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Impact of Fish, Mollusk and Seafood Consumption before Sample Donation on Urinary and Toenail Metal Levels in Workers Exposed to Heavy Metals

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Abstract: Introduction: We assessed the impact on metal levels of seafood, mollusk and fish consumption (SMFc) before urine and toenail sample donation among workers exposed to metals. Methods: This is a cross-sectional epidemiological study with 101 workers from the chemical and metal industry and 40 unexposed workers from the services sector. We measured urinary (As, Ba, Be, Cd, Co, Cu, Hg, Li, Mo, Pb, Se, Sr, Tl, V, W and Zn) and toenail (same plus Al, Cr, Fe, Mn, Ni and U) metal levels. Results: Urinary arsenic levels were higher among workers eating seafood or mollusks (102 ppm vs. 55.4 ppm; $p = 0.042$) or fish (109 ppm vs. 48 ppm; $p = 0.007$) 8 h before sample donation. Urinary mercury was associated with consumption of blue fish (11.865 ppm) and canned sardines (19.125 ppm) ($p = 0.028$). With respect to toenails, fish consumption was associated with aluminum (17 ppm vs. 8.6 ppm; $p = 0.012$) and beryllium (5 ppb vs. 1 ppb; $p = 0.017$). Arsenic urinary levels were associated with numbers of hours prior to sample collection since latest SMFc ($p = 0.001$). Conclusion: Among workers exposed to metals, seafood, mollusk and fish consumption is an important determinant of urinary arsenic levels, as sea fish for urinary mercury, but not for other metals.

Keywords: fish, mollusk and seafood consumption; urine; toenail; metal levels; workers; heavy metals

1. Introduction

Human exposure to toxic metals is increasing, which has led to a growing interest from the scientific community to investigate their health effects [1]. Toxic metals, including "heavy metals and metalloids", are individual metals and metal compounds that can affect negatively people's health [2,3].

Metals can be separated into essential and non-essential according to their effects on biological systems. Essential elements are either needed to maintain physiologically important functions or part of organic structures with vital functions in humans [4]. However, in larger amounts, they become toxic [2,3]. The list of essential elements includes Cu, Co, Cr, Fe, Mn, Mo, Se and Zn. Furthermore, non-essential trace elements, including Al, As, Cd, Cr, Hg, Ni, Pb, Sb, Sn and U, do not have any established biological function in humans, and they could exert harmful long-term health effects even at low concentrations [5].

Humans have developed mechanisms to achieve metal balance in the body: a general metal response pathway, which responds to toxic conditions for both essential and non-essential elements, and other specific response pathways to transport and regulate

homeostasis of a particular essential element [6,7]. Moreover, toxic metals do not remain in the body easily; the majority of them are expelled from the body through sweat, urine and feces. However, if organic/inorganic compounds have been formed, they accumulate mainly in bones, myocardial tissue, internal parenchymal organs, skin, nails and hair [1,8]. Thus, the effects on the human body vary depending on both the characteristics of each metal and the affinity of each tissue [9,10]. As, Pb and Hg lead the podium of the Agency for Toxic Substances and Disease Registry (ATSDR) 2022 substance priority list [11]. Cd ranks seventh, and it is the third inorganic substance. Among the most important human health effects due to exposure to metals, we can find cancer [12], cardiovascular diseases [13] and neurotoxicity [14].

These elements can enter the human body via three main routes, namely inhalation, transdermal adsorption and ingestion [15,16]. Dietary intake, including food, drinking water, soil and dust ingestion, are the main source of exposure to metals in the general population [17], although recent studies have reported that levels of ingestion in daily foods are lower compared to inhalation exposure via airborne particles, the main exposure pathway [15,16]. Among metal, chemical and mining workers, occupational exposure would be the most important source through the inhalation and transdermal adsorption of dust and airborne particles [18]. Occupational exposure to metals is more frequent than what initially could be expected, in both the environmental and the occupational settings [19,20].

Hair, nails, blood, umbilical cord urine, feces, placenta and breast milk are commonly used biomarkers in research of contamination risks to human health [21]. Urine, saliva and blood samples offer short-term exposure (24 h), and are largely influenced by diet [22–24]. Urine biomonitoring of metal levels among workers is a simple, non-invasive and common way to monitor levels of such elements in the occupational environment [25]. On the other hand, nails and hair have been used as internal exposure indicators and may reflect the long-term (6 $\pm$ 18 months) patterns of mineral metabolism [26–28]. Furthermore, the advantage of using toenails as a biomonitoring tool is that unlike hair or fingernail samples, it reduces the likelihood of external contamination [27–30].

