28-36 days [19, 20], followed by Cd and Cr – with about 40 months [21-23], and only for As the half-life is measured in 10-20 hours [24].

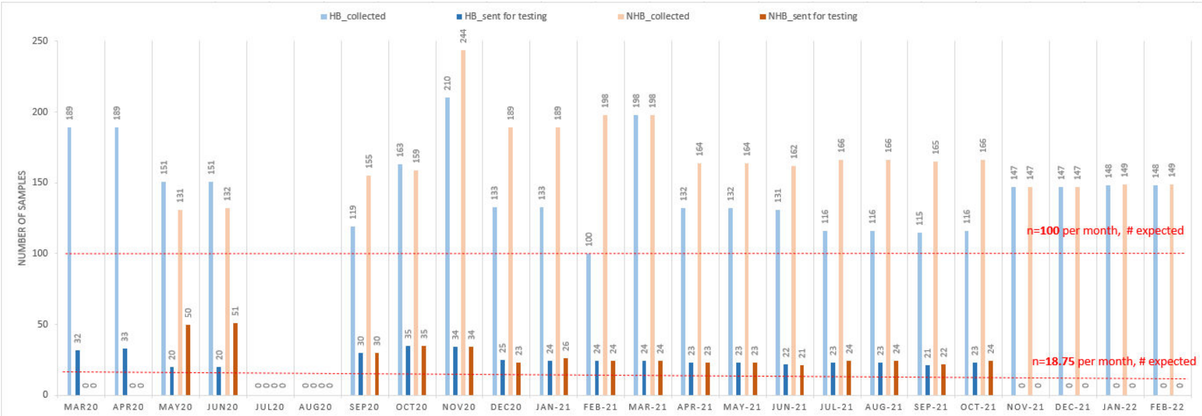
Results

Enrollment

The enrollment of samples started in March 2020 and was finalized 24 months later, in February 2022. In all, we collected close to 6230 samples and out of these, 916 samples were sent to the laboratory and 911 were successfully tested for the 4 selected metals. The enrollment numbers exceeded those stated in the proposal, where we planned to collect 4800 samples and testing 900. Figure 1 shows the final enrollment log of samples' collection in the study. The bars in blue colors are assigned to donors from Haifa Bay (HB) locations, while the brown colored bars stand for all other non-Haifa Bay (NHB) regions in Israel. Bars in dark colors show samples that were tested and lighter bars – samples that were only collected. The expected counts of enrollment were 18.75 and 100 samples per month in each of the HB and NHB regions, for tested and collected samples, respectively.

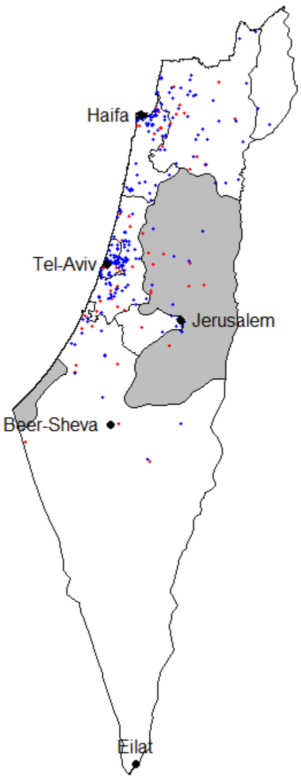
The enrollment log demonstrates an even temporal coverage of the 14 months between Sep 2020-Oct 2021. The gaps in samples collection during the first 6 months of the study are mostly explained by the logistic obstacles in enrollment at the very beginning of the COVID-19 pandemic, that started with the beginning of the project. The active testing of samples has been finalized by Nov 2021, owing to the high enrollment rate in general.

Figure 1. Enrollment log over the study period



The information on the demographical characteristics of the donors of all collected and stored samples has been verified ץThe report will focus on the information recorded for the subset of 911 samples that were tested. This group represents a random subset of the 6230 donors. The spatial coverage of the samples is reflected in figure 2 showing the locations of 911 samples tested to metals, whereas the blue dots are assigned to donations' sites and red – to the donors' residential addresses. The map demonstrates a good spatial distribution of the samples, although the Southern part of Israel accounting for approximately 1mln residents (1/10 of the entire population in the country) seems somewhat underrepresented. This can be explained by the main focus of the current analysis being on the northern part of the country.

Figure 2. Spatial distribution of tested samples in the study, by location of residence and donation site



Red – location of blood donors' residence
Blue- location of donation sites

In the analysis, we inspect both types of locations while the residence location is deemed to reflect a cumulative chronic exposure and the donation site – a more recent short-term exposure. In most cases the geographic area of residence and donation site did not vary. Half of the donors in Haifa Bay lived less than 3.7 km from their donation site. The largest distance was in Gush Dan area, featured by median distance of 3.9 km between the two locations.

Table 1. Residential location of the blood donors and geographical location of donation sites

Geographic Area	Residence (N=911)	Donation site (N=911)	Both in the same area (N=911)	Median distance between donation site and residential address, by residential location, km
Haifa Bay	40.8 (369/905)	41.9 (347/829)	39.8 (329/827)	3.7
Gush Dan	20.7 (187/905)	24.7 (205/829)	17.7 (146/827)	3.9
Jerusalem	4.8 (44/905)	5.8 (48/829)	3.9 (32/827)	1.6
All other regions	33.7 (305/905)	27.6 (229/829)	23.6 (195/827)	2.6

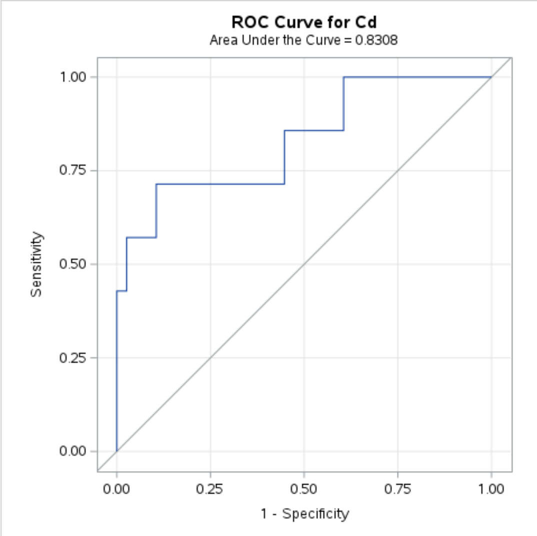
Defining smoking status based on the Cadmium levels

Smoking is an important contributor to high levels of metals in blood, especially to the levels of Cadmium. As smoking habit is not reported on the donors' questionnaire, the smoking status had to be assumed or modeled based on other possible proxies available for the analysis.. In the following analysis we explored the association between Cotinine and Cadmium in attempt to classify smokers based on Cadmium levels alone when Cotinine test is not available. A threshold of Cd>1µg/L has been derived by Schulz et al in 2011, based on a German HBM survey [25]. This rule, however, required validation in the Israeli sample of donors. For this purpose, we tested a sub-sample of 45 blood donors for both biomarkers, and further suggested a rule for determining a smoking status based on Cd values alone. A threshold for defining smoking status was obtained based on Receiver Operating Characteristic (ROC) Curve analysis and maximal Youden index. To collect more information on potential smokers, we artificially ensured the number of donors with Cd>1µg/L in the training subsample to be 20%, by a random sample of 9 subjects with Cd>1µg/L and all the rest being randomly sampled from a stratum with Cd≤1µg/L.

The geometric mean (GM) of Cotinine was 0.88 ppb, with 95%CI: 0.30; 2.54. The lowest values of Cotinine in the sample were at the level below detection and the maximal value was equal 295.6ppb. It has been shown by others, that smokers rarely test lower than 10ppb [26] On the other hand, a threshold of 100ppb for Cotinine in blood has been more frequently used for active smokers [27-29]. Therefore, the higher threshold of 100ppb was used to determine a smoking status.. In all. 15.6% (7/45) of the sample chosen for this analysis had Cotinine>100ppb.

Cadmium appeared to be a good biomarker for smoking with a high potential to correctly discriminate between smokers and non-smokers, with a Receiver Operating Characteristic (ROC) Curve featured by the area under the curve (AUC)=0.831 (Figure 3).

