Elemental analysis of particulate matter in a metal workshop and of biological samples from exposed workers

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Abstract

During metal welding and cutting, large amounts of particulate matter (PM) are produced that might represent a significant health risk for the exposed workers. In the present pilot study, we performed an elemental analysis of fine PM collected in a metal workshop. Also, elemental analysis of the hair and nail samples collected from workers exposed to the workshop dust and control group was done. Concentrations of 15 elements in PM were measured with X-Ray Fluorescence (XRF) and Particle Induced X-ray Emission (PIXE), whereas Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to determine 12 elements in hair and nail samples. Mean 8-h concentrations of $PM_{2.5}$, Fe, and Mn in the vicinity of welders were up to 1803 μg/m^3, 860 μg/m^3, and 30 μg/m^3, respectively, whereas in the nearby city, daily $PM_{2.5}$ concentrations are on average 11 μg/m^3. We found that several elements, especially Fe and Mn, had substantially higher concentrations in hair and nail samples of exposed workers than in the control group, which indicates the accumulation of metals in workers' tissues, although limit values were not exceeded.

Key words: metal workshops, particulate matter, XRF, PIXE, ICP-MS

1. Introduction

Particulate matter (PM) is a mixture of particles and liquid droplets suspended in the atmosphere and it stands as one of the most important air pollutants, especially its fine fraction ($PM_{2.5}$), which consists of particles less than 2.5 μm in diameter. These fine particles can enter the human respiratory

system and can, therefore, have harmful effects on human health [1]. Many studies have been conducted so far to monitor PM outdoors, but since people spend most of their time indoors, it is especially important to investigate indoor pollution. It is known that metal processing techniques such as welding, cutting, turning, grinding, and polishing produce significant amounts of PM.

Limit values (LV) for different pollutants are set for indoor working environments in many countries. For selected pollutants that are measured in the present study, we have found in the literature LVs from several countries (Croatia, Estonia, Germany, Hungary, Poland, and the USA). Overall, LVs from different countries are comparable, being of the same order of magnitude. However, some exceptions exist, among which the most pronounced differences are for Mn [2,3]. Therefore, potentially inadequate LVs are not country-specific but are typically a global problem.

In Croatia, where this study was performed, regulation for indoor pollutants in working environments sets LVs for many components of particulate matter [4], and those considered in this study are listed in Table 1. LVs are defined as mean concentrations during an 8-h working shift.

In metal workshops, welding and cutting are the most important sources of PM. The International Agency for Research on Cancer (IARC) classified welding fume as carcinogenic for humans (Group 1) [5]. Hazardous by-products of welding are many: fume components (fluorides, Al, SiO, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Mo, Cd, Pb), gases (CO_2, CO, NO_x, NO_2, O_3), radiant energy (UV, visible, IR), and other (heat, noise, vibration) [6]. Moreover, PM can also contain all components from outdoor sources, including organic, but these components depend on outdoor air quality, since they are not produced by metal processing. All these components can have a negative effect on human health, depending on their solubility, metal content, surface area, and reactivity [1], but here we focus on metal components as the most characteristic ones for the workshop. Health effects can be investigated through epidemiological, in vitro, or animal studies, but also by analysing tissue samples of the exposed workers. Tissue samples can

give some valuable information on different paths that metals take in organisms, especially on their possible deposition in the body.

Several studies have found a connection between occupational exposure to metals and metal levels in biological samples, but in many cases, the analysed samples were blood and/or urine of exposed workers [7–9], which show recent and acute exposures. In other studies, hair and/or nail samples were analysed [10–19], which are more appropriate biological samples for this purpose, since they can give information on the long-lasting accumulation of metals in human tissues [20,21]. In order to assess the connection between work exposure and accumulation of metals in tissues, it is important to monitor both metal levels in tissues and in PM, which was done only in three studies [12,14,17]. However, we have found only one study with a control group [14], but only the concentration of Mn was measured.

The aim of the present study was to determine the level of workers' exposure to metals in a metal workshop by elemental characterisation of indoor particulate matter and by evaluation of elemental concentrations in biological samples. The results of the pilot study with measurements of 15 elements in $PM_{2.5}$ and 12 elements in hair and nail samples are presented. This paper is a continuation of our earlier work [22], where we published concentrations of some elements in particulate matter samples.

2. Materials and Methods

2.1. Sample collection

Fine particulate matter was collected inside the metal workshop located in the suburb of the City of Rijeka, Croatia. The workshop has a ground and a first floor. The main metal processing activities in the workshop are welding (on the ground floor) and plasma cutting (on the first floor) of iron and steel. The ground floor is mostly confined. The workshop was neither ventilated nor heated during the sampling. The usual working shift starts at 6 am and finishes at 2 pm, but there is often some residual work to be done even after regular working time. Workers usually take a half-hour break for lunch around 11 or 12 am.

The sampling was performed during two separate periods. In total, 66 samples were collected, out of which 30 were 12-h and 36 were 1-h samples. The first sampling period lasted 13 days (Saturday, May 14–Friday, May 27, 2016). Twelve-hour samples were taken during the daytime (5 am–5 pm) and night-time (5 pm–5 am). The sampler was positioned on the ground floor in a separate room that is used as a storeroom and not for metal processing. The sampler inlet was positioned at 1.7 m above the floor, as an approximation of the average breathing height.

In order to obtain a better time resolution during working hours, in the second sampling campaign (Sunday, November 13–Thursday, November 17, 2016), hourly samples were taken from 6 am to 6 pm. For hourly samples collected during the night, it would be difficult to perform elemental analysis due to the low amount of collected deposit, so a single 12-h sample was taken from 6 pm to 6 am for each sampling day. In total, 36 hourly and four 12-h samples were taken. This time, the sampler was moved to the main working room where welding was performed, at about 5 m distance from the closest welding process. The sampler inlet was again at 1.7 m.