There is not a single biological matrix that is the best option for metal biomonitoring. Urine is the most commonly used biomarker of exposure across metals with some exceptions like lead exposure [21,28]. While most urinary markers reflect recent metal exposure, there are some exceptions like those for Cd and Sn, reflecting medium/long-term exposure to these metals [31,32]. If metal exposures vary over time (e.g., due to fluctuations in diet, changes in the environment, occupational exposure and implementation of regulatory policies), biomarkers like nails or hair that reflect integrated exposures over longer durations may be preferable. Nails and hair are good biomarkers of exposure to As, organic Hg, U and essential metals such as Mn and Se [21,27,28]. However, for other metals, such as Ba, Be, Cd, Li, Mo, Ni, Pb, Sr, Tl, V, W or Zn, there is not yet an established biomarker that reliably reflects environmental exposure [21,27,28]. Furthermore, the use of toenails as metal exposure biomarkers has increased over time because of their indubitable logistical advantages for large epidemiological studies and because they are supposed to represent longer-term exposures compared with other biomarkers, which is an essential feature to study the involvement of certain agents in chronic diseases, but there is still excessive heterogeneity among the studies and analytical methods [21,27,28]. Although toenails share many characteristics with fingernails, they have specific advantages that make them more desirable as a biomarker of exposure. Toenail growth rates are slightly slower than fingernail growth rates; thus, toenails can reflect exposures that occurred further in the past. In addition, toenails are less frequently externally contaminated, although in any case, both toenails and fingernails need to be pretreated before being analyzed [33].

Fish and seafood consumption can influence the levels of some metals, interfering with the biomonitoring in the environmental or occupational setting. In the general population, the most common or massive accumulation of some metals occurs specifically through the consumption of fish and shellfish [34]. The United States Food and Drug Administration

(1993) noted that fish and other shellfish represent 90% of total arsenic exposure [35]. Moreover, seafood consumption has been reported to influence the levels of arsenic among steel and iron smelting in China [36] and a fertilizer factory in Italy [37]. Thus, arsenic and mercury in nails (which reflect a longer exposure window) and urine (recent exposure) are good examples of biological markers [38,39]. Nevertheless, the impact of seafood and fish for cadmium and lead have mixed results in the scientific literature [38,40,41], and little information is available for other carcinogenic metals like beryllium, chromium and nickel. For instance, while a recent small randomized controlled intervention trial identified fish consumption as a positive driver for UE of Cd and Pb [42], a cohort study of Spanish children from a heavily industrialized area showed a correlation between seafood and lead but not with cadmium [43]. Urinary levels of cadmium in Korean adult women from agricultural areas was associated with smoking but not with fish consumption [44].

We assessed the impact on short-term (urine) and long-term (toenails) metal biomarkers' levels of seafood, mollusk and fish consumption (SMFc) before urine and toenail sample donation in a group of workers occupationally exposed to metals.

2. Population and Methods

2.1. Participants

We conducted a cross-sectional epidemiological study to determine whether there is biocumulative exposure to heavy metals in workers in the chemical, mining and metal industries of Southwestern Spain. Between March 2007 and December 2008, we recruited 121 workers from the target industries and 48 workers from services industries (post offices and railway transportation) not related to metal exposure in the provinces of Huelva, Cadiz and Seville. Inclusion criteria included a minimum enrollment in the company of approximately 3 years. The inclusion criteria included metal-exposed workers aged between 21 and 65 years old with availability to provide sociodemographic and epidemiological information on lifestyle and to provide biological samples (toenails, blood and urine).

The participating workers were scheduled for a first appointment at the University of Huelva, or at the participating hospital closest to their place of residence. The workers were exhaustively informed about the study, and once they agreed to participate, they were invited to read and sign the informed consent form. Participants were invited to provide socio-demographic information, a food frequency questionnaire and epidemiological information on lifestyle and to provide biological samples (nails, blood, urine and semen). A clinical examination was also performed by a certified Medical Doctor (respiratory, cardiovascular, renal, hepatic, endocrine, hormonal, reproductive, immunological and nervous systems). Likewise, tests on neurotoxicity and spirometry were carried out. Finally, the tips of the toenails were cut, and a spot urine sample was obtained. At the time of sample donation, a specific questionnaire on recent exposures (8 h before the sample donation) related to metal exposure (including recent fish, mollusk and seafood consumption, smoking, physical activity, coffee, medications and vitamins) was filled out prior to obtaining samples. Upon leaving, the worker was provided with the instructions and necessary material that they should take to the next appointment.

