**Iournal** of

DOI: 10.23937/2572-4061.1510060

Volume 10 | Issue 1 Open Access



# **Toxicology and Risk Assessment**

**REVIEW ARTICLE** 

# Contamination Levels of Heavy Metals and Assessment of Potential Health Risks in Food Samples in the Democratic Republic of Congo (DRC): A Systematic Review

Mputu Malolo Lievins-Corneille<sup>1</sup>, Mankulu Kakumba Jocelyn<sup>2\*</sup> , Ndelo Matondo Patrick<sup>1</sup>, Nuapia Belo Yannick<sup>1,3</sup> and Ndelo-di-Phanzu Josaphat<sup>1</sup>

<sup>1</sup>Toxicology and Food Hygiene Laboratory, Faculty of Pharmaceuticals Sciences, University of Kinshasa, D.R. Congo

<sup>2</sup>Laboratory of Analysis and Quality Control of Food and Drugs, Faculty of Pharmaceutical Sciences, University of Kinshasa, D.R. Congo



<sup>3</sup>Department of Pharmacy, School of Healthcare Sciences, Faculty of Health Sciences, University of Limpopo, South Africa

\*Corresponding author: Jocelyn MANKULU KAKUMBA, Laboratory of Analysis and Quality Control of Food and Drugs, Faculty of Pharmaceutical Sciences, University of Kinshasa, Po.Box 212 Kinshasa XI, D.R. Congo

## **Abstract**

Food safety and human health are both negatively impacted when heavy metals are present in food. The objective of this review was to summarise the available data on food contamination with heavy metals (HMs) in the Democratic Republic of the Congo and offer suggestions for developing the field of HMs risk assessment. We searched PubMed/ MedLine, Google Scholar, Sciencedirect, and EMBASE extensively for articles on exposure levels to trace elements in DR C (2011-2021). Ten documents in all, five of which were located in Kinshasa (50%) and three of which were in Katanga (33%), with the remaining two (20%) in Kongo Central. Seventy percent of the studies that could be located and that reported HMs levels involved vegetable samples, fish (30%), beef (10%), and aquatic invertebrates (10%). Five studies (50%) that used the metrics estimated daily intake (EDI), targeted hazard quotient (THQ), metal pollution index (MPI), and hazard index (HI) to link data on heavy metals contamination to risk assessment. The Common recommandation made by the reviews was the HMs monitoring in various foods from all over the Democratic Republic of the Congo and independantly to their source, in order to precisely estimate the risks to human health.

# Keywords

Accumulation, Contamination, Food chain, Risk assessment, Heavy metals

#### Introduction

The potential risks that pollution poses to the environment and public health have drawn increasing attention from government authorities in recent years. Indeed, the cause of heavy metal pollution in our environment is human activity-agriculture, urbanisation, and industry. These activities are growing at an exponential rate. Heavy metals cannot be broken down, despite the fact that many organic molecules can, and their concentrations in soils and waterways are continuously rising. Due to their accumulation in food, heavy metals pose a toxic risk to humans and expose the food chain to ever-higher concentrations of these pollutants (Bourrelier P.H. and Berthelin J., 1998). Globally, there is an increasing risk of HM contamination [1,2].

Food is a major source of nutrition for the human body. It provides vitamins, minerals, proteins, and carbs. The presence of heavy metals in food is giving rise to worries about its safety and quality. Heavy metals' lengthy half-lives in soil and challenging post-ingestive metabolism are reasons for growing concern. According to Huff [3] and Andujar [4], they



**Citation:** Lievins-Corneille MM, Jocelyn MK, Patrick NM, Yannick NB, Josaphat NP (2024) Contamination Levels of Heavy Metals and Assessment of Potential Health Risks in Food Samples in the Democratic Republic of Congo (DRC): A Systematic Review. J Toxicol Risk Assess 10:060. doi.org/10.23937/2572-4061.1510060

Accepted: July 15, 2024: Published: July 17, 2024

**Copyright:** © 2024 Lievins-Corneille MM, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

can therefore rapidly accumulate in metabolic organs such as the liver and kidney, leading to a range of toxic symptoms. Metals can cause damage to the kidney, bones, liver, brain, and other organs by interfering with a variety of biochemical processes. In addition, various systems like the circulatory, neurological, and reproductive systems [5,6]. According to Gollenberg, et al. [7], prepubescent girls had disturbed reproductive hormones, which resulted in decreased fertility upon reaching reproductive age [8], marked by irregular menstruation [9].

Eating food, including plants for energy intake and animals for protein intake, is one of the main ways that metal pollutants enter the human body [10]. However, the majority of the world's population eats fruits and vegetables. A daily intake of 400g or more of fruits and vegetables is also advised by the World Health Organisation (1990), [11].

The degree to which an animal is exposed to heavy metals during its growth will depend on its environment of raising and, in particular, on the food it eats. As a result, the animal may absorb heavy metals. Depending on their concentrations and chemical speciations, these pollutants may eventually find their way into the body of the animal, where they may undergo varying degrees of degradation. The Japanese Minamata disease, which was caused by mercury poisoning of fish and the subsequent human exposure to mercury, serves as an excellent example of the phenomenon of bioaccumulation through the food chain. The fact that metals can linger in the environment and thus encourage bioaccumulation in a number of target organs, including the liver and kidneys, is one of the main issues with them.