The sampling of $PM_{2.5}$ was performed using a cyclone sampler based on the ANSTO ASP sampler [23]. $PM_{2.5}$ was collected on thin polytetrafluoroethylene (PTFE) membrane filters (d = 25 mm).

Samples of hair and nails were collected from two groups of people. The experimental group for biological samples included 8 male workers from the metal workshop, aged from 25 to 69 years (median: 55 years) and with 5 to 45 years of working experience. Since the composition of hair and nails can depend on age, sex, ethnicity, and environmental pollution [21,24,25], the control group included 9 male volunteers aged from 26 to 63 years (median: 43 years) who lived in the same area as the workshop workers, but none of them was exposed to PM from metal workshops. The hair was cut into approx. 0.3 cm pieces, washed with 1:200 dilution of Triton X-100, after that rinsed with acetone and water, drained, and dried in an oven at 45 °C. Fingernails were cleaned with acetone for 20 minutes in an ultrasonic bath and then by ultrapure water for 20 minutes, dried, and cut into small pieces.

This study was done in accordance with the Helsinki Declaration and approved by the Ethical Committee for Biomedical Research of the Faculty of Medicine in Rijeka. The examined participants agreed to be involved in the research at their free will. Each volunteer signed an informed consent form prior to participating and filled out a short survey that covered basic questions including gender, age, smoking status, duration of work at this workplace, type of profession, and the most common activity during working hours.

2.2. Analysis

$PM_{2.5}$ concentrations were obtained by gravimetric measurements with a Mettler Toledo XA105 Dual Range balance (readability 10 μg) at the Laboratory for Macromolecular Research (University of Rijeka, Centre for Micro and Nano Sciences and Technologies). Each Teflon filter was weighed before and after sampling to obtain the total $PM_{2.5}$ mass. Before weighting, all samples were conditioned for at least 24 h, at a temperature of 22 °C and relative humidity of 20%. Since there was no specially equipped weighing room available for this purpose, the samples were conditioned in a desiccator, where the relative humidity of 20% was the most stable achievable one.

Elemental analysis of the $PM_{2.5}$ samples was performed with the Energy-Dispersive X-Ray Fluorescence technique (ED-XRF) at the University of Rijeka, Department of Physics. A rhodium X-ray tube was used under 1 mA and 50 kV, with a 2 mm diameter collimator placed perpendicularly to the sample. A silicon drift detector was positioned at 45° to the sample. An area of 8 × 8 mm^2 was scanned to avoid possible inhomogeneities in the sample. The analysis was performed in air. The QXAS program was used to analyse the spectra [26]. With this technique, concentrations of S, Cl, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, and Pb were obtained.

Additional elemental analysis was performed with Particle Induced X-Ray Emission (PIXE) at the Ruđer Bošković Institute to determine concentrations of several additional elements. A proton beam of 1.8 MeV was used for the excitation and the samples were placed in a vacuum chamber. A silicon drift

detector was used for low Z (Na-S) and a Si(Li) detector was used for higher Z elements (K-Pb). Analysis of the spectra was performed with the GUPIX software [27]. With this technique, concentrations of Na, Mg, Al, Si, P, S, Cl, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, and Pb were obtained.

In order to determine concentrations of Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, and Zn in biological samples, the Inductively Coupled Plasma Mass Spectrometer (ICP-MS) NexION 300X (PerkinElmer Instruments, Waltham, MA, USA) was used at the Teaching Institute of Public Health of Primorsko-goranska County in Rijeka. Approx. 0.1 g of nail or hair sample was digested using an Anton Paar Multiwave 3000 microwave system (Anton Paar GmbH, Graz, Austria) equipped with pressurised vessels, using 5 mL of 65% nitric acid (HNO_3 Suprapur, Merck, Germany), 4 mL of 30% hydrogen peroxide (H_2O_2 Suprapur, Merck, Germany), and 1 mL of 30% hydrochloric acid (HCl Suprapur, Merck, Germany) per sample over a 20-minute operation cycle at 1400 W. The digested samples were transferred to 25 mL volumetric flasks and filled to the mark with ultrapure water (Siemens Water Technologies Corp, Warrendale, PA, USA). Analytical blanks were prepared and run in the same way as the samples. The mass fractions of metals were determined using external standards, with standard solutions prepared in the same acid matrix. Standards for the instrument calibration were prepared on the basis of a multielement certified reference solution for ICP (PerkinElmer Instruments). The calibration curves with $r^2 > 0.999$ were accepted for concentration calculation.

Considering the small-size biological samples, we tested the normality using the Kolmogorov-Smirnov test. Since the variables were not normally distributed, we used the nonparametric Mann-Whitney U test to test the differences between the experimental and control group.

3. Results

3.1. Particulate matter samples

Since the elements from S to Pb were measured with both XRF and PIXE, the obtained elemental concentrations were compared. The results were consistent, with the systematic error between the

methods comparable to the measurement uncertainties. The correlations between the two results were very good ($r^2 > 0.8$). For future analysis, PIXE concentrations were used for Na to P and XRF concentrations for all other elements. The concentrations of Cl, K, Sc, V, Co, and Pb were usually lower or very close to the limit of detection (LOD) or hardly detectable because of interference with other, usually much stronger lines in the spectra (Co), which is why these elements were not considered (except K for the first sampling period). Uncertainties of measurement were calculated from the uncertainty of flow measurement during the sampling, uncertainty of spectra fitting (statistical and systematic), uncertainty of mass, and uncertainty of system calibration. Overall uncertainties were estimated to be less than 10% for concentrations well above the LOD. For trace elements like Na or Mg, the uncertainties were 20% or higher.

During the first sampling period, the 12-h concentrations of elements and $PM_{2.5}$ (Figure 1) showed a pattern clearly connected to the working activities. The concentrations during the night and weekends were almost constant, but the daytime concentrations during the weekdays were on average 6 times higher for $PM_{2.5}$ and up to 27 times higher for the concentrations of some elements. Most of the elements followed a similar trend as $PM_{2.5}$. Manganese, an element that typically occurs in the workshop dust during welding operations, had the biggest day-night increase. The time series for S, on the other hand, showed no relationship with the working pattern, which was expected, since S mostly originates from outdoor sources.