Figure 3: Receiver Operating Characteristic (ROC) Curve for classifying smokers based on the Cadmium concentrations in blood donors (n=45)

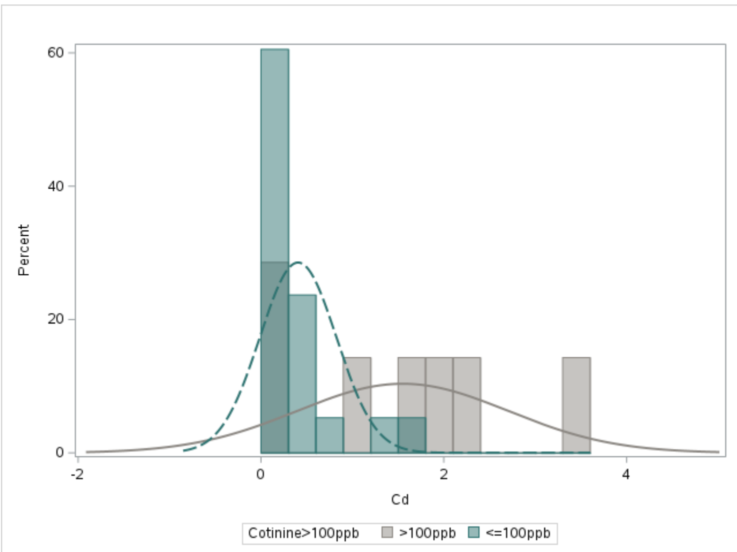


The distribution of Cadmium among smokers (donors with Cotinine>100ppb) was substantially different from non-smokers, as it appears in Table 2 and Figure 4 below.

Table 2. Distribution of Cadmium concentrations among blood donors, by their smoking status

	Cadmium, µg/L										
Status by Cotinine>100ppb	Min; Max	Mean	Percentiles								
			1st	5th	10th	25th	Median	75th	90th	95th	99th
Non-smokers (n=38)	0.07; 1.80	0.41±0.42	0.07	0.07	0.10	0.18	0.24	0.43	1.27	1.57	1.80
Smokers (n=7)	0.22; 3.43	1.55±1.15	0.23	0.23	0.23	0.26	1.65	2.30	3.43	3.43	3.43

Figure 4. Distribution of Cadmium concentrations (µg/L) by smoking status (n=45)



Based on the findings above a cut off at Cadmium>1μg/L will provide a Sensitivity (Se)= 71.4% (5/7) and Specificity (Sp)= 89.5% (34/38) in defining a smoker.
To add a degree of likelihood to the definition of smokers in the analysis, we will consider using a predicted chance of a donor to be a smoker, a by-product of the ROC curve analysis presented earlier. This approach is expected to yield a better adjustment to a donor's personal exposure. The likelihood of a donor to be a smoker in the study population is (mean±sd) 0.08±0.11 within the range of 0.03; 0.99. The estimate of 8% of smoking in the study population is lower than the prevalence of smoking in general population in Israel. According to the national survey conducted in 2011, the overall active smoking rate in the adult (21 years and older) population based on self-report, was 20.6%[30].

Study population

The main thrust of the study was the comparison of metal concentrations between donors from Haifa Bay with other geographic regions in Israel. This comparison can be meaningful if the donors from different regions are similar on their potentially confounding characteristics, and/or adjusted to those confounders in case of imbalance between the study groups.
From inspection of table 3, donors in Haifa Bay and Gush Dan areas are older by approximately 3 years as compared to the donors in Jerusalem and other regions in Israel (p-value<0.05). In Haifa Bay area, half of the donors are younger than 34.7 years as compared to 29.8-33.0 years in other areas. The distribution of gender and smoking status (defined by Cd<1 μg/L) was similar across the geographic areas.

Table 3. Demographic characteristics of the tested samples, by town of residence

Donors' characteristics	Haifa Bay (N=369)	Gush Dan (N=187)	Jerusalem (N=44)	All other regions (N=305)	p-value for comparing	
					All regions	Haifa Bay vs. others
Age, years Mean±SD (n) Median Min; Max	37.0±13.6 (369) 34.7 17.6; 71.4	37.3±14.5 (187) 33.0 18.1; 79.3	33.7±13.4 (44) 30.2 18.2; 67.9	34.1±13.6 (305) 29.8 17.5; 71.3	0.015	0.046
Age grouped, years % (n/N) <35 35-60 60+	50.1 (185/369) 42.8 (158/369) 7.1 (26/369)	54.0 (101/187) 37.4 (70/187) 8.6 (16/187)	59.1 (26/44) 34.1 (15/44) 6.8 (3/44)	58.0 (177/305) 37.4 (114/305) 4.6 (14/305)	0.307	0.149
Males, % (n/N)	63.4 (234/369)	59.9 (112/187)	70.5 (31/44)	65.3 (199/305)	0.500	0.904
Residing in Arabic town, % (n/N)	0.0 (0/369)	0.0 (0/187)	0.0 (0/44)	13.1 (40/305)	<0.001	<0.001
Smoker (based on Cd>0.6 μg/L), % (n/N)	11.4 (42/369)	10.7 (20/187)	13.6 (6/44)	12.5 (38/305)	0.908	0.798

The *concentrations of metals* in the blood donors' population in Israel (table 4) are comparable to the general population sampled for the biomonitoring surveys in Europe over the last two decades ([31, 32]), and on a lower side with respect to Pb.

Table 4. Metals' concentrations by demographic characteristics

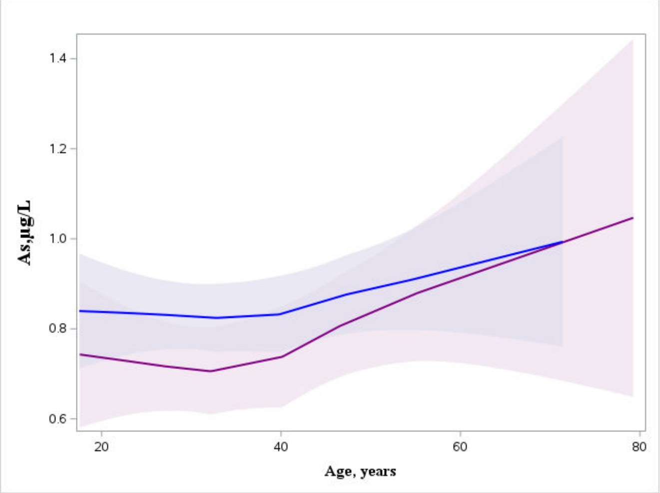
Metal	GM (95%CI)	Median	Min; Max	GMs in blood for EU adults) [31]	HBM4U[32] Medians of concentrations in surveys using whole blood matrix
As, µg/L (As) (n=911)	0.53 (0.50; 0.57)	0.55	0.07; 5.65	Minimal GM: 0.50 Maximal GM: 0.74	Minimal median: 0.62 Maximal median: 1.81
Cd, µg/L (Cd) (n=911)	0.22 (0.21; 0.24)	0.22	0.001; 3.85	Minimal GM: 0.28 Maximal GM: 0.60	Minimal median: 0.15 Maximal median: 0.60
Cr, µg/L (Cr) (n=910)	0.98 (0.95; 1.02)	0.13	1.05; 8.55	Not in the report	Not in the report
Pb, µg/dL (Pb) (n=911)	6.68 (6.37; 7.00)	7.05	0.07; 51.06	Minimal GM: 9.5 Maximal GM: 33.0	Minimal median: 9.9 Maximal median: 41.3

In the following analysis we inspect the possible differences in metals concentrations by gender and age (Table 5). This is followed by a visual illustration of the same comparisons (Figure 5).

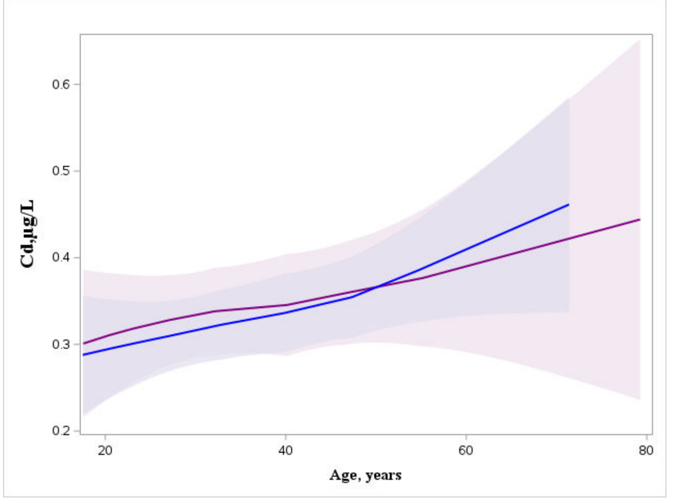
Table 5: Metals concentrations by gender and age. Results of a univariable analysis.