In a second appointment, the participants delivered two containers of urine, previously collected at home (first urine in the morning) to the Hematology and Clinical Analysis service and filled out again the same specific questionnaire on recent exposures related to metal exposure. One of the containers was analyzed in the hospital for the systematic study of microalbuminuria and creatinine, and the other was kept at 4 °C in a refrigerator for less than 24 h and sent to the University of Huelva, where an aliquot of 10 mL was obtained. Both the container and the aliquot were stored at −80 °C to analyze markers of oxidative stress in the case of the urine in the container and to measure levels of metals in subsequent studies (10 mL aliquot).

The study protocol was approved by the ethical committee of the University of Huelva and was conducted following the Declaration of Helsinki principles.

2.2. Occupational Exposure Assessment

A complete work history was collected on the epidemiological questionnaire completed by the workers at the first appointment and was evaluated by an expert hygienist. An industrial hygiene expert assessed the likelihood of occupational exposure, exposure intensity, and confidence of his assessment. The probability of exposure was classified as null (less than 5% probability of exposure in any occupation-related task), low (between 5% and 50% probability of being exposed) or high (greater than 50% probability of exposure). The intensity of exposure was classified as low when the level of exposure in working conditions in the Spanish state was usually less than 50% of the environmental limit value (ELV), or high when it was usually greater than 50% of the ELV. The confidence of the evaluation was considered low when the evaluation was made only based on the expert's experience as a hygienist and high when the assignment was made based on objective data in the literature. The evaluation by the hygienist was carried out for a closed list of exposures that included herbicides, insecticides, fungicides, plastic fumes, factory fumes, combustion fumes, polycyclic aromatic hydrocarbons, solvents, organic solvents, glues, aluminum, arsenic, cadmium, zinc, chromium, nickel and lead.

2.3. Metal Analyses

Samples were blinded and randomly distributed to the laboratory. We measured urinary (As, Ba, Be, Cd, Co, Cu, Hg, Li, Mo, Pb, Se, Sr, Tl, V, W and Zn), and toenail (same plus Al, Cr, Fe, Mn, Ni and U) levels. Determination of levels of metals in toenails and urine was carried out by the Department of Analytical Chemistry of the University of Huelva, using the recommendations established by the International Atomic Energy Agency [45].

Urine samples were collected in urine collection polypropylene containers, which had been previously washed with nitric acid. Samples were frozen within 1–2 h of collection and stored at $-80\,^\circ C$ until analysis after being blinded and randomized. Trace metals in urine were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) with dynamic reaction cell after diluting 1 mL of the sample five times with a 5% (v/v) solution of ultrapure nitric acid in ultrapure water and filtering through a 0.45 μm polytetrafluoroethylene (PTFE) membrane filter before analysis. Quality control of the analysis was based on the following operations: (a) analysis of two reference materials, Clincheck (RECIPE) urine control for trace elements—level I and Standard Reference Material (2670a)—toxic elements in freeze-dried urine (LGC)—high level, in each sample batch, with a mean accuracy of 90% maintained $\pm5\%$; (b) monitoring of the ICP-MS response during the time period by measurement of metal concentrations at a point on the calibration curve (2 ng mL^{-1}), with every 20 samples analyzed, which guaranteed a good evaluation of the instrument response; (c) instrumental drift correction by addition of Rh (100 ng mL^{-1}) as an internal standard to all the samples and calibrants, with the samples whose response differed $\pm\,10\%$ with respect to the internal standard measured again; (d) analysis of every 5 samples of reagent blanks containing 5% (v/v) HNO$_3$ (Suprapur quality), 1% (v/v) HCl and Rh 100 ng mL^{-1} in Milli-Q water; (e) analysis of duplicate samples every 2.5 h of the sequence; and (f) spiked sample analysis, by spiking the reference materials with the analytes under study (50 ng mL^{-1}). Finally, potential interferences from molybdenum and tin, frequently present in urine, were removed by operating the ICP-MS system in helium collision mode (He flow: 4 mL min^{-1}). The creatinine concentration in urine was determined by the classic Jaffé method.

