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Toenail Metal Exposures in Fishermen from Bodo City, Nigeria

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Abstract
We conducted exposure assessment using toenails from 20 fishermen living in Bodo City, a community of the Niger delta region in Nigeria. This community has been affected by over 4000 oil spills and environmental disasters. Fishing is the primary source of food and income for individuals in this community. Previous research in Bodo City found elevated metal levels in fish. Toenails were used as a biomarker to investigate the feasibility for use in risk assessment studies in developing countries. The toenails collected had significantly higher manganese levels (median 5.8 µg/g) and lead levels (median 0.98 µg/g) than those reported in more developed countries, comparable levels to those from other low-middle incomes countries. These exposure levels are likely a direct result of exposures from the environmental disasters the community has experienced and would be related to increased risks for many diseases previously associated with heavy metal exposures.

Keywords Nigeria · Metals · Oil spills · Manganese · Lead · Toenails

Heavy metals are often used for industrial purposes, and several, specifically, lead (Pb), mercury (Hg), arsenic (As), chromium (Cr), and manganese (Mn), are known to affect nearly every organ system in the body (Caito and Aschner 2015), including the nervous and cardiovascular systems. (Nigra et al. 2016). Furthermore, Pb, As, Cr, and Cd have been suggested as a potential carcinogens, particularly for breast, gastrointestinal, stomach, ovarian, skin, and brain cancers (Alatise and Schrauzer 2010; Canaz et al. 2017; Lin et al. 2018; Mayer and Goldman 2016; Nunez et al. 2016; Welling et al. 2015; Wu et al. 2012). With newly industrialized countries and vulnerable occupational communities, exposure from heavy metals is an ongoing problem worldwide. Environmental contaminations from industry coupled with environmental disasters, such as oil spills, can have a significant impact ecologically, and the communities surrounding affected areas are subjected to the prolonged strain associated with the reduced availability of sustainable, safe natural resources. Oftentimes, the locations of these events lead to difficulty in collecting or analyzing biomarkers indicative of environmental exposure.

The Niger Delta region of Nigeria is one such area; the United States Department of Energy estimates that well over 4000 oil spills have occurred in the Niger Delta in the past 5 decades (Boele et al. 2001; Nkpaa et al. 2013b; Nwaichi et al. 2014; Osuji and Adesiyan 2005). Moreover, limited efforts for cleanup and remediation have been implemented in this community. As a consequence, a recent study measuring soil and vegetables found significant accumulations of heavy metals in soils and vegetables typically consumed in Bodo City, a region in the Niger Delta (Zabbey et al. 2017). Another study investigating heavy metal concentrations in fish in Bodo City found elevated levels of lead, cadmium, zinc, manganese, and iron, which were two-fourfold higher than the US Environmental Protection Agency (EPA), World Health Organization (WHO), and Food and Agriculture Organization (FAO) permissible levels for human consumption of 0.3, 0.01, 5.0, 0.02, and 0.3 mg/kg respectively (US Food and Drug Administration and Centre for Food Safety and Applied Nutrition 2001; Nkpaa et al. 2016; Food and Agriculture Organization 1983; World Health Organization 1984). As evident from previous studies, communities in the Niger Delta lack access to clean food and drinking water, yet human biologic uptake has not been studied due to limited access to funding and biomarkers in the community (Kponee et al. 2015).

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Toenails have been used in previous studies looking at environmental exposure to metals, with most focusing on toenails alongside other biomarkers to assess health impacts (Grashow et al. 2014; Mordukhovich et al. 2012; Reis et al. 2015). Toenails have been shown to reflect a more remote exposure window than blood, capturing exposures occurring between 6 months and 1 year prior to sample collection of standard clipping sizes (Grashow et al. 2014; Mordukhovich et al. 2012; Reis et al. 2015). Due to their non-invasive collection procedures and ease of storage and shipping, toenails serve as a much more accessible biomarker for environmental health studies than blood, which requires invasive collection procedures, specialized collection vials, and expensive shipping and storage (Specht et al. 2018).Toenails were also shown to be a stable biomarker for ongoing environmental exposures with strong correlations over time (Wu et al. 2018).

In this pilot study, we examined toenail metal concentrations in a population near the Niger Delta due to the aforementioned elevated heavy metal levels found in fish from the community (Nkpaa et al. 2016). Bodo City has a population of about 69,000 people. As about 60% of the male individuals living in Bodo City are fishermen, we sought to assess their environmental elemental exposures through toenail measurements in a subsample of fishermen (Nkpaa et al. 2013b, 2016). In addition, since the fish collected in Bodo City makeup about 30% of the total seafood market in the Niger delta, a region with a total population of 37 million, this study aimed to give preliminary results of potential widespread exposures in the region. We compared the metal concentrations found in toenails from Bodo City to those found in similar studies around the world.