***As:** GMs (95%CI)*	**Females (N=332)**	**Males (N=579)**	**Total**	**p-value**
<35 (N=495)	0.48 (0.42; 0.55)	0.57 (0.52; 0.63)	0.54 (0.49; 0.58)	0.818
35-60 (N=357)	0.43 (0.36; 0.52)	0.57 (0.51; 0.65)	0.52 (0.47; 0.58)	
60+ (N=59)	0.65 (0.47; 0.90)	0.57 (0.42; 0.78)	0.60 (0.48; 0.76)	
Total	**0.47 (0.42; 0.53)**	**0.57 (0.53; 0.62)**		
p-value	0.004			
***Cd:** GMs (95%CI)*				
<35 (N=495)	0.21 (0.19; 0.24)	0.19 (0.17; 0.21)	0.20 (0.18; 0.22)	<0.001
35-60 (N=357)	0.27 (0.24; 0.30)	0.23 (0.21; 0.69)	0.25 (0.23; 0.27)	
60+ (N=59)	0.34 (0.27; 0.44)	0.36 (0.28; 0.46)	0.35 (0.30; 0.42)	
Total	0.24 (0.22; 0.26)	0.22 (0.20; 0.23)		
p-value	0.077			
***Cr**: GMs (95%CI)*	**Females (N=329)**	**Males (N=558)**	**Total**	p-value
<35 (N=495)	0.99 (0.91; 1.08)	0.97 (0.91; 1.04)	0.98 (0.93; 1.03)	0.866
35-60 (N=357)	0.97 (0.87; 1.08)	1.01 (0.93; 1.09)	0.99 (0.93; 1.06)	
60+ (N=59)	0.82 (0.65; 1.03)	1.09 (0.89; 1.33)	0.97 (0.83; 1.14)	
Total	0.97 (0.91; 1.03)	0.99 (0.94; 1.04)		
p-value	0.586			
***Pb**: GMs (95%CI)*				
<35 (N=495)	5.05 (4.58; 5.57)	6.59 (6.05; 7.17)	5.98 (5.60; 6.39)	<0.001
35-60 (N=357)	6.52 (5.79; 7.35)	7.75 (7.09; 8.47)	7.29 (6.78; 7.83)	
60+ (N=59)	8.69 (7.08; 10.66)	10.86 (8.81; 13.37)	9.95 (8.55; 11.59)	
Total	5.79 (5.38; 6.23)	7.25 (6.82; 7.70)		
p-value	<0.001			

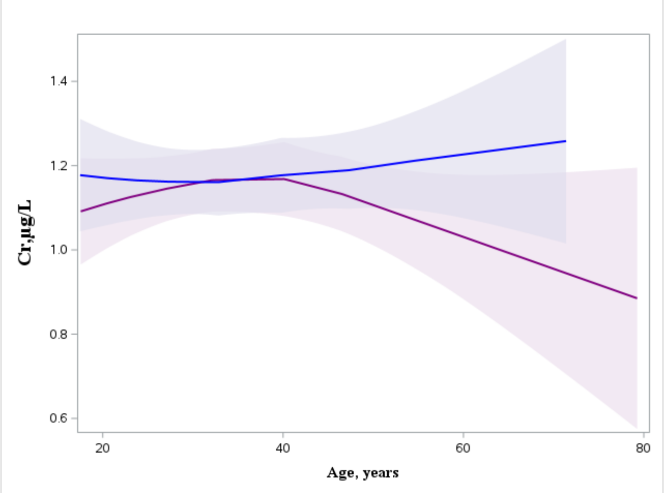
Figure 5. Metals concentrations, by gender and age. Results of a univariable analysis[1]



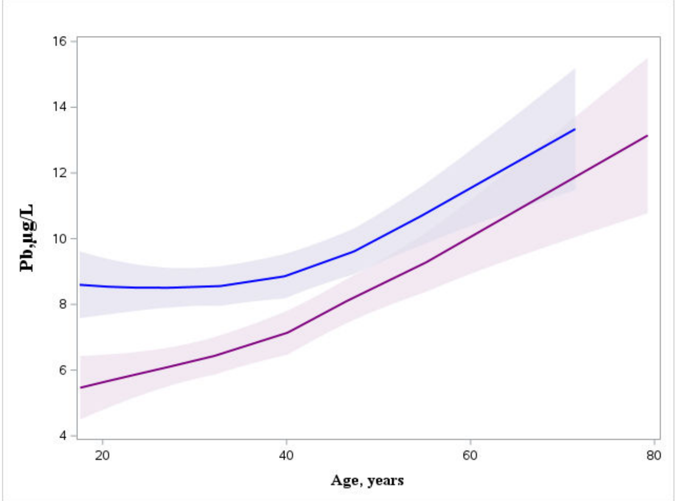
p-value (sex)= 0.004; p-value (age)=0.494



p-value (sex)= 0.077; p-value (age)<0.001



p-value (sex)= 0.586; p-value(age)=0.918



p-value (sex)<0.001; p-value (age)<0.001

[1] Male participants are shown in blue color and female – in purple. The shaded area around the curves represents its 95%CI.

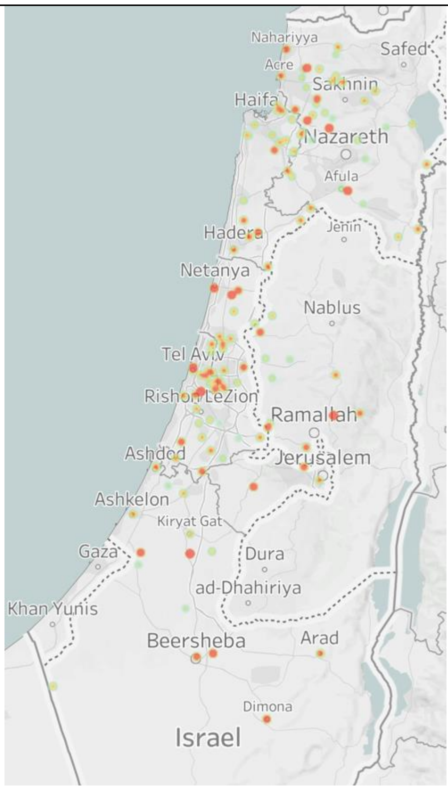
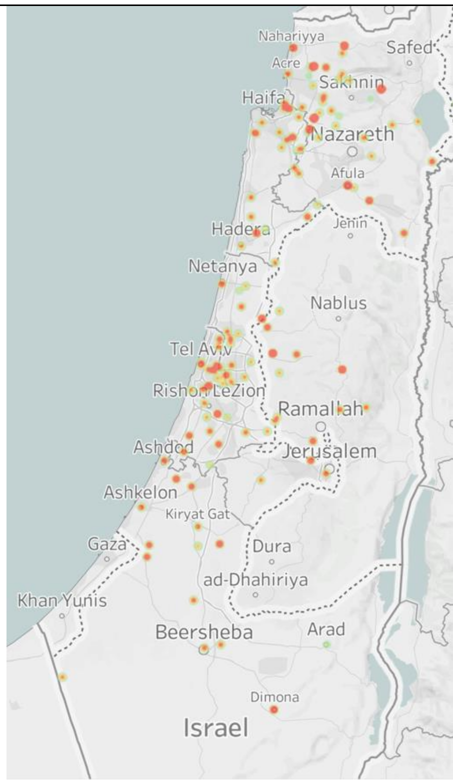
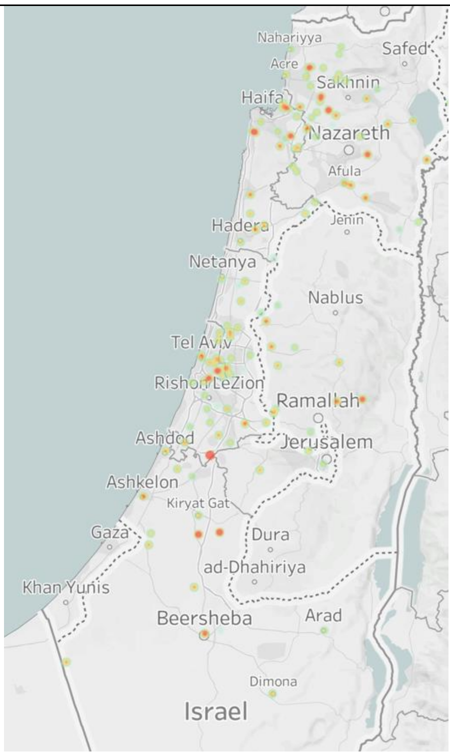
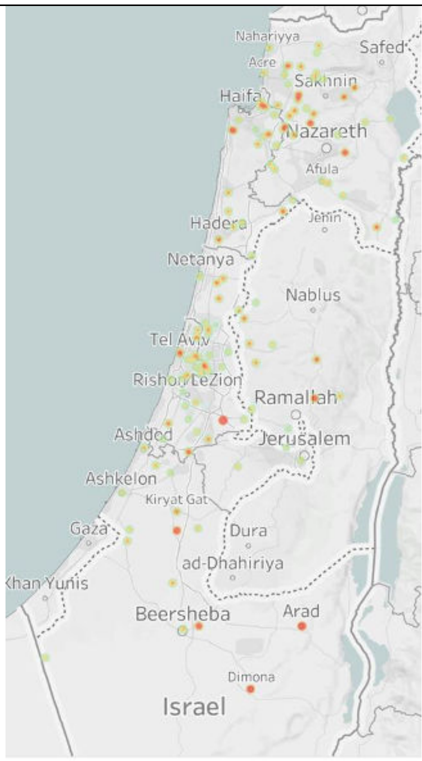
With respect to age, shown previously to be statistically higher among Haifa Bay donors, we may conclude that Cd and Pb are the two elements that tend to be higher with age. The same might be true for As, however, the trend was not statistically significant. Cr element is likely to change over time, but increasing for males and decreasing for females, and hence its overall change appears to be not significant, although also the interaction between age and gender. These findings warrant for age-adjustment in future comparisons of metals between Haifa Bay and non-Haifa Bay donors.