Toenail samples (50 to 100 mg) were first cleaned twice using 2 mL of a 5% (weight/volume) Triton water solution, then twice with 2 mL of Milli-Q water and finally twice with 2 mL of acetone with additional ultrasound treatment for 5 min. Then, samples were air dried and digested with 800 μL of a (4:1) mixture of HNO$_3$ and H$_2$O$_2$ of ultra trace metals grade quality, in a Teflon reactor for microwave-assisted attack. Mineralization was performed at 400 W, starting from room temperature, ramped up to 160 $^\circ C$ for 15 min and held for 20 min at this temperature. Finally, the extracts were filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane filter before analysis.

Analyses of nail extract were performed by ICP-MS, similarly to those described for urine samples. In this case, 100 mg of human hair was used as the reference material (NSC DC73347a) in order to correct the instrumental variability, following a quality control procedure similar to that used in urine analysis.

2.4. Statistical Analyses

Samples with values under the limit of detection were assigned half of the value, which is specific to each metal. We compared the median for each metal between those with and without seafood or fish consumption by using the Mann–Whitney U test. We used the median test when comparing by type of fish/seafood (more than two categories).

We used linear regression models adjusted for age, sex and industry to assess the association between hours before SMFc and each metal level. A *p*-value < 0.05 was considered as statistical significance. All statistical analyses were performed with SPSS version 27.

3. Results

3.1. Sociodemographic Characteristics

Selected sociodemographic characteristics of the subjects included in this study are shown on Table 1. We obtained information on the exposures before sample donation and the levels of metals either in toenails or urine in 111 out of 121 (91.7%) metal workers and 42 out of 48 (87.5%) service workers. The mean age of participants was 45.56 ± 10.29 years, with 131 (85.62%) men and 22 women (14.38%). Most (77.12%) of the participants were from the Huelva province, and 51.63% were never smokers.

Table 1. Characteristics of the study population (n = 153), demographics and lifestyle factors.

Item	n	%
Gender		
Males	131	85.62
Females	22	14.38
Type of participants		
Metal occupationally exposed workers	111	72.55
Non occupationally exposed workers	42	27.45
Geographic area		
Huelva	118	77.12
Other	35	22.88
Smoking habits		
Ever smokers	74	48.37
Never smokers	79	51.63
Age (years) [a]	45.56 ± 10.29	
Height (m) [a]	1.72 ± 0.07	
Weight (kg) [a]	80.40 ± 14.00	

[a] Mean ± standard deviation.

3.2. Urine Sample Donation

Table 2 shows the comparison of the medians of the metal levels by type of fish ingested in the previous 8 h before urine sample donation. Only arsenic (107.3 ppm vs. 47.7 ppm; *p* = 0.009) and cobalt (0.463 vs. 0.292 ppm; *p* = 0.037) showed higher levels among those who had consumed any type of fish in the 8 h prior to sample donation. For the rest of the metals, differences did not reach statistical significance, although they tended to be higher among those who had consumed fish (Table 2). When taking into account the type of fish, differences for arsenic were mainly due to consumption of sea fish, and an association also became apparent for mercury, related to consumption of blue fish (11.865 ppm) and canned sardines (19.125 ppm) (*p* = 0.028). Lead showed higher levels among those who consumed white fish, though the differences were not statistically significant (Table 2).

Table 2. Comparison of the median metal levels (ppb) in urine samples according to fish consumption in the 8 h prior to sample donation. Statistically significant results are highlighted in bold.