The better time resolution during the second sampling period, which included both 12-h and 1-h samples, provided more detailed information. Much higher concentrations were measured compared to the first period because the sampler was moved closer to the welders. The temporal changes in elemental and $PM_{2.5}$ concentrations are shown in Figure 2. At the beginning of the working shift (grey areas), the concentrations of $PM_{2.5}$ and most elements increased rapidly compared to the non-working hours. The concentrations of elements varied according to the changes in the work intensity and at the end of the

working shift, there were clearly visible decreases of concentrations on the first and third day. However, on the second day, those decreases were not so pronounced for many elements due to additional work performed at the workshop. The variations of Fe and Mn concentrations follow variations in $PM_{2.5}$. The hourly Mn concentrations were up to 250 times higher than during the night.

In order to compare these concentrations with the limit values, 8-h equivalent concentrations were calculated for each element and each sampling day, and the results are presented in Table 1. These 8-h concentrations can be compared to the LVs for the respirable fraction, if existing, also reported in Table 1. Our 8-h concentrations did not exceed the LVs. More precisely, they were for at least one order of magnitude lower than the LVs. However, the Mn concentrations in the second and third day exceeded the German and ACGIH limit value of 20 $\mu g/m^3$.

3.2. Biological samples

Table 2 shows the median values and ranges of metal concentrations in hair and nail samples of workshop workers and controls. Samples with concentrations below the LOD were replaced with 1/2 LOD for calculating median values. Elemental concentrations are shown in Figures 3 and 4. A comparison of these two groups was done with the Mann-Whitney U test (Table 2). Several elements had substantially higher concentrations in hair and nails of workers than in the control group, but the lowest *p* values were obtained for Fe and Mn in hair samples and for Ti, Fe, and Mn in nail samples. Concentrations of Cr and Mn in hair and of Mn in nails in almost all samples of the control group were below the LOD, whereas for workers, they were around two orders of magnitude larger than the LOD.

4. Discussion

4.1. Particulate matter samples

As expected, elemental and $PM_{2.5}$ concentrations in the workshop were substantially higher than in the outdoor air. In the recent outdoor study in the City of Rijeka, medians of daily $PM_{2.5}$, Fe, and Mn

concentrations were found to be 11 μg/m^3, 0.1 μg/m^3, and 0.0053 μg/m^3, respectively, whereas their third quartiles were 14 μg/m^3, 0.18 μg/m^3, and 0.0079 μg/m^3, respectively [28].

Comparing the two sampling sites in the workshop, the mean concentrations were about 10 times higher in the working room than in the storeroom during the working hours, but in non-working periods the concentrations were comparable for all elements except for Fe, which was still several times higher in the working room. This could mean that, during the non-working time, most of the particles deposit relatively quickly and the remaining particles are mixed through all the rooms.

Elements typical for steel processing, such as Fe, Mn, Cu, and Si, have shown the greatest day-night increase. Other elements had lower production in the workshop but higher outside sources or resuspension inside. Resuspension can be an important source of PM even in residential houses [29]. The only element that did not show any day-night increase in the first period was S, but when the sampling was performed close to the welding in the second period, S showed some small daily variation, which points to at least some production of S from steel.

We believe that the days that have been selected for the second period are representative of the workshop. Although on some working days PM concentrations can be very low, as the last two days in the first sampling period, days like these are not so common.

The mean elemental concentrations during the second sampling period were not higher than the LVs in Croatia for any of the elements, but the Mn levels exceeded the German and USA ACGIH LV of 20 μg m^{-3} [2,3] in the second and the third day (Table 1). The Croatian LV for Mn is set only for the inhalable fraction as 500 μg m^{-3}, whereas the German LV for inhalable Mn is 200 μg m^{-3}. In our opinion, the currently valid limit values in Croatia are not adequate in two major points. The LVs are not set for thee respirable fraction for most of the species, although this fraction should be of major concern for its health effects. In addition, the present LVs are very high for specific species, as high as PM concentrations in a sand storm. This implies that the overall allowed PM concentration is even higher

than that when all species are included. Moreover, the current regulation does not follow the recent concern about welding fume being carcinogenic nor the concern about Mn, which recently led to lowering the ACGIH LV for $PM_{2.5}$ and Mn from 200 to 20 μg m^{-3} in the USA.

It is important to note that the LVs are set as 8-h means through the working period, but in this study, high concentrations were prolonged even after official working hours, so it is possible that the workers were often exposed to high concentrations of Mn and other elements for periods longer than 8 h per day (in the case of overtime work). Moreover, in the second period, the sampler was moved closer to the welders but was still several meters from them, so the actual exposure was probably even higher than the measured one, as one study [30] found that PM concentrations in the vicinity of the welding process can be up to 40% lower than concentrations obtained by a personal sampler.

During all three days in the second period, elemental and $PM_{2.5}$ concentrations showed a clear pattern, closely connected to the working activities (Figure 2). The concentrations of P, Ti, Cr, Mn, Fe, Ni, and Cu showed almost the same changing behaviour as $PM_{2.5}$. The most interesting components of $PM_{2.5}$ are Fe and Mn, since Fe makes about 50% of the total mass, probably in the form of oxides, and Mn is a characteristic compound of welding fume as it is one of the constituents of steel and welding electrodes.

Mean concentrations during the working shifts were within previously reported ones found in the literature [7,8,30–35]. In most of these studies, personal sampling was performed, which usually yields higher concentrations than stationary ones [30,36], but even in this way, it can be concluded that the workshop in the present study is not an extremely polluted one compared to the others.