Materials and Methods

This study took place in Bodo City, a community from the Niger Delta region of Nigeria. The Bodo City fish makeup about 30% of the entire seafood market in the Niger Delta region. The majority of individuals in this community, including men, women and children use fishing as a primary source of food and income. In this community, seafood, vegetation, and drinking water are subjected to pollution by various contaminants from the previous environmental disasters, including heavy metals (Nkpaa et al. 2013a, 2018; Peters et al. 2018). About 60% of the males living in Bodo City are fishermen, thereby exposing them to environmental toxicants via oral and dermal contact pathways (Nkpaa et al. 2013b; 2016).

We collected samples from 20 fishermen. Potential participants were excluded if they were not fishermen, not 18 years of age or older, unwilling to remove toenail polish, or unwilling to donate their toenail samples. During site-visits at the dock area, all study participants clipped their own toenails from all ten toes using wide-jaw toenail clippers, which were caught on a sheet of white paper and deposited into coin envelops. Each envelope was labelled with a sequential subject ID and date of sample collection. No demographic data was collected from study subjects, and envelopes were shipped to Boston, Massachusetts, USA for analysis.

The study protocol was explained in local language to both Ogoni chiefs and study participants. Because toenails are considered discarded tissue and no identifiable information was collected, this study was determined to be exempt from full review by the Harvard T.H. Chan School of Public Health Institutional Review Board, and received approval from University of Port Harcourt Institutional Review Board. The study had verbal consent with all participating subjects receiving a consent form and verbal explanation of the study procedures prior to participation in the study.

Inductively coupled plasma mass spectrometry (ICP-MS) measurements were performed for the toenails after a rigorous cleaning regimen including 1% triton, deionized (DI) water, and acetone. All clippings were digested in 3 mL of trace metal grade nitric acid and 1 mL of hydrogen peroxide at room temperature for 48 h. Then the samples were diluted with 6 mL of DI water prior to analysis. The analysis was done using an internal standard of scandium, yttrium, and terbium. For further quality control and assessment, we used ERM-DB001, a certified standard for human hair, with varying concentrations of a continuous calibration standard, digestion duplicates, and NIST1643f standard for trace elements in water. In our analysis, there were no metals analyzed that were below the detection limits. In analysis duplicates, the standard error was 6.5% on average for all metals analyzed. In ERM-DB001, the average recovery percentage was found to be 85%. Our calibration standards had recovery on average of 94% for all metals, and the NIST1643f standard had an average recovery for all metals of 90%. Toenail samples had a mass with a mean $\pm$ standard deviation (SD) of 91.6 ± 0.49 mg. The elements analyzed in this sample did not show signs of interference through observed changes with use of internal standards for matrix effects and instrumental reduction with kinetic energy discrimination.

For total Hg analysis, we added 0.2 mL of bromine monochloride to the sample tubes and refrigerated them for storage prior to analysis. We used a Tekran 2600 interfaced with the ICP-MS utilizing cold vapor atomic fluorescence spectroscopy (CVAS) to measure Hg in the toenails. We used the ERM-DB001 certified human hair standard, various concentration total Hg standard, digestion duplicates, and an Hg standard (SPEX certified reference standard). Average recovery of standards for mercury was 107%. Digestion duplicates had a standard error of 7% on average for mercury.

Results and Discussion

Notably, all samples met detectable limits for each metal analysis. Detailed descriptive statistics for metal concentrations found in the toenails of study participants are displayed in Table 1.

Table 1 Descriptive statistics for metal concentrations from toenails of 20 Nigerian fishermen

Metal concentrations (µg/g)	Mean	Standard deviation	Median	Minimum	Maximum
Mn	8.04	6.16	5.76	2.72	28.32
Pb	0.98	0.49	0.93	0.20	2.57
Hg	0.17	0.04	0.16	0.10	0.27
Cu	4.09	1.77	3.4	2.28	8.15
Fe	126.75	40.47	135.34	50.48	196.79
V	0.16	0.05	0.16	0.04	0.25
Cr	0.51	0.46	0.35	0.09	2.08

This pilot study is the first to identify toenail metal concentrations specific to the Niger Delta region of Nigeria. In the following, findings for investigated metals with the most prominent concentrations are discussed. Table 2 summarizes select literature on exposure assessment with nails. The review indicates that Mn and Pb are elevated in Bodo City.

We found elevated levels of Mn compared to other industrial populations and occupational samples. These results suggest that there is a significant source of Mn exposure in this population of fishermen. Manganese is an essential element, but studies have indicated that excessive Mn exposures may be related to deficits in cognition and potential increased risk of neurodevelopmental diseases such as autism spectrum disorder and manganese induced Parkinson's disease (manganism) (Dickerson et al. 2017; Vollet et al. 2016). Inorganic Mn compounds can permeate communities near industrial facilities (Michalke and Fernsebner 2014). Much of the conflicting results found in literature are evaluated at environmental exposure levels with an unknown negative effect threshold for manganese; however, most of the studies agree that too much manganese is bad for cognition (Michalke and Fernsebner 2014; Vollet et al. 2016).