	No SMFc [1]	Any Type	Mann–Whitney [2]	White Fish River	White Fish Sea	Blue Fish	Canned Tuna	Canned Sardines	Median Test [3]
	n = 113	n = 33	*p*	n = 4	n = 6	n = 8	n = 11	n = 2	*p*
As	47.7	107.3	**0.009**	45.7	189.9	106.0	103.5	124.3	**0.025**
Ba	1.774	2.239	0.400	4.132	1.846	1.399	2.543	0.809	0.218
Be	0.015	0.025	0.547	0.035	0.020	0.069	0.001	0.018	0.257
Cd	0.190	0.122	0.158	0.177	0.114	0.078	0.021	0.074	0.453
Co	0.292	0.463	**0.037**	0.139	0.543	0.534	0.375	1.151	0.206
Cu	7.293	8.506	0.392	8.037	7.640	6.963	9.247	8.749	0.453
Hg	6.030	7.475	0.232	4.911	2.413	11.865	6.332	19.125	**0.028**
Li	14.0	16.0	0.719	16.8	18.6	10.8	18.3	18.2	0.078
Mo	33.8	28.4	0.320	29.1	36.5	32.7	27.2	17.3	0.148
Pb	2.570	3.630	0.100	11.260	2.286	4.416	3.283	18.730	0.103
Se	42.2	47.4	0.311	63.1	60.8	53.7	38.0	46.9	0.391
Sr	152.1	154.2	0.724	112.7	232.5	145.8	137.0	143.0	0.584
Tl	0.046	0.071	0.619	0.064	0.062	0.069	0.122	0.117	0.359
V	0.281	0.175	0.118	0.155	0.179	0.170	0.175	0.125	0.453
W	0.204	0.187	0.898	0.218	0.129	0.191	0.187	0.176	0.703
Zn	416.7	409.2	0.465	537.3	368.2	523.8	283.8	409.5	0.970

[1] No fish consumption in the 8 h prior to donating the urine sample. [2] No fish consumption vs. any type of fish consumption. [3] No fish consumption vs. type of fish consumption.

The comparison of the medians of the metal levels by type of seafood and mollusks ingested in the previous 8 h before urine sample donation is shown on Table 3. Only arsenic showed statistically significant higher levels among those who consumed any type of seafood or mollusks (112.2 ppm vs. 55.1 ppm; *p* = 0.009) with respect to those who did not. When taking into account the type of mollusks, an association became apparent for lithium, showing higher levels for shell mollusks and seafood (17.4 ppm) vs. no-shell mollusks (10.0 ppm; *p* = 0.046). Arsenic, molybdenum and lead showed higher levels among those who consumed shell mollusks, though differences were not statistically significant (Table 3).

Table 3. Comparison of the median metal levels (ppb) in urine samples according to seafood and mollusk consumption in the 8 h prior to sample donation. Statistically significant results are highlighted in bold.

	No SMFc [1]	Any Type	Mann–Whitney [2]	Shell Mollusks	No-shell Mollusks	Shrimps, Prawn, etc.	Median Test [3]
	n = 131	n = 16	*p*	n = 1	n = 5	n = 8	*p*
As	55.1	112.2	**0.009**	274.6	72.3	102.8	0.144
Ba	1.837	2.762	0.675	5.034	2.458	1.425	0.637
Be	0.020	0.003	0.205	0.035	0.006	0.009	0.637
Cd	0.183	0.106	0.435	0.006	0.216	0.076	0.353
Co	0.326	0.412	0.135	0.274	0.274	0.506	0.353
Cu	7.533	8.635	0.938	10.370	8.764	6.902	0.738
Hg	6.234	6.832	0.894	7.688	7.331	5.540	0.705
Li	14.49	15.605	0.859	17.4	10.0	17.4	**0.046**
Mo	33.82	27.975	0.333	72.2	22.3	31.1	0.083
Pb	2.834	4.192	0.162	12.430	5.322	2.210	0.083
Se	44.58	41.345	0.955	30.5	45.3	54.2	0.637
Sr	152.1	156.9	0.879	84.3	73.9	199.6	0.348
Tl	0.058	0.005	0.357	0.000	0.000	0.026	0.323
V	0.262	0.135	0.303	0.002	0.068	0.266	0.753
W	0.197	0.265	0.437	0.283	0.551	0.175	0.339
Zn	414.1	351.75	0.668	823.4	827.8	233.8	0.362

[1] No shellfish or mollusk consumption in the 8 h prior to donating the urine sample. [2] No shellfish or mollusk consumption vs. any type of shellfish or mollusk consumption. [3] No shellfish or mollusk consumption vs. type of shellfish or mollusk consumption.

3.3. Toenail Sample Donation

The comparison of the median of the metal levels by type of fish ingested in the previous 8 h before toenail sample donation is shown in Table 4. Only aluminum (17.6 ppm vs. 8.0 ppm; $p = 0.003$) and beryllium (0.0005 vs. 0.0001 ppm; $p = 0.009$) showed higher levels among those who had consumed any type of fish in the 8 h prior to sample donation. On the opposite direction, strontium levels were lower among those who did not consume fish (1.620 vs. 0.616 ppm; $p = 0.017$). When taking into account the type of fish, differences for beryllium were mainly due to consumption of blue and white fish (0.0005 and 0.0007 ppm, respectively; $p = 0.013$). Aluminum showed higher levels among those who consumed white river fish and blue fish; meanwhile, arsenic revealed its highest levels after white fish and canned sardine consumption, though differences were not statistically significant (Table 4).