4.2. Biological samples

In the present study, in almost all cases, the median metal concentrations were higher in the workers' group than for the control one. When regarding both hair and nail samples, this difference is the most significant for Fe and Mn, which were also the most abundant elements in $PM_{2.5}$ in our case. This

suggests that Mn and Fe were accumulated because of the overexposure to these metals in $PM_{2.5}$ at the workplace. It is known that Mn-overexposure can lead to Mn-neurotoxicity and symptoms such as motor and cognitive deficiencies, tremors, gait problems, memory loss, psychotic behaviour, etc. [37,38] and, because Mn and Fe share some important membrane transporters [39,40], the two metals can influence each other's homeostasis and toxicity [38,39,41]. For example, it has been shown that iron loading could alter the physiological function of Mn in the brain and could further modify Mn-induced brain dysfunction [42].

Furthermore, higher levels of Fe and Mn were found in hair and nail samples, although Mn and Fe concentrations in $PM_{2.5}$ were not even close to current Croatian LVs. However, at the same time, the German LV for Mn was exceeded during two out of three sampling days, which could suggest that the German regulations are possibly better adjusted, at least in the case of working environments with significant metal processing. Metal workshops could be of special concern regarding Mn production, not only because of high Mn production but also because of the simultaneous high production of Fe, which can further enhance absorption of Mn within the body. At this stage, we have not examined a possible link between the accumulation of metals and potential health problems of examined workers, but we think that such a study could be interesting. If further studies confirm negative impacts on health, we suggest lowering the LVs in Croatia and many other countries with similar regulations.

We were able to find only one study which analysed both PM and hair samples of exposed workers [12]. Similar to our results, the estimated exposure to Mn in this study was in the range of 0.2–44.7 μg/m^3, whereas Mn in hair was in the range of 0.1–51.5 μg/g. In the study of PM and nail samples [14], Mn in PM_4 was in the range of 8–477 μg/m^3 with a mean value of 129 μg/m^3, whereas Mn in toenails was in the range of 3.53–15.56 μg/g with a mean value of 6.87 μg/g. Although our airborne Mn was much lower than this one, much higher Mn levels were found in the nails.

The elemental concentrations in hair samples for the control group were compared to the values previously reported in the literature for the unexposed, healthy population [11,15,43–48]. As expected, the reported ranges vary considerably. In almost all cases, our results are inside the reported ranges. It can, therefore, be concluded that our control group is typical.

The concentrations of Cr, Mn, and Fe in the hair samples taken from workshop workers are significantly higher in the present study than in similar studies that can be found in the literature [13,15,16,18,19], whereas other elements have comparable levels. Since these studies did not perform $PM_{2.5}$ sampling, one possible reason for this could be that these workshops had lower metal pollution than the one in the present study. Other studies reported Fe concentrations in hair in the range of 15–27 μg/g [16], 4.35–61.1 μg/g [15], 32.0–380 μg/g [18], and 40.1–277.8 μg/g [19]. Our results for Mn concentrations in hair are similar to those reported by [19], where Mn was in the range of 7.5–29.7 μg/g. Other studies reported Mn in the range of 0.26–2.4 μg/g [16], 0.07–9.85 μg/g [15], and 4.10–10.9 μg/g [18]. Information about concentrations of Al, Ti, and V for exposed workers was not found in the literature.

The concentrations for the nail samples from the control group for most of the elements were found to be within the previously reported data [11,14,45,49–51], with few samples outside these ranges, and only V concentrations were much higher than values that can be found in the literature.

The concentrations for the nail samples of workers can hardly be compared to the values from the literature. We found only four studies that analysed nail samples from welders, which were just for Mn, Ni, Cd, and Pb [10,11,14,17]. Our results for Mn and Ni are much higher, but for Cd and Pb they are within the range reported in those studies.

5. Conclusions

Occupational exposure to metals can have an adverse impact on health, and studies on metal accumulation in biological samples of workers could be the first step in metal overload investigation.

However, so far, such investigations are scarce and not many can be found in the literature. We believe that the study presented here gives a valuable contribution to the field of occupational health because we simultaneously measured the total amount and concentration of elements in the PM together with the appropriate biological samples (hair and nails) of exposed workers and a control group.

$PM_{2.5}$ and elemental concentrations in the chosen metal workshop have shown a clear pattern connected to the workshop activities, being significantly higher during the working shift than during the night. The most abundant elements were Fe, constituting about one half of the total mass, and Mn, typical for arc welding. In hair and nail samples, the differences between the experimental and control group were the most significant for Fe and Mn.

Since elevated values of Fe and Mn were determined in our study in both air and biological samples (hair and nails), it could be suggested that the accumulation in the examined tissues could come from the overexposure to both metals at the working place during working hours.

Elevated concentrations of Fe and Mn were found in tissues although the LVs were not exceeded for these elements (the measured concentrations were at least one order of magnitude lower than the LVs), according to Croatian regulations. Moreover, the regulations in Croatia, as in many other countries, do not consider Mn as a neurotoxic element, nor they consider welding fume as carcinogenic. We believe that regulations should be revised and more studies on this topic should be performed.

This study is a pilot study because only one workshop was involved in PM sampling and only 8 workers and 9 controls participated with their biological samples. For a thorough examination of air quality in workshops and deposition of metals in tissues, both more workshops and more participants should be included. Therefore, we are continuing the investigation in additional metal workshops.

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Declaration of interest

The authors declare that they have no conflicts of interest to disclose.

References

[1] C. A. Pope III, D. W. Dockery, *J. Air Waste Manag. Assoc.* **2006** ; *56* , 707. DOI: 10.1080/10473289.2006.10464484.

[2] Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, *Technische Regeln Für Gefahrstoffe: Arbeitsplatzgrenzwerte* **2018**.

[3] OSHA, "OSHA Annotated Table Z-1," available at https://www.osha.gov/dsg/annotated-pels/tablez-1.html, **2020**.

[4] Official Gazette 13/09 and 75/13, *Official Gazette 13/09 and 75/13*, Http://Narodne-Novine.Nn.Hr/Clanci/Sluzbeni/2009_01_13_300.Html, Republic of Croatia **2009**.

[5] WHO, *Welding, Molybdenum Trioxide, and Indium Tin Oxide*, Vol. 118 **2018**.