Even though gender appears to have an impact on the metals' concentrations, usually higher for males than for females (As, Cd and Pb), its distribution did not vary between the regions and hence, adjustment to it is not required in future analysis.

Metals concentrations by geographic area

Figures 6a,b demonstrate the geographic distribution of metals' concentration among donors, adjusted to age, gender and smoking predicted probability. The points in more intense red colors assigned to values higher than 1 and more intense green for values below. Figures 7a,b statistically compare the donors from the Haifa Bay region to the rest of the country.

Figure 6a. Point heatmap of ratios of metals' concentrations observed over concentrations expected based on age, gender and smoking status of the donors, by *residence location*

<table>
<tr><th>As</th><th>Cd</th></tr>
<tr><td>
</td><td>
</td></tr>
<tr><th>Cr</th><th>Pb</th></tr>
<tr><td>
</td><td>
</td></tr>
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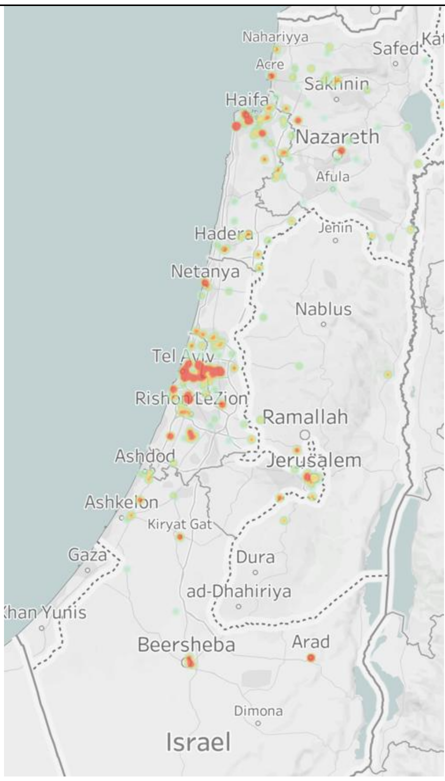
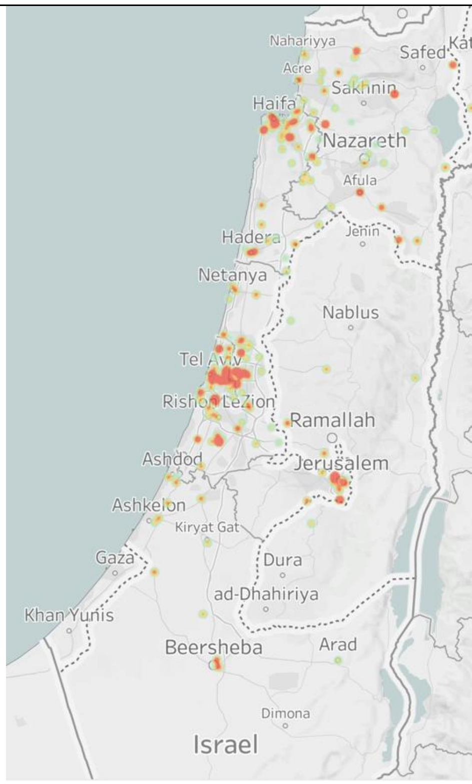
The colors of the points in the map range for from green through yellow to red corresponding to values below, around and above 1.

Figure 6b. Point heatmap of ratios of metals' concentrations observed over concentrations expected based on age, gender and smoking status of the donors, *by donation site*

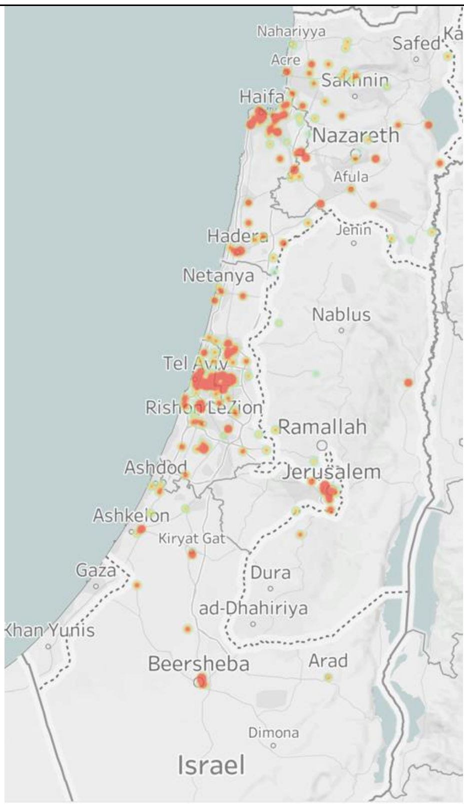
As



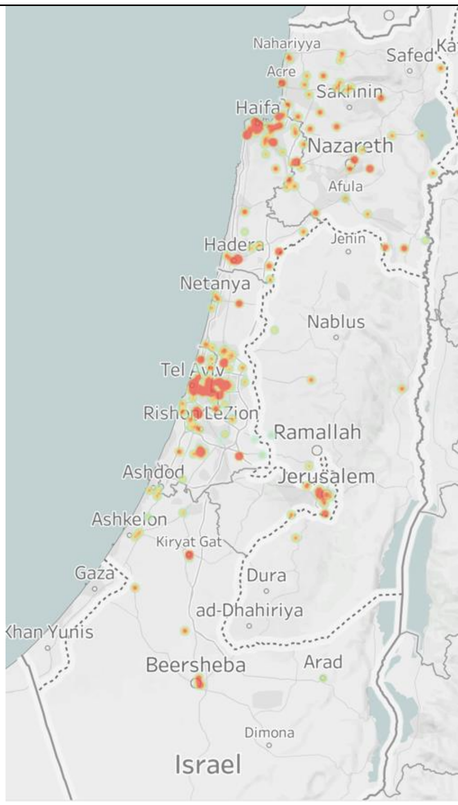
Cd



Cr



Pb



The colors of the points in the map range for from green through yellow to red corresponding to values below, around and above 1.