Table 4. Comparison of the median metal levels (ppb) in toenail samples according to fish consumption in the 8 h prior to sample donation. Statistically significant results are highlighted in bold.

	No SMFc [1]	Any Type	Mann–Whitney [2]	White Fish River	White Fish Sea	Blue Fish	Canned Tuna	Canned Sardines	Median Test [3]
	n = 73	n = 24	*p*	n = 4	n = 4	n = 7	n = 7	n = 1	*p*
Al	8.0	17.6	**0.003**	24.5	16.3	20.2	12.9	13.1	0.354
As	0.109	0.090	0.467	0.066	2.448	0.148	0.054	1.169	0.488
Ba	1.250	0.984	0.316	0.964	1.880	0.735	1.110	1.444	0.436
Be	0.0001	0.0005	**0.009**	0.0047	0.0003	0.0005	0.0007	0.0003	**0.013**
Cd	0.031	0.053	0.417	0.020	0.050	0.058	0.021	0.587	0.622
Co	0.017	0.016	0.874	0.027	0.024	0.015	0.016	0.014	0.266
Cr	1.190	0.749	0.208	0.832	1.015	0.813	1.188	0.341	0.409
Cu	5.200	3.277	0.320	4.007	6.732	2.329	4.212	12.178	0.270
Fe	25.9	32.9	0.096	48.0	34.2	32.1	38.1	26.1	0.790
Hg	0.220	0.189	0.198	0.187	0.315	0.215	0.123	0.175	0.169
Li	0.028	0.021	0.879	0.039	0.028	0.018	0.036	0.018	0.425
Mn	0.392	0.384	0.907	0.641	0.501	0.211	0.518	0.209	0.469
Mo	0.045	0.058	0.273	0.060	0.088	0.069	0.048	0.056	0.583
Pb	2.190	2.798	0.472	3.285	1.556	3.430	5.555	1.449	0.473
Ni	0.575	0.857	0.110	1.242	0.853	0.902	0.527	6.602	0.583
Se	0.811	0.784	0.947	0.926	1.082	0.747	0.788	1.141	0.322
Sr	1.620	0.616	**0.017**	0.717	2.211	0.509	0.569	0.452	0.234
Tl	0.003	0.002	0.163	0.003	0.008	0.002	0.003	0.018	0.562
U	0.004	0.005	0.744	0.010	0.005	0.003	0.005	0.001	0.222
V	0.038	0.032	0.457	0.038	0.027	0.033	0.045	0.023	0.790
W	0.005	0.010	0.326	0.016	0.005	0.026	0.006	0.027	0.620
Zn	96.3	102.5	0.598	92.5	156.4	102.0	87.6	125.5	0.256

[1] No fish consumption in the 8 h prior to donating the toenails sample. [2] No fish consumption vs. any type of fish consumption. [3] No fish consumption vs. type of fish consumption.

Table 5 resumes the comparison of the medians of the metal levels by type of seafood and mollusk ingested in the 8 h before toenail sample donation. None of the metals showed different levels depending on whether seafood or mollusks were consumed. According to the type, only aluminum revealed high levels after shell mollusk intake with no statistically significant relation (Table 5).

With respect to the association between hours before SMFc and each metal level, only arsenic urinary levels were associated with numbers of hours prior to sample collection since latest SMFc ($p = 0.001$).

Table 5. Comparison of the median metal levels (ppb) in toenail samples according to seafood and mollusk consumption in the 8 h prior to sample donation. Statistically significant results are highlighted in bold.