[6] J. M. Antonini, *Crit. Rev. Toxicol.* **2003** ; *33* , 61. DOI: 10.1080/713611032.

[7] D. G. Ellingsen, L. Dubeikovskaya, K. Dahl, M. Chashchin, V. Chashchin, E. Zibarev, Y. Thomassen, *J. Environ. Monit.* **2006** ; *8* , 1078. DOI: 10.1039/b605549d.

[8] B. Pesch, T. Weiss, B. Kendzia, J. Henry, M. Lehnert, A. Lotz, E. Heinze, H. U. Käfferlein, R. Van Gelder, M. Berges, et al., *J. Expo. Sci. Environ. Epidemiol.* **2012** ; *22* , 291. DOI: 10.1038/jes.2012.9.

[9] B. Sjogren, V. Lidums, M. Hakansson, L. Hedstrom, *Scand. J. Work. Environ. Health*. **1985** ; *11* , 39. DOI: 10.5271/sjweh.2255.

[10] R. Grashow, J. Zhang, S. C. Fang, M. G. Weisskopf, D. C. Christiani, J. M. Cavallari, *J. Occup. Environ. Hyg.* **2014** ; *11* , 397. DOI: 10.1080/15459624.2013.875182.

[11] R. Mehra, M. Juneja, *Indian J. Biochem. Biophys.* **2004** ; *41* , 53.

[12] B. Reiss, C. D. Simpson, M. G. Baker, B. Stover, L. Sheppard, N. S. Seixas, *Ann. Occup. Hyg.* **2016** ; *60* , 139. DOI: 10.1093/annhyg/mev064.

[13] A. Sukumar, R. Subramanian, *Biol. Trace Elem. Res.* **2000** ; *77* , 139. DOI: 10.1385/BTER:77:2:139.

[14] E. J. Ward, D. A. Edmondson, M. M. Nour, S. Snyder, F. S. Rosenthal, U. Dydak, *Ann. Work Expo. Heal.* **2018** ; *62* , 101. DOI: 10.1093/annweh/wxx091.

[15] A. Khalique, M. H. Shah, M. Jaffar, N. Shaheen, S. R. Tariq, S. Manzoor, *Toxicol. Environ. Chem.* **2006** ; *88* , 697. DOI: 10.1080/02772240600883627.

[16] M. Teresa, S. D. Vasconcelos, H. M. F. Tavares, *Sci. Total Environ.* **1997** ; *205* , 189. DOI: 10.1016/S0048-9697(97)00208-8.

[17] W. Laohaudomchok, X. Lin, R. F. Herrick, S. C. Fang, J. M. Cavallari, D. C. Christiani, M. G. Weisskopf, *J. Occup. Environ. Med.* **2011** ; *53* , 506. DOI: 10.1097/JOM.0b013e31821854da.

[18] V. V. S. Ramakrishna, V. Singh, A. N. Garg, *Sci. Total Environ.* **1996** ; *192* , 259. DOI: 10.1016/S0048-9697(96)05316-8.

[19] M. C. Yebra-Biurrun, R. M. Cespón-Romero, *Anal. Bioanal. Chem.* **2007** ; *388* , 711. DOI: 10.1007/s00216-007-1264-5.

[20] I. M. Kempson, E. Lombi, *Chem. Soc. Rev.* **2011** ; *40* , 3915. DOI: 10.1039/c1cs15021a.

[21] K. He, *Eur. J. Clin. Invest.* **2011** ; *41* , 98. DOI: 10.1111/j.1365-2362.2010.02373.x.

[22] M. Čargonja, G. Žauhar, I. Orlić, *RAD Assoc. J.* **2017** ; *2* , 220. DOI: 10.21175/radj.2017.03.044.

[23] D. D. Cohen, E. Stelcer, D. Garton, J. Crawford, *Atmos. Pollut. Res.* **2011** ; *2* , 182. DOI: 10.5094/APR.2011.023.

[24] Z. Meng, *Biol. Trace Elem. Res.* **1998** ; *61* , 79. DOI: 10.1007/BF02784043.

[25] B. J. Kim, J. I. Na, W. S. Park, H. C. Eun, O. S. Kwon, *Int. J. Dermatol.* **2006** ; *45* , 1435.

[26] P. Van Espen, K. Janssens, J. Nobels, *Chemom. Intell. Lab. Syst.* **1986** ; *1* , 109. DOI: 10.1016/0169-7439(86)80031-4.

[27] J. L. Campbell, N. I. Boyd, N. Grassi, P. Bonnick, J. A. Maxwell, *Nucl. Instruments Methods Phys. Res. Sect. B Beam Interact. with Mater. Atoms*. **2010** ; *268* , 3356. DOI: 10.1016/j.nimb.2010.07.012.

[28] M. Čargonja, D. Mekterović, D. Mance, G. Žauhar, I. Bogdanović Radović, I. Zamboni, *X-Ray Spectrom.* **2019** ; *48* , 561. DOI: 10.1002/xrs.3052.

[29] T. L. Thatcher, D. W. Layton, *Atmos. Environ.* **1995** ; *29* , 1487. DOI: 10.1016/1352-2310(95)00016-R.

[30] M. Lehnert, B. Pesch, A. Lotz, J. Pelzer, B. Kendzia, K. Gawrych, E. Heinze, R. Van Gelder, E. Punkenburg, T. Weiss, et al., *Ann. Occup. Hyg.* **2012** ; *56* , 557. DOI: 10.1093/annhyg/mes025.

[31] M. Wallace, S. Shulman, J. Sheehy, *Appl. Occup. Environ. Hyg.* **2001** ; *16* , 771. DOI: 10.1080/10473220117155.

[32] A. Smargiassi, M. Baldwin, S. Savard, G. Kennedy, D. Mergler, J. Zayed, *Appl. Occup. Environ. Hyg.* **2000** ; *15* , 746. DOI: 10.1080/10473220050129383.