Figure 7a. Metals' concentrations by area of residence

<table>
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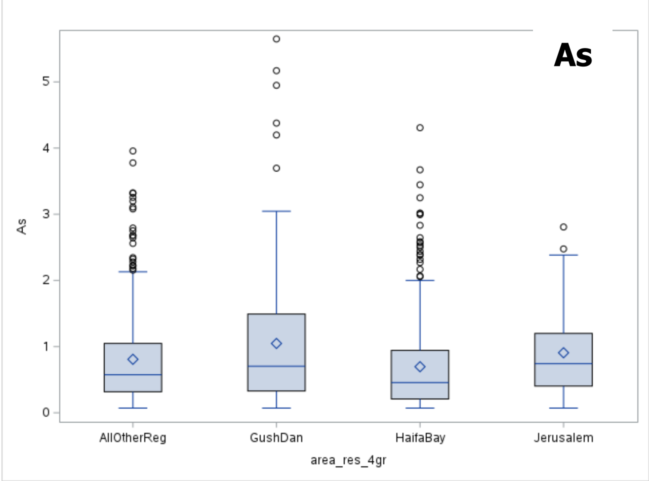
<td>
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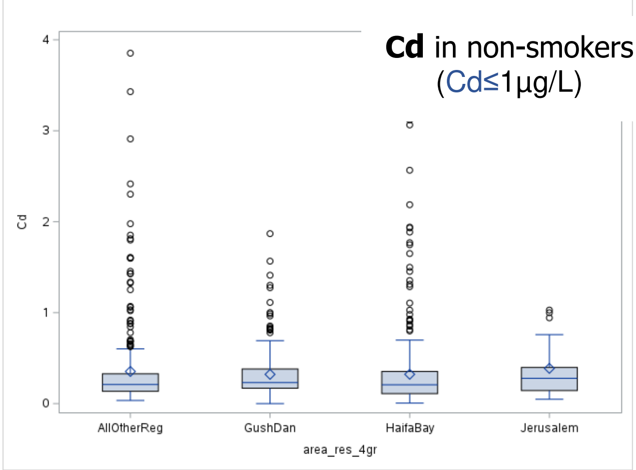
<td>
</td>
</tr>
<tr>
<td>Gush Dan: 0.69 (0.60; 0.79)
Haifa Bay: 0.43 (0.38; 0.48)
Jerusalem:0.66 (0.51; 0.85)
All other regions: 0.57 (0.52; 0.63)
p-value (crude comparison of HB to other regions) <0.001
p-value (comparison of HB to other regions, adj to age, gender and smoking) <0.001, PR=0.69</td>
<td>Gush Dan: 0.22 (0.18; 0.21)
Haifa Bay: 0.19 (0.18; 0.27)
Jerusalem:0.26 (0.20; 0.33)
All other regions: 0.20 (0.18; 0.21)
p-value (crude comparison of HB to other regions) =0.126
p-value (comparison of HB to other regions, adj to age and gender) =0.029, PR=0.90</td>
</tr>
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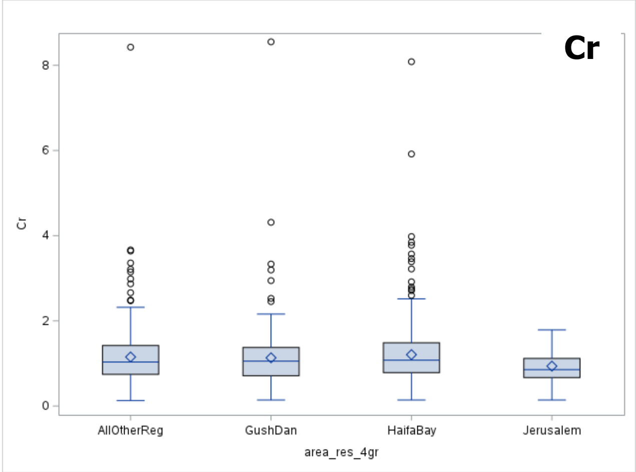
<td>
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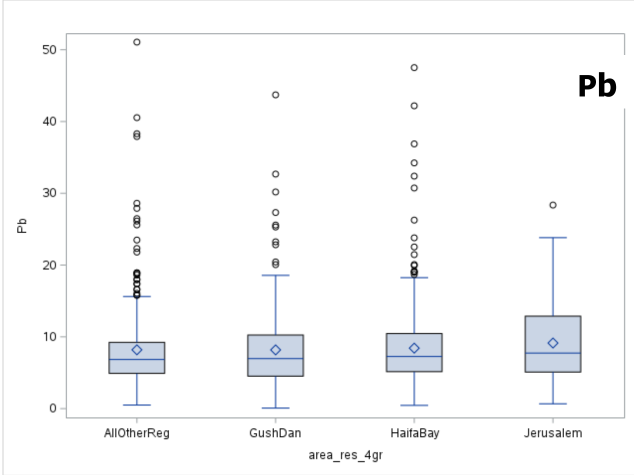
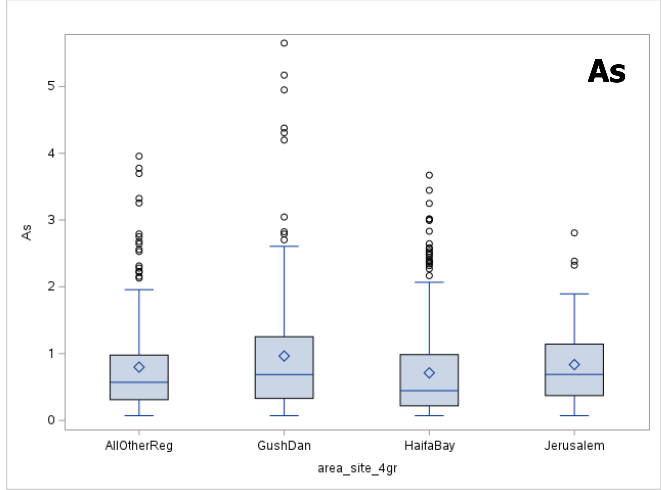
<td>
</td>
</tr>
<tr>
<td>Gush Dan: 0.95 (0.87; 1.04)
Haifa Bay: 1.03 (0.97; 1.09)
Jerusalem: 0.79 (0.65; 0.97)
All other regions: 0.98 (0.92; 1.05)
p-value (crude comparison of HB to other regions) =0.072
p-value (comparison of HB to other regions, adj to age, gender and smoking) =0.067, PR=1.08</td>
<td>Gush Dan: 5.92 (5.18; 6.77)
Haifa Bay: 7.10 (6.68; 7.55)
Jerusalem: 6.87 (5.28; 8.94)
All other regions: 6.59 (6.10; 7.12)
p-value (crude comparison of HB to other regions) =0.028
p-value (comparison of HB to other regions, adj to age, gender and smoking) =0.048, PR=1.10</td>
</tr>
</table>

Donors residing in the Haifa Bay area had lower levels of As and Cd as compared to the rest of the donors in Israel, and higher levels of Cr and Pb. The later concentrations were times 1.18 and 1.10 higher than in other regions (although with borderline significance - p-value=0.067 for Cr), after adjusting to age and smoking.

Figure 7b. Metals' concentrations by area of donation site

<table>
<tr>
<td>
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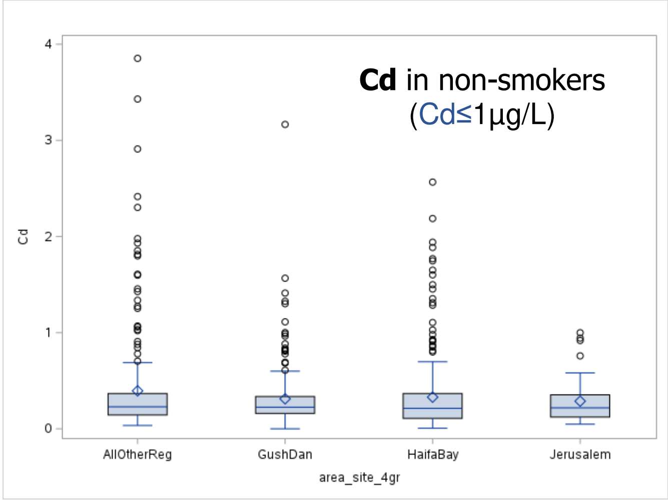
<td>
</td>
</tr>
<tr>
<td>Gush Dan: 0.63 (0.52; 0.71)
Haifa Bay: 0.44 (0.39; 0.49)
Jerusalem:0.62 (0.50; 0.84)
All other regions: 0.57 (0.52; 0.64)
p-value(crude comparison of HB to other regions)<0.001
p-value(HB vs other regions, adj to age, gender and smoking)<0.001, PR=0.72</td>
<td>Gush Dan: 0.21 (0.19; 0.24)
Haifa Bay: 0.19 (0.17; 0.24)
Jerusalem: 0.22 (0.17; 0.27)
All other regions: 0.20 (0.18; 0.22)
p-value(crude comparison of HB to other regions)=0.167
p-value(HB vs other regions, adj to age and gender)=0.074, PR=0.91</td>
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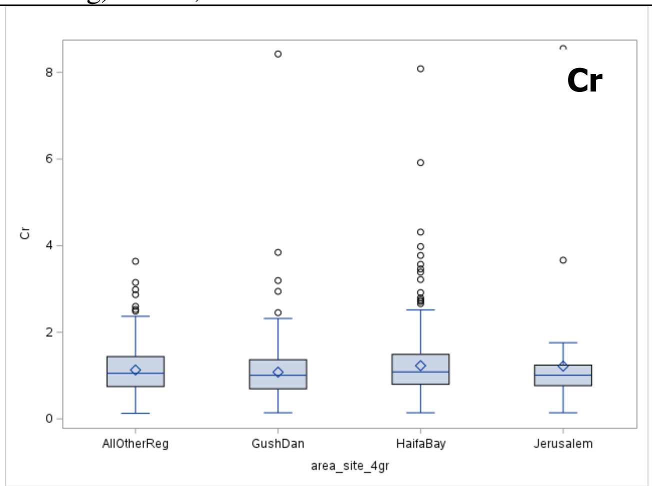
<td>
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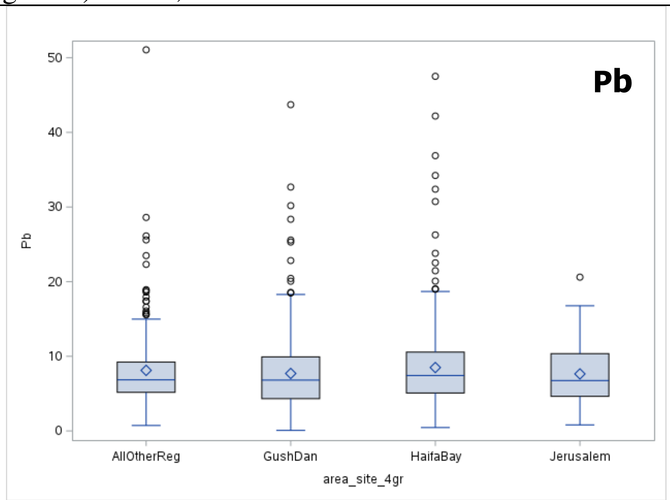
<td>
</td>
</tr>
<tr>
<td>Gush Dan: 0.91 (0.84; 0.99)
Haifa Bay: 1.06 (1.00; 1.124)
Jerusalem: 0.97 (0.81; 1.17)
All other regions: 0.96 (0.88; 1.04)
p-value(crude comparison of HB to other regions)=0.004
p-value(HB vs other regions, adj to age, gender and smoking)=0.004, PR=1.13</td>
<td>Gush Dan: 5.46 (4.78; 6.22)
Haifa Bay: 7.18 (6.75; 7.64)
Jerusalem: 6.14 (4.97; 7.60)
All other regions: 6.93 (6.43; 7.46)
p-value(crude comparison of HB to other regions)=0.002
p-value(HB vs other regions, adj to age, gender and smoking)=0.004, PR=1.15</td>
</tr>
</table>