	No SMFc [1]	Any Type	Mann–Whitney [2]	Shell Mollusks	No-Shell Mollusks	Shrimps, Prawn, etc.	Median Test [3]
	n = 87	n = 11	*p*	n = 1	n = 4	n = 6	*p*
Al	9.5	11.2	0.884	29.4	4.8	12.6	0.053
As	0.109	0.069	0.555	0.069	0.077	0.131	0.551
Ba	1.110	0.925	0.478	0.726	0.722	1.598	0.444
Be	0.0002	0.0005	0.502	0.0086	0.0002	0.0007	0.621
Cd	0.031	0.022	0.809	0.018	0.036	0.036	0.808
Co	0.016	0.017	0.475	0.025	0.031	0.016	0.551
Cr	1.017	0.818	0.293	0.354	1.319	0.693	0.621
Cu	4.940	4.212	0.870	2.429	3.267	5.286	0.444
Fe	27.5	30.3	0.464	54.6	29.8	28.9	0.798
Hg	0.206	0.196	0.609	0.166	0.268	0.186	0.798
Li	0.028	0.021	0.528	0.019	0.014	0.035	0.127
Mn	0.360	0.584	0.710	0.421	0.140	0.691	0.189
Mo	0.050	0.060	0.818	0.037	0.074	0.027	0.128
Pb	2.190	3.156	0.879	3.156	2.080	2.699	0.798
Ni	0.595	1.170	0.140	0.543	1.036	1.500	0.428
Se	0.811	0.746	0.690	0.792	0.926	0.729	0.621
Sr	1.050	0.967	0.831	0.467	1.273	1.360	0.798
Tl	0.003	0.001	0.298	0.000	0.002	0.002	0.798
U	0.004	0.005	0.748	0.004	0.004	0.005	0.287
V	0.035	0.027	0.282	0.028	0.024	0.027	0.399
W	0.005	0.005	0.393	0.002	0.009	0.006	0.818
Zn	100.0	95.2	0.657	82.0	89.1	126.2	0.551

[1] No shellfish or mollusk consumption in the 8 h prior to donating the toenail sample. [2] No shellfish or mollusk consumption vs. any type of shellfish or mollusk consumption. [3] No shellfish or mollusk consumption vs. type of shellfish or mollusk consumption.

4. Discussion

We assessed the impact on short-term (urine) and long-term (toenails) metal biomarkers levels of seafood, mollusk and fish consumption before urine and toenail sample donation in a group of workers occupationally exposed to metal and controls. We found that the impact of SMFc is different depending on the metals for urine and toenail samples. We found that among workers exposed to metals, when urine is used as a matrix for biomonitoring, arsenic and cobalt can be influenced by any type of fish consumption. Arsenic showed statistically significant relationships with sea fish (white and blue fish and canned sardines), and mercury showed significance after blue fish and, especially, canned sardines. Moreover, arsenic and lithium levels are statistically related with seafood or mollusk consumption, while the other studied metals (Ba, Be, Cd, Cu, Hg, Pb, Se, Sr, Tl, V, W and Zn) did not reflect any statistical relation. On the other hand, when using a biomarker for long-term exposure (like toenails), recent intake of fish and seafood or mollusks did not have any impact except for aluminum and beryllium after fish consumption.

Toenails seem to be a good biomarker to evaluate metal bioaccumulation because of their advantages for large epidemiological studies and because they represent longer-term exposures compared with other biomarkers, which is an essential feature to study the involvement of certain occupational exposures. As our study has shown, matrices that capture long-term exposure, such as toenails, are better bioindicators of workers' occupational metal exposure because they are less influenced by specific factors that could distort metal exposure levels, like SMFc. In metal-exposed workers, urine does not appear to be a good biomarker of occupational exposure to total arsenic or cobalt, as matrices that capture short-term contamination are more sensitive to any specific interference. Alternatively, when biomonitoring occupational short-term exposure to total arsenic, seafood consumption

should be taken into account, or alternatively, speciation should be performed to separate organic (mainly dietary) from inorganic (mainly occupational) forms. When interested in relevant long-term or cumulated exposure, the role of occupation as a source of exposure using toenails as biomarker has been assessed in several studies. Concerning occupational exposures, there is some evidence of increased toenail As concentrations in relation to residing in specific industrial areas and with exposure in the workplace, which appears to affect mainly workers in mines or smelting operations [46–49]. For the other metals, data were obtained from a few studies that were associated with increased toenail Al, Cr and Cu levels in galvanizers [50]; with Co and Cu in workers of a phosphate fertilizer plant [51]; with Fe in miners [52]; with Cd, Ni and Pb in welders [53]; and with Hg in workers in chemical processing plants that use mercury [54]. In contrast, toenail Cd, Cr, Ni, Pb [46,47] and Hg levels [47] were not higher among miners, nor were toenail Al levels higher in workers from a fertilizer plant [51]. Furthermore, no association with Co [50–52] or V [50,52] was found, and there were contradictory results for Mo and Zn [51,52].