[33] M. R. Flynn, P. Susi, *Stoch. Environ. Res. Risk Assess.* **2010** ; *24* , 377. DOI: 10.1007/s00477-009-0327-x.

[34] R. E. Korczynski, *Appl. Occup. Environ. Hyg.* **2000** ; *15* , 936. DOI: 10.1080/104732200750051175.

[35] D. Mattorano, J. Harney, C. Cook, K. Roegner, *Appl. Occup. Environ. Hyg.* **2001** ; *16* , 339. DOI: 10.1080/10473220117911.

[36] A. L. Insley, J. R. Maskrey, L. A. Hallett, R. C. D. Reid, E. S. Hynds, C. Winter, J. M. Panko, *J. Occup. Environ. Hyg.* **2019** ; *16* , 410. DOI: 10.1080/15459624.2019.1603389.

[37] S. L. O'Neal, W. Zheng, *Curr. Environ. Heal. Reports*. **2015** ; *2* , 315. DOI: 10.1007/s40572-015-0056-x.

[38] A. C. Pfalzer, A. B. Bowman, *Curr. Environ. Heal. Reports*. **2017** ; *4* , 223. DOI: 10.1007/s40572-017-0136-1.

[39] Q. Ye, J. E. Park, K. Gugnani, S. Betharia, A. Pino-Figueroa, J. Kim, *Metallomics*. **2017** ; *9* , 1028. DOI: 10.1039/C7MT00079K.

[40] T. E. Gunter, B. Gerstner, K. K. Gunter, J. Malecki, R. Gelein, W. M. Valentine, M. Aschner, D. I. Yule, *Neurotoxicology*. **2013** ; *34* , 118. DOI: 10.1016/j.neuro.2012.10.018.

[41] J. A. Roth, *Biol. Res.* **2006** ; *39* ,. DOI: 10.4067/S0716-97602006000100006.

[42] A. C. G. Chua, E. H. Morgan, *Biol. Trace Elem. Res.* **1996** ; *55* , 39. DOI: 10.1007/BF02784167.

[43] J. P. Goullé, L. Mahieu, J. Castermant, N. Neveu, L. Bonneau, G. Lainé, D. Bouige, C. Lacroix, *Forensic Sci. Int.* **2005** ; *153* , 39. DOI: 10.1016/j.forsciint.2005.04.020.

[44] H. Faghihian, H. Rahbarnia, *J. Radioanal. Nucl. Chem.* **2002** ; *251* , 427. DOI: 10.1023/A:1014882125256.

[45] I. Rodushkin, M. D. Axelsson, *Sci. Total Environ.* **2000** ; *262* , 21. DOI: 10.1016/S0048-9697(00)00531-3.

[46] P. O. Onuwa, L. A. Nnamonu, I. S. Eneji, R. Sha'Ato, *J. Anal. Sci. Methods Instrum.* **2012** ; *02* , 187. DOI: 10.4236/jasmi.2012.24029.

[47] I. Baranowska, L. Barchański, M. Bąk, B. Smolec, Z. Mzyk, *Polish J. Environ. Stud.* **2004** ; *13* , 639.

[48] M. Jergović, Prisutnost Metala i Drugih Rijetkih Elemenata i Utjecaj Na Zdravlje Stanovništva Istočne Hrvatske **2011**.

[49] G. Karimi, S. Shahar, N. Homayouni, R. Rajikan, N. F. A. Bakar, M. S. Othman, *Asian Pacific J. Cancer Prev.* **2012** ; *13* , 4249. DOI: 10.7314/APJCP.2012.13.9.4249.

[50] A. R. Aguiar, M. Saiki, *J. Radioanal. Nucl. Chem.* **2001** ; *249* , 413. DOI: 10.1023/A:1013235123875.

[51] R. Masironi, S. R. Koirtyohann, J. O. Pierce, R. G. Schamschula, *Sci. Total Environ.* **1976** ; *6* , 41. DOI: https://doi.org/10.1016/0048-9697(76)90005-X.

Tables

Table 1. Eight-hour working-shift mean concentrations of elements and $PM_{2.5}$ for each day in the second sampling period, and limit values (LV) for inhalable (I) and respirable fraction (R) of PM valid in Croatia. All concentrations are expressed in μg/m^3. Results are rounded to two significant digits.

	November 14th	November 15th	November 16th	LV(I)	LV(R)
Na	1.9	2.6	2.8		
Mg	0.27	0.39	0.48		
Al	1.9	2.2	2.5	10 000	4 000
Si	8.4	14	22	10 000	4 000
P	0.31	0.40	0.46	100	
S	1.0	1.5	1.8		
Ca	6.3	5.5	4.1		
Ti	0.73	0.89	1.1		
Cr	0.42	0.66	0.82	2 000	
Mn	15	22	30	500	
Fe	500	600	860		
Ni	0.39	0.44	0.54	500	
Cu	6.0	7.1	9.2	1 000	
Zn	0.70	0.71	0.83		
$PM_{2.5}$	990	1200	1800		

Table 2. Median values and ranges of metal concentrations in hair and nail samples of metal workers (n = 8) and controls (n = 9), along with the p values obtained from the comparison with the Mann-Whitney U test.