Donors donating blood in the Haifa Bay area had lower levels of As as compared to the rest of the donors in Israel, and higher levels of Cr and Pb. The later concentrations were times 1.13 and 1.15 higher than in other regions (
with p-value=0.004 and 0.004, respectively), after controlling for age and smoking.

External Exposures and Metals Concentrations

The next step of the analysis is aimed to reveal possible sources of exposure to metals. Here, we will inspect external factors like ambient pollution measured by the monitoring stations, as well as the proximity to industries working with the metals at study, also quarries and power plants.
The following table (table 6) inspects the mixtures of metals that are likely to be found together in one subject. We split the analysis by smoking status, to adjust for a chronic exposure that is likely to impact the entire analysis.

Table 6. Association[1] between metals, by smoking status (Cd>1 µg/L)

Non-smokers (N=863)				**Smokers (N=48)**			
	Cd	Cr	Pb		Cd	Cr	Pb
As	-0.02 (0.579)	-0.09 (0.001)	0.08 (0.019)	As	-0.10 (0.513)	-0.24 (0.095)	0.04 (0.772)
Cd		0.03 (0.345)	0.37 (<0.001)	Cd		0.11 (0.445)	0.07 (0.636)
Cr			0.27 (<0.001)	Cr			0.01 (0.924)

[1]Spearman rho (p-value)

Among non-smokers, Pb concentrations were positively associated with Cd and Cr, indicating a possibly mutual source of environmental pollution. Pb was also negatively correlated with Cr.
Among smokers, we recorded a borderline positive association between As and Cd.
Concluding the table 6 above, the possible environmental mixtures not related to smoking may contain Pb+Cd, Pb+Cr , Pb+As or As alone.

Association between ambient exposures and metals

In exploring the association between the ambient exposures and metals' concentrations, the important factor to be taken into consideration is the relevant window period when the air pollution may be absorbed by the human body and become visible in blood. The analysis below is a univariable investigation of the associations between the metals in blood and pollutants averaged over a week and a month preceding the blood donation. In the analysis we also differentiate between levels of pollution at the residence location of a donor and at the place of the donation site.

Table 7. Correlation between ambient pollutants and metals' concentrations in blood, by location and window period

By residence[1]

Rho p-value	PM_{10} 7d	PM_{10} 30d	$PM_{2.5}$ 7d	$PM_{2.5}$ 30d	NO_2 7d	NO_2 30d	CO 7d	CO 30d	SO_2 7d	SO_2 30d
As	-.07 **.057**	-.04 .278	.00 .953	.04 .322	**.11** **.001**	**.11** **.003**	-.05 .208	-.05 .198	.00 .964	.00 .898
Cd	-.02 .661	.01 .720	-.05 .167	-.02 .599	.06 **.082**	.06 .093	-.01 .831	-.01 .820	.05 .160	.06 **.077**
Cr	.03 .352	**.07** **.053**	-.09 **.015**	-.05 .113	-.04 .293	-.01 .672	-.05 .200	.00 .975	.05 .204	**.09** **.012**
Pb	-.01 .884	**.09** **.012**	-.13 **.000**	-.10 **.004**	-.06 **.085**	-.05 .189	.01 .752	.04 .248	**.10** **.005**	**.13** **<.001**

[1]Statistically significant results with p-value<0.1 are shown in bold. Positive correlations are shown in red color.

By site of donation[1]

Rho p-value	PM_{10} 7d	PM_{10} 30d	$PM_{2.5}$ 7d	$PM_{2.5}$ 30d	NO_2 7d	NO_2 30d	CO 7d	CO 30d	SO_2 7d	SO_2 30d
As	-.01 .883	.04 .241	.01 .689	.01 .705	**.08** **.016**	**.08** **.016**	-.04 .320	-.01 .702	.01 .748	-.02 .653
Cd	-.03 .470	-.01 .752	.00 .943	.01 .798	**.06** **.100**	**.06** **.080**	.00 .932	.01 .758	.04 .254	.04 .258
Cr	-.03 .362	-.02 .658	-.06 **.074**	-.01 .763	-.03 .362	.01 .843	-.07 .063	.02 .470	.03 .392	**.08** **.027**
Pb	-.06 **.075**	.01 .790	-.04 .224	-.01 .867	-.03 .376	-.02 .642	-.01 .701	.04 .336	**.09** **.016**	**.14** **<.001**

[1]Statistically significant results with p-value<0.1 are shown in bold. Positive correlations are shown in red color.

From analyzing the table above, it becomes apparent that:

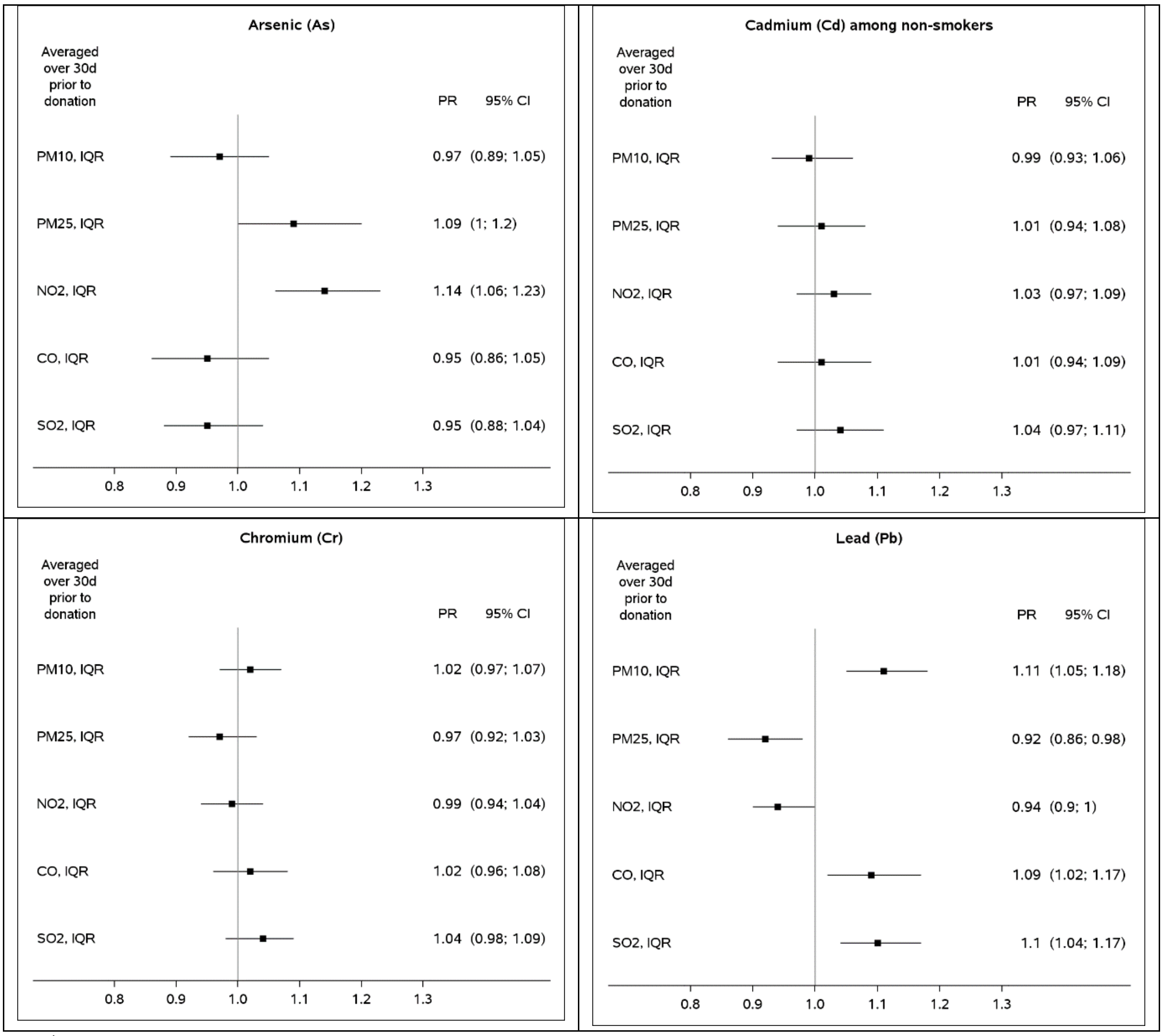
- exposure by site of donations does not fully reflect the real ambient exposure, as opposed to the one measured at the donors' residence address.
- comparison between the cumulative 7 and 30 days indicated a response of higher magnitude in exposure averaged over the 30 preceding days rather than 7 days.
- According to these findings, the estimates of 30 days at the residential location will be used in further analyses.

The following forest plots (figure 8) demonstrate the associations between metals and ambient measurements using the residential address and averaging the pollution over the 30 days prior to the donation. The ambient pollutants are expressed in intra-quartile range units. All associations are adjusted to age, sex and smoking status (defined by predicted probability of smoking).

Based on the findings, pollutants like $PM_{2.5}$ and NO_2 , are likely to be associated with higher concentrations of As, irrelevant of their demographic status and smoking. To be precise, an increase of interquartile range (IQR) in $PM_{2.5}$ was associated with a 9% and 14% increase in As (although with only borderline significance for $PM_{2.5}$-As link, p-value=0.062). Conversely, PM_{10} and SO_2 and CO are more likely to be associated with higher Pb. Specifically, increase of IQR in $PM_{10,}$ CO and SO_2 were associated with an 11%, 9% and 10% increase in Pb.

In all cases, the associations are adjusted to age, sex and predicted probability of smoking.

Figure 8. Association between ambient exposure at residence location and metals' concentrations in blood

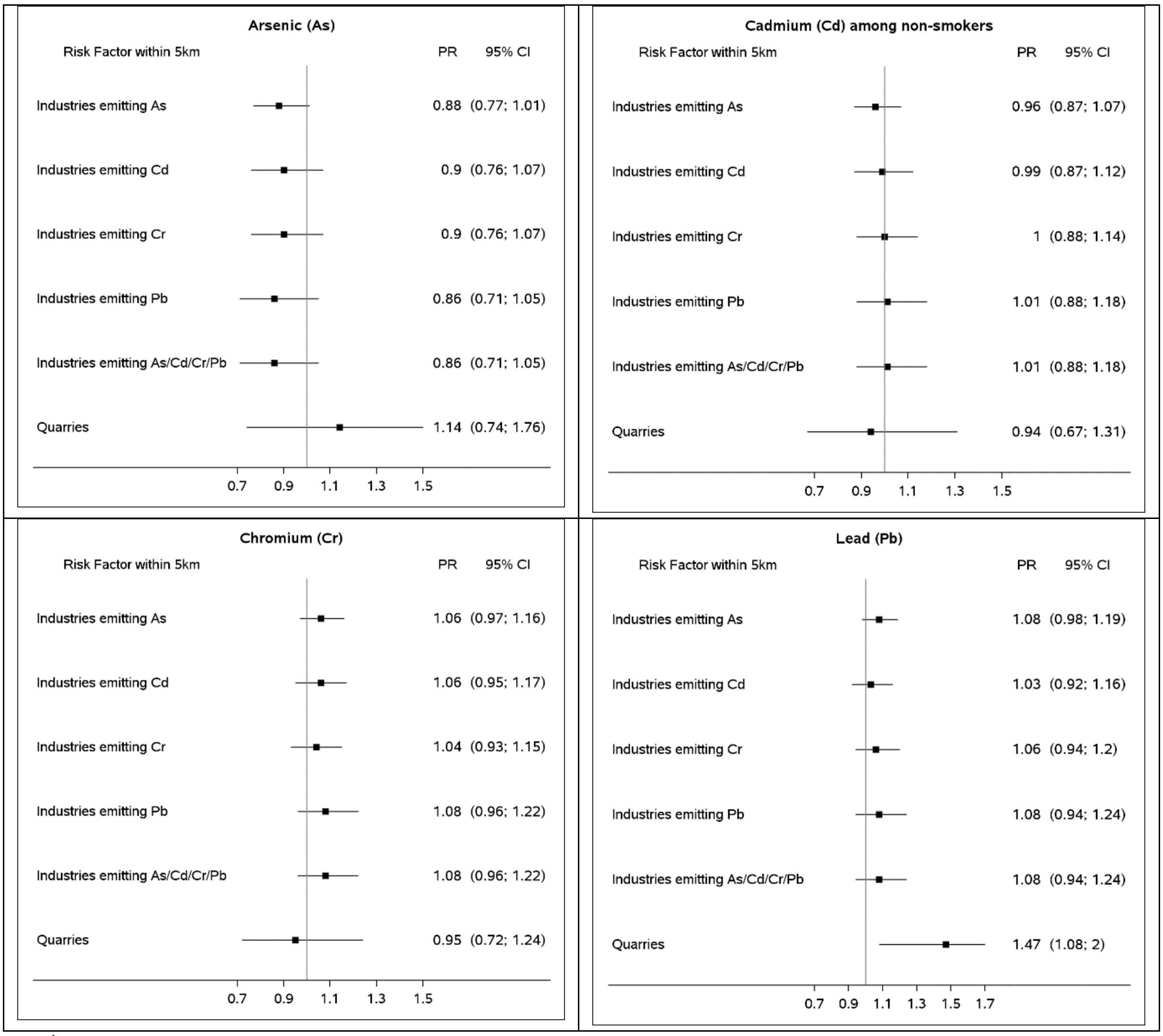


[1] All estimates are adjusted to age, sex and predicted probability of smoking, with the exception of the model of Cd concentrations, where analysis was performed in a stratum of non-smokers, i.e. Cd≤1.0 µg/L.

To estimate the possible *contribution of industries* to the overall environmental exposure reflected in metals' concentrations, we added an indication of plants and factories within the 5 km radius from the donors' residence, to the analysis. In particular, we consulted with the findings of the MIFLAS report prepared by the Ministry of Environmental Protection, which helped us to identify industries working with As, Cd, Cr and Pb metals in close proximity to the donors' residential addresses. In the analysis, we defined exposure to industries as a binary indication of presence of factories working with the metals at study within the 5km radius.

All the analyses were adjusted to age, gender and predicted probability of smoking (Figure 9). In the analysis of Cd, we excluded donors with Cd>1 µg/L, this is to be able to focus on factors not related to smoking.