Our results reveal how SMFc influences the levels of accumulated metals in non-exposed workers, who do not receive a relevant source of exposure. Even for those workers who suffer occupational exposure, diet is important for some metals, such as arsenic, cobalt or mercury in urine, but for the vast majority of metals, it is insignificant, especially in toenail samples. Metals in urine samples are likely to vary over time because of the variable exposure driven by changes in diet [55–58]. These investigations have examined the temporal variability in urinary levels of As, Co, Hg and Li, with some differences between the results due mainly to differences in diet, principally fish, seafood and drinking water consumption. However, the results showed, as our study did, that participants with higher levels of arsenic and mercury in urine consumed fish more frequently. On the other hand, some studies have estimated exposure through diet with the corresponding metal levels in toenails. The most studied element was Se, with some reports showing a direct relationship between toenail levels and global Se intake [59–63] and other studies reporting no association [64–68]. For the other three metals, most of the reports did not show a good correlation between dietary intake and toenail levels. For non-essential elements, several authors have supported a positive association between Hg in toenails and seafood, mollusk and fish intake in the general population [69–72], particularly tuna and saltwater fish [73]; tuna, dark-meat fish and other fish [74]; whale or shark meats [75]; and shellfish, dark-meat fish and tuna in men [76]. With regard to other metals, a cross-sectional study performed in suburban areas in Pakistan found that toenail As and Co were positively associated with SMFc frequency [77]. On the other hand, some studies have evaluated how SMFc affects workers who have occupational exposure to metals. Research developed on Chinese metal-exposed workers revealed the statistically significant relationship between SMFc and As/Hg level in urine [78,79]. Similarly, seafood consumption has been reported to influence the levels of arsenic among steel and iron smelting in China [36] and a fertilizer factory in Italy [37]. Another study evaluated the association between cadmium exposure and seafood consumption in cadmium-exposed workers in China [80]. The results showed that workers who consumed shellfish most frequently had the highest levels of cadmium in urine despite being highly occupationally exposed to Cd [80]. However, to our knowledge, there are no studies in the literature regarding the correlation between SMFc and metal level in toenails of workers occupationally exposed to metals.

For metal biomonitoring studies based on the non-exposed population, it is relevant to obtain data on diet, especially fish, mollusk or shellfish consumption, depending on the metals of interest. However, for those studying metal exposure levels in workers with occupational metal exposure, it might not be necessary for most metals, with exceptions like arsenic and cobalt.

The present study has several limitations, including its cross-sectional design with only one spot measurement, which hampers the study of causality. The second limitation was the relatively small sample size of the population and the relatively smaller toenail sample size compared to the urine sample size, although this study had a much bigger

sample size than many studies reported in the literature. We consider that the robustness of the toenail data and urine data should be evaluated under an absolute approach for each one rather than relatively by comparing one to the other. With 146 subjects included in the urine analyses, the precision and power of our study is higher for urine analyses than for toenail analyses (97 subjects). However, with 97 subjects with toenails, in the worst-case scenario (lowest prevalence of exposure (i.e., any type of seafood)), with 87 unexposed (to seafood) subjects and 11 seafood consumers, we have 80% power to detect differences (increase or decrease) of 29% between the means of the two groups assuming an SD of 1/3 of the mean and a type I error of 5%, which we believe is more than acceptable. We assessed multiple metals, which led to multiple comparisons; however, our results are in agreement with the international scientific literature. Some degree of residual confounding could be present due to lack of information on the geographic origin of the fish, mollusks and seafood being collected.

The results of our study are also reinforced by some methodologic strengths. A large number of metals in both urine and toenails and their relationship with fish, mollusk and seafood consumption were included in the study. Moreover, a rigorous standardized protocol for toenail/urine preparation and analysis was applied to detect chemical levels after SMFc by taking into account information on the subjects' work activity and other relevant data. Information on fish, mollusk and seafood intake in the 8 h before sample donation was directly obtained from participants.

5. Conclusions

Seafood, mollusk and fish consumption is an important determinant of urinary arsenic levels, as well as sea fish for urinary mercury, but not for other metals among workers exposed to metals. An unexpected association was observed between aluminum and beryllium toenail metal levels and recent consumption of fish, but not for mollusks or seafood.

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