	Hair samples			Nail samples		
	Median [range]		p	Median [range]		p
	Workers	Controls		Workers	Controls	
Al	180 [31–930]	13 [<10–170]	0.003	120 [<10–1200]	53 [<10–560]	0.470
Ti	31 [12–38]	11 [4.6–33]	0.011	73 [38–870]	12 [8.6–39]	< 0.001
V	2.5 [<5–41]	2.5 [<5–160]	0.962	100 [<5–7000]	25 [11–150]	0.194
Cr	11 [<0.5–36]	0.25 [0.5–4.5]	0.014	58 [<0.5–140]	1.3 [<0.5–17]	0.008
Mn	8.1 [3.6–32]	0.25 [<0.5–0.79]	< 0.001	36 [<0.5–130]	0.25 [<0.5]	0.003
Fe	650 [250–1300]	37 [15–180]	< 0.001	5500 [2300–15000]	78 [24–170]	< 0.001
Co	0.25 [0.16–6.1]	0.18 [<0.1–1.6]	0.194	1.6 [<0.1–2.7]	0.050 [<0.1–0.18]	0.003
Ni	2.4 [<0.5–11]	0.78 [<0.5–3.6]	0.112	20 [<0.5–72]	1.0 [<0.5–87]	0.124
Cu	27 [21–42]	20 [6.5–95]	0.194	31 [20–72]	11 [9.1–200]	0.008
Zn	190 [99–290]	220 [56–890]	0.163	160 [130–1600]	130 [83–190]	0.024
Cd	0.14 [0.078–0.36]	0.076 [<0.05–0.28]	0.470	0.36 [0.060–0.73]	0.076 [<0.05–0.19]	0.011
Pb	2.0 [0.83–27]	0.40 [0.11–4.7]	0.024	3.8 [<0.1–11]	0.61 [<0.1–12]	0.054

Figure captions

Figure 1. Weekly variations of $PM_{2.5}$ and elemental concentrations during the first sampling period.

Figure 2. Temporal variations of elemental and $PM_{2.5}$ concentrations during the second sampling period. Grey areas indicate working hours; dashed lines indicate one missing sample.

Figure 3. Metal concentrations in hair samples of exposed workers and control group. Concentrations lower than the limit of detection (LOD) are replaced with ½ LOD and indicated by squares.

Figure 4. Metal concentrations in nail samples of exposed workers and control group. Concentrations lower than the limit of detection (LOD) are replaced with ½ LOD and indicated by squares.

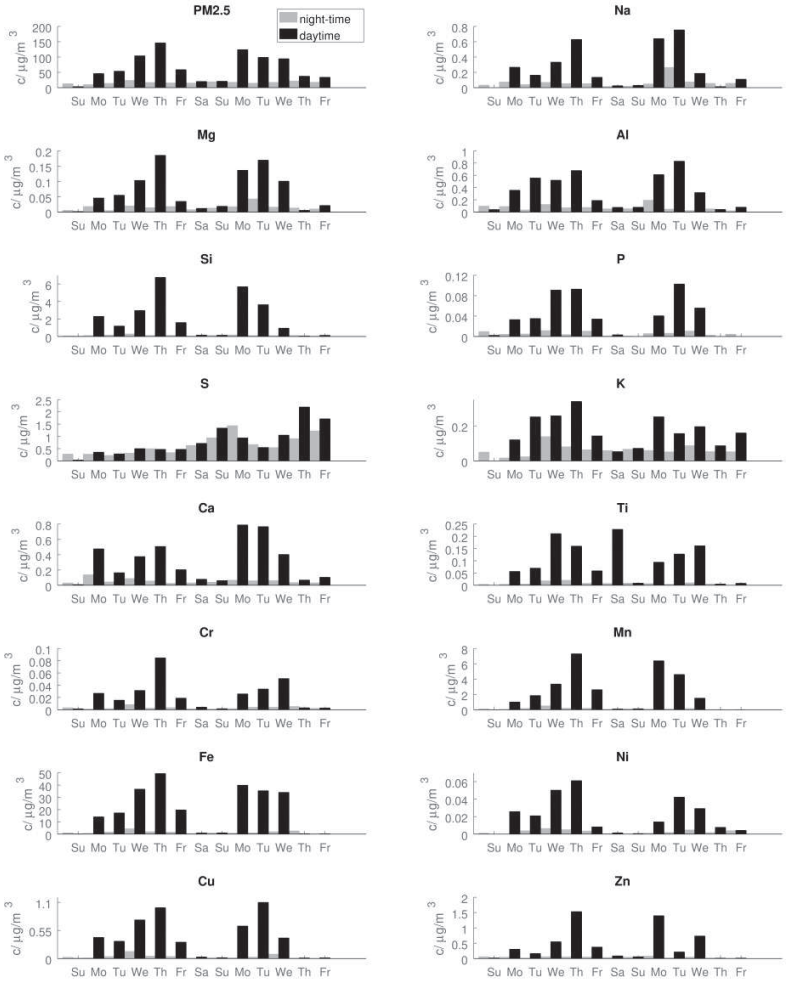

Figure 1. Weekly variations of $PM_{2.5}$ and elemental concentrations during the first sampling period.

116x145mm (1200 x 1200 DPI)

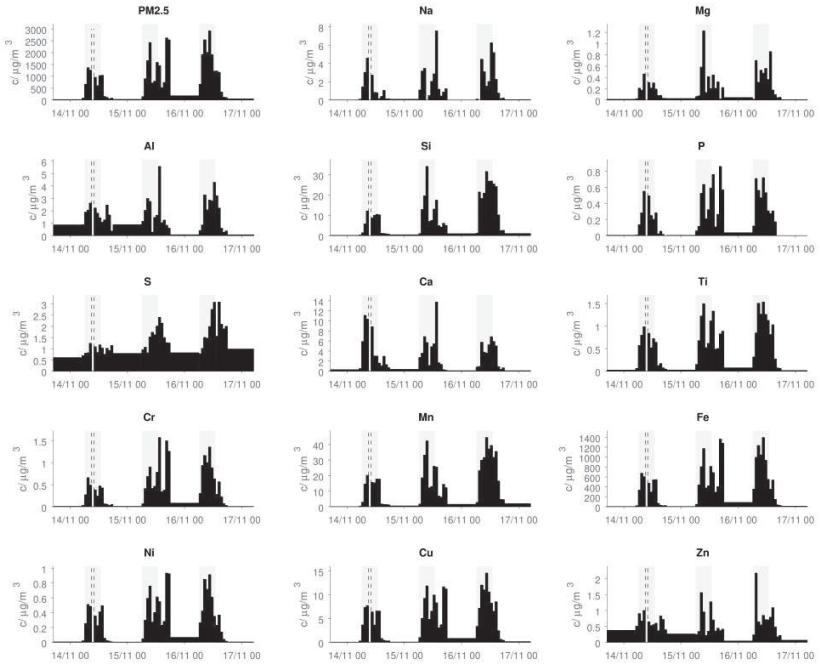

Figure 2. Temporal variations of elemental and $PM_{2.5}$ concentrations during the second sampling period. Grey areas indicate working-hours; dashed lines indicate one missing sample.