Figure 9. Association between industries within 5 km from the residential location and metals' concentrations in blood

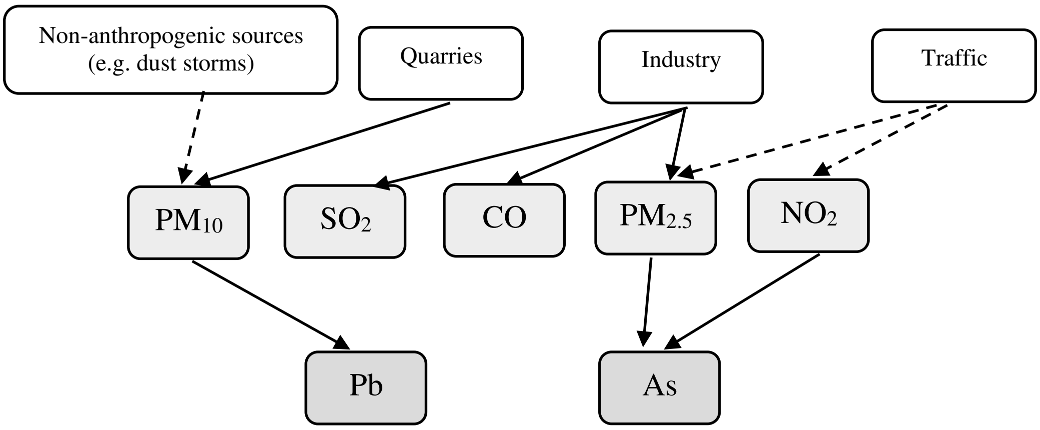


[1] All estimates are adjusted to age, sex and predicted probability of smoking, with the exception of the model of Cd concentrations, where analysis was performed in a stratum of non-smokers, i.e. Cd≤1.0 µg/L.

The forest plots demonstrate no adverse association of industries with As and Cd. In fact, donors with higher levels of As in blood were likely not to reside in high proximity to industries (although none of the protective factors were significant). All association estimates between industries and Cr levels were negative, however none of these was statistically significant. Analysis of Pb concentrations revealed the same pattern. The donors' proximity to quarries was likely to increase the Pb levels times 1.47 (p-value=0.013).
An inspection of association between the donors' *ambient pollution* levels and *industries* located within 5 km from their residence, revealed that presence of industries was positively correlated with $PM_{2.5}$ and CO and negatively correlated with PM_{10}. The latter was positively associated with quarries (Table 3, supplementary analysis).
We further investigated a joint impact of ambient pollutants and presence of any industries working with metals on metals' concentrations, adjusted to age, gender and smoking (analysis not shown). The analysis indicated a possible adverse impact of NO_2 on As levels (p-value<0.001), PM_{10} and SO_2 on Cr (p-value=0.023 and borderline p-value=0.093, respectively) and PM_{10} on Pb levels (borderline p-value=0.068).

The analysis presented in the tables above along with additional univariable testing (not shown here) can be concluded in the following diagram (Figure 10). The dashed lines represent the associations assumed based on literature and not tested in the current analysis.

Figure 10. Associations between exposure sources and concentrations of metals in blood, based on the analysis[1]



[1] Dashed arrows are assigned to assumed associations not tested in the current analysis.

Discussion and Conclusions

The first objective of the current project was to compare concentrations of metals in blood of donors residing in the Haifa Bay region and the rest of the country. Based on the analysis adjusting to age, gender and smoking status, we came to conclusion Haifa Bay residents are exposed to statistically lower levels of As and Cd. On the other hand, the levels of Cr and Pb appeared to be 1.08-1.10 times higher among Haifa Bay residents than in the rest of the country (although with borderline significance of 0.067 for Cr). These metals were 1.13 and 1.15 times higher for those who donated blood in the Haifa Bay region but not necessarily resided in the area..

What could explain the difference in metals concentrations between Haifa Bay region and the rest of the country? In general, elevated concentrations of metals like As and Cr can result from a diet, although an assumption of different dieting patterns between geographic regions seems extremely unlikely. The list of anthropogenic sources of As, Cd, Cr and Pb in the environment is long and many of these sources are not specific to a metal. For instance, Arsenic can be a product of automobile exhaust, wood preservatives, pesticides and dyes [33]. Cadmium can result from exposure to refined petroleum, paint, plastics, but also pesticides. The possible sources for Chromium includes chromeplanting, petroleum refining, electroplanting industry, textile manufacturing, and for Lead – petrol-based materials, and also pesticides and mobile batteries [33]. To indicate a source of pollution for each of the metals, a comprehensive investigation of potential industrial sources is warranted in future projects.

The findings of higher Cr and Pb and lower As and Cd, in the Haifa Bay area fit a pattern of an industrial metropolitan area not as much subjected to traffic-related pollution, as compared to Tel-Aviv area.

The second objective focused on the association of metals with ambient pollutants, in attempt to reveal an environmental source contributing to high metal concentration. Investigation of possible sources of the high levels of Pb, pointed at their association with CO, SO_2 and PM_{10}. The latter was found associated with proximity to quarries.

As concentrations were independently associated with $PM_{2.5}$ and NO_2.
An in-depth analysis of *industrial sources* was out of scope of the current project, yet it is highly warranted for the future analyses. Likewise, testing of collected but not yet tested samples must provide more granulated information on exposures and more certainty in identification of pollution sources and their possible control and/or elimination.

As a third objective, we considered developing a platform for a nation-wide human biomonitoring effort, featured by high temporal and spatial resolution. With this in mind, we attempted to maximally simplify the procedures of collecting and testing, that would be readily applicable in Israel and similar settings worldwide in future. Judging by (i) enrollment numbers exceeding the expectations and (ii) high spatial resolution of randomly chosen samples, this objective was met. Moreover, the temporal distribution over the span of 2 years of enrollment was minimally affected by the COVID-19 pandemic that started exactly with the study onset. The overall success of the study team in developing the platform supports the underlying idea of the current project, in taking advantage of Magen David Adom Blood Services, the national organization with well-developed infrastructure and procedures for samples collection. Likewise, collaboration with the national laboratory of public health granted the study the ability to conduct high quality tests among general population featured by low pollutants' concentrations.

The study has a few limitations. For instance, donors do not fully represent the general population. This is mainly related to donors being volunteering, usually featuring subjects with higher socio-economic level and higher health-related compliance. With that been said, this selection bias can hardly interfere with the main objective of human biomonitoring intended to monitor human exposures to chemicals, similarly to monitoring stations indicating the levels of ambient pollution. Furthermore, healthy and active donors not exposed to medications of any sort or behavior-related risk factors helps to provide reliable estimates of exposure clean from possible chemical substances.
Another limitation would be the inability to collect study-customized information on blood donors, such as diet or occupation For instance, an engineer in a factory might be more exposed to hazardous environment than a teacher. Accounting for occupation is warranted for valid comparisons between the regions. Nevertheless, prospective collection of additional information on donors would enormously complicate the enrollment and possibly harm the blood collection process itself. We therefore chose to compromise on working with an available information.

To conclude, the residents in Haifa Bay area are featured by low levels of As and Cd, and by high levels of Cr and Pb, as compared to the rest of the country. Donors with high Pb concentrations are likely to live close to quarries and be exposed to higher levels of PM_{10}, CO and SO_2. In general, ambient levels of pollution were found associated with internal metals' concentrations, confirming their contribution to the pathological pathway between air pollution and morbidity.

Applicability of the study results in Israel

The study provided an answer to the main thrust of the current study, which was to compare internal exposures of the Haifa Bay residents to the rest of the country. The information on elevated levels of Cr among donors from the Haifa Bay as compared to the general population in Israel provides an indication of a possible hazardous exposure. Identifying sources of Cr and Pbexposure can potentially reduce emission levels and thus eliminate chronic exposure of Haifa Bay residents to hazardous and carcinogenic chemical.
Another important consequence of the current study is the establishment of a framework of dynamic national biomonitoring by assessing the exposure to selected chemicals in Israel. The successful accomplishment of the research objectives and enrollment in numbers exceeding those planned in the study, prove that the national blood bank in Israel (MDA Blood Services) represents one of the best platforms for samples collection. The spatial coverage of the country in its entirety and routine collection of samples scheduled on all working days throughout the year, ensures a steady and uninterrupted supply of the samples available for the human biomonitoring purposes. This was demonstrated on enrollment numbers unaffected even by the COVID-19 pandemic that started exactly with the project onset.

Recommendation for future research

The study findings urge for more and immediate research in the following directions.

1. An extensive investigation of industries in Haifa Bay and other areas in the country featured by high levels of metals is warranted and will be conducted by the study researchers.
2. Additionally, testing of the remaining samples collected in the study seems to be of highest importance and has a potential to reveal possible sources of exposure and their elimination in future.
3. Association with morbidity indices, especially related to Cr exposure, should be explored.

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