Table 2 Review of toenail exposures in other studies with similar metals measured

	Country	Mn (µg/g)	Pb (µg/g)	Hg (µg/g)	Cr (µg/g)
Specht et al. (2018)	Nigeria	8.04	0.98	0.17	0.51
Amaral et al. (2012)	Spain		0.469		
Ayodele and Bayero (2010)	Nigeria	0.68			
Bai (2015)	USA				0.552
Chanpiwat (2015)	Cambodia	25.46	1.3		1.64
Coelho (2014)	Portugal	2.84; OE: 1.98	1.25; OE: 1.16	0.46; OE: 0.51	2.17; OE: 0.91
Gibb (2011)	Ukraine			0.35	
Gutierrez (2017)	Spain				0.61
Hashemian (2017)	Iran			0.018	0.77
He (2013)	USA			0.266	
Hinners (2012)	USA			0.587	
Johnson (2011)	USA				0.27
Kuiper (2014)	Qatar	2.48	0.51		
Laohaudomchok (2011)	USA	0.8			
Meramat (2017)	Malaysia		0.55		3.83
Mohmand (2015)	Pakistan	23.6	10.7		3
Mordukhovich (2012)	USA	0.28	0.31	0.22	
Ndilila et al. (2014)	Zambia		21.4		
Park and Seo (2016)	South Korea			0.4	
Qayyum and Shah (2014)	Pakistan	2.72	68.4		20.6
Reis (2015)	Portugal	0.34			
Rodrigues (2018)	Brazil	1.48			
Sureda (2017)	Spain			0.549	0.554
Wong (2015)	USA	OE: 3.72	OE: 0.87		

Occupational exposures are labelled (OE), unlabeled measures are environmental exposures. Vanadium (V) was removed as it had no comparison studies. Iron (Fe) and copper (Cu) were removed, as they have unclear health associations in toenail

Toenail Pb levels were also notably higher than that seen in other studies assessing Pb using this biomarker. As Pb is a well-known toxicant and oxidative stressor that affects neurologic and cardiovascular health (Navas-Acien et al. 2007; Shih et al. 2007), development of progeny (Allen 2015; Dickerson et al. 2017), and has also been suggested as a potential carcinogen (Alatise and Schrauzer 2010; Lin et al. 2018; Weisskopf et al. 2009; Wu et al. 2012). Thus, the elevated concentrations seen in this population are of concern.

Due to ease of absorption of Pb and Mn^{2+} ions, and subsequent Ca^{2+} replacement in soil and plants, vegetables, starchy grains, and fruits are common sources of Mn intake, vegetables and can be easily exchanged with Ca^{2+}, making fruits, vegetables, and grains primary nutritional sources of manganese in Nigeria (Nduka et al. 2008). Previous studies in the Niger Delta region have shown high levels of Cr, Mn an Fe in starchy root vegetables (cassava, taro, and yams) ranged between 0.2 and 0.84 ug/g, 2.11 and 11.8 ug/g, and 6 and 118 ug/g which were substantially higher than their permissible limit (0.2, 2 and 5 ug/g respectively) (Nkpaa et al. 2018; Peters et al. 2018). Moreover, another source of nutritional exposure to metals is through bioaccumulation in fish and seafood (Bonsignore et al. 2018). Notably, previous studies have shown highly elevated levels of Pb and Mn in fish in the Bodo City area, with metals in fish on the order of 20ug/g for Pb and 60ug/g for Mn (Nkpaa et al. 2013b, 2016), compared to fish concentrations in other countries of ≤ 1.5 ug/g for Pb and ≤ 20 ug/g for Mn (Ahmed et al. 2016; Malhat 2011). Thus, the increased metal exposures are most likely from dietary sources in this community, though some may also be occupational. The dietary exposure route for this population could lead to greater biological uptake. This is especially concerning, as the fish from Bodo City makeup more than 30% of the fish market for the entire Niger Delta region, which has a population of more than 37 million people.

We acknowledge that our study does have some limitations. First, our sample was a convenience sample, and a very small portion of the community in this region. Because study subjects were all male, we cannot assess differences by sex. Additionally, because we were unable to collect demographic information in our sample, we could not adjust for potentially confounding factors such as age and socioeconomic status. Furthermore, because all study participants were fishermen and no dietary assessments were administered, we cannot rule out the possibility that occupational exposures may contribute, at least in some part, to the metal levels observed in our study. Despite these limitations, in being the first study to provide exposure assessment data in this highly unique region of Nigeria, this pilot study adds to the body of knowledge regarding exposure levels in low- and middle-income countries plagued by industrial disasters by using non-invasive and temperature-stable biological markers of exposure, and a novel method of analysis that eliminates the need to destroy these valuable samples.

In summary, heavy metal exposures in this community are significantly elevated and potentially the result of the environmental disasters that persist in this region. Based on previous studies, the elevated heavy metals observed in this study may have adverse impacts on the health of highly exposed members of this community. However, the study was limited by the sampling methodology, number of subjects, and lack of demographic information. Subsequent studies conducted in this population should seek to elucidate health outcomes and demographic predictors that may be associated with the exposure, determine the extent of exposure among the population in the Niger Delta region, and identify appropriate procedures to minimize harmful toxicants from food and water sources in the community. Further studies are needed to determine the concentrations of other metals associated with crude oil spills that could adversely affect health.

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