145x116mm (1200 x 1200 DPI)

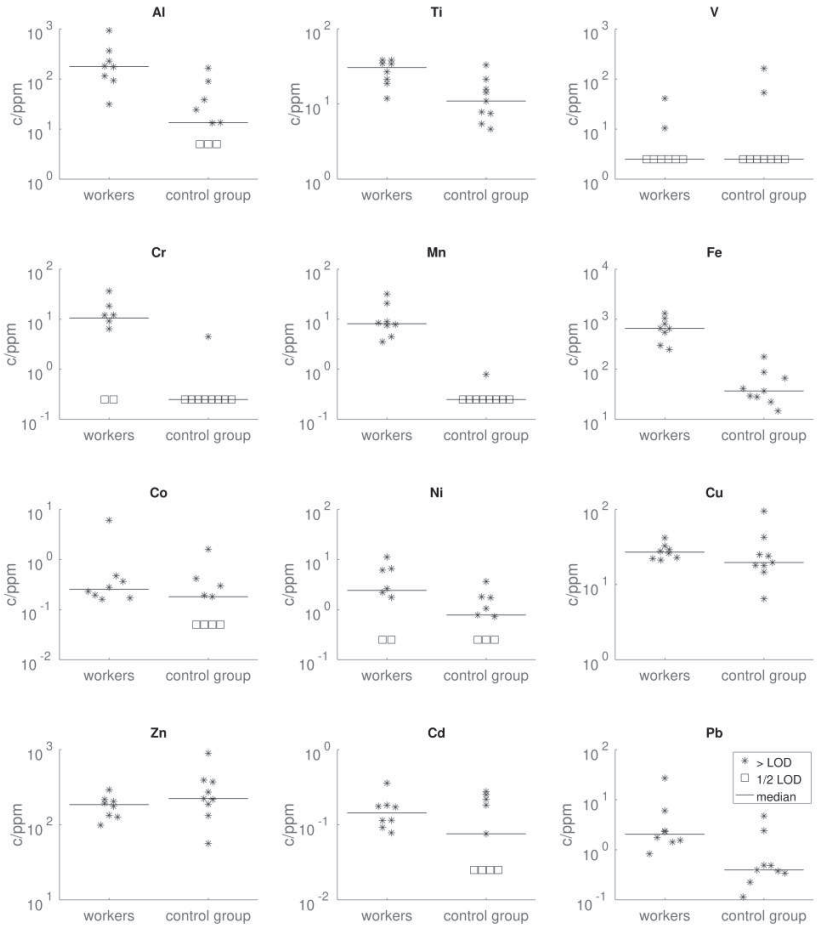

Figure 3. Metal concentrations in hair samples of exposed workers and control group. Concentrations lower than the limit of detection (LOD) are replaced with ½ LOD and indicated by squares.

116x130mm (1200 x 1200 DPI)

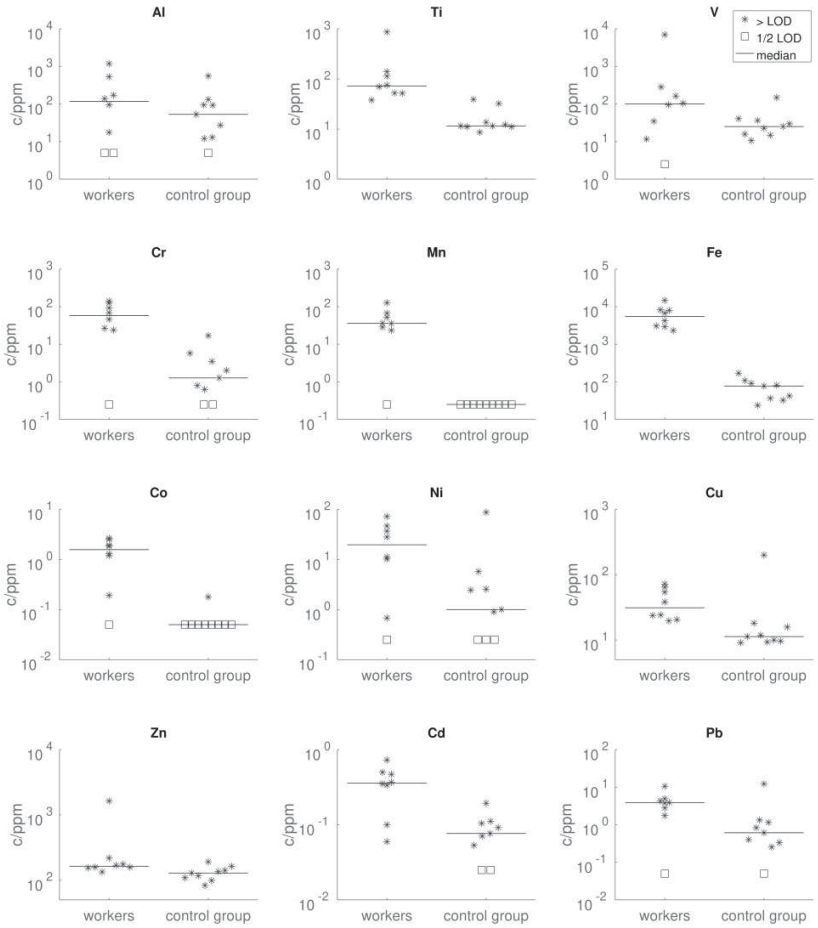

Figure 4. Metal concentrations in nail samples of exposed workers and control group. Concentrations lower than the limit of detection (LOD) are replaced with ½ LOD and indicated by squares.

116x130mm (1200 x 1200 DPI)