The same principle applies to plants: Based on their growth environment, they will essentially accumulate any metals present in the soil or nearby atmosphere [12,13]. The chemical speciation and compartmentation in the target will affect the bioavailability and toxicity in the event of food consumption, regardless of the living organism (plant or animal) in which the metals will accumulate. In the end, the amount of pollution ingested, its bioavailability, and its concentration in the matrix will determine the extent of human exposure. When computing quantitative health risk assessments, these different influences are integrated.

#### **Methods**

# Search strategy

The present study is based on screening a vast number of literature that documented about contamination levels of heavy metals in some foods in DRC. Thus, a systematic search and review were conducted on the PubMed/MedLine, Google Scholar, ScienceDirect and EMBASE databases in order to obtain information for the period between January 2011 and December

2021. In addition, Relevant texts published by Science Direct and google scholar between January 2013 and December 2021 were cited in this article.

# **Data collection**

The following eligibility criteria were applied while evaluating entire texts and abstracts: research on HMs contamination that have been printed in reputable scientific publications; - Written in either English or French; - Measuring the concentrations of HMs in food and classifying them according to the publication year, the location, the food of interest, the analyte (study year), the analytical methods, and the mean (min-max) concentrations of HMs. Studies that failed to disclose these selection criteria were disqualified.

Each article's publication year, location, food of interest, analyte (research year), analytical techniques, and mean (min-max) amounts of HMs were all taken out (Table 1 and Table 2). The DRC results were compared to permitted values established by literature and a few research [14-22].

# **Results**

A total of 10 food contamination by HMs studies carried out in 2011-2021 were examined in this article (Table 1). The earliest study (2013) was undertaken in Katanga and the latest (2021) study was in Kinshasa. The majority were conducted in Kinshasa (50%). Among all retrieved studies reporting HMs levels, 70% were in vegetables, 30% in fishes, 10% in beef and 10% in aquatic invertebrate samples. This was upper to 100% because one of the studies treated together vegetables, beef and fish samples. Amaranthus sp was the vegetable well studied (71.42%). All of these studies focused on contamination to trace elements but only 50% of them focus on risk assessment. For HMs measured, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used as principal method and Atomic Absorption Spectrophotometry (AAS) was used for Hg determination and in one of study for all metals. Heavy metals determination in vegetable samples were in the decreasing order of Cu, Cd, Pb and Zn < As < Co, Cr < Ni < Hg, Mn, U < Al < Mg, Fe and in fish and aquatic invertebrate samples, the decreasing order were Cu, Cr, Cd, Pb, Se, Hg and Zn < Ni, Sb and Mn < Al, As, Fe and Co.

# Heavy metals in vegetables

# a) Amaranthus sp

Mpumbu reported that Cu, Co, Cd, Pb, and Zn are present in *A. hybridus*. Metal concentrations of 21.9 (Cu), 1.2 (Co), 1.2 (Cd), 1.72 (Pb), and 101.33 mg kg<sup>-1</sup> (in mg kg<sup>-1</sup>) were determined. (Zn). In every market, the vegetables had copper contents that exceeded the set standard of 10 mg/kg. Certain markets did not meet the allowed maximum concentration (AMC) established in France for other trace elements (Pb: 3 mg/kg MS and Cd: 2 mg/kg MS) [14].

DOI: 10.23937/2572-4061.1510060 ISSN: 2572-4061

Table 1: HMs contaminants analysis using advanced analytical tools.

Author	Location	Vegetable samples	Analytical methods	Analyte study year	Trace metals investigated
Mpumbu [14]	Former Katanga	Amaranthus hybridus and Spinacia oleracea	AAS	-	Cu, Co, Cd, Pb and Zn
Mudimbu [15]	Former Katanga	Manihot esculanta, Amaranthus hybridus and Psidium guajava L.	ICP-MS	-	Mg, Al, Cr, Fe, Co, Ni, Cu, Zn, Cd, Pb and U
Nuapia [16]	Kinshasa	Cabbage, beans, fish and beef from markets	ICP-MS, ICP-OES and Mercury analyseur	From July till October 2016	Al, As, Cd, Cr, Cu, Hg, Mn, Pb, Se, Zn
Suami [17]	Kongo Central	Fishes from Atlantic Coast	ICP-MS and AAS	August 2016	Cr, Cu, Zn, Ni, Sb, Cd, Pb, Se and Hg
Suami [18]	Kongo Central	Oysters and Shrimp	ICP-MS and AAS	November 2017	Hg, Cr, Ni, Cu, Zn, Se, Cd, Sb, Pb, Mn, Co and Fe
Ngweme [19]	Kinshasa	Amaranthus viridis from gardens	ICP-MS and AAS	July 2018	Cr, Co, Cu, Zn, As, Cd, Pb and Hg Cr, Ni, Cu, Zn, As, Cd,
Mata [20]	Kinshasa	Ledermaniella schlechteri	ICP-MS and AAS	March 2019	Pb and Hg Cr, Co, Cu, Zn, As, Cd, Pb and Hg
Ngweme [21]	Kinshasa	Amaranthus viridis from markets	ICP-MS and AAS	In Febuary 2019 and August 2019	Mn, Co, Ni, Cu, Zn,
Ambayeba [22]	Former Katanga	Amaranthus hybridus, Cucurbita maxima, Manihot esculanta, Ipomea batatas, Lycopersican esculentum and Phaseolus vulgaris	ICP-MS and ICP-OES	-	As, Cd, Pb and U

According to Kalonda, *A. hybridus* contains metal concentrations of Mg, Al, Cr, Fe, Co, Ni, Cu, Zn, Cd, Pb, and U. (in mg kg<sup>-1</sup>) ranged from 20290-23000 (Mg), 5173-8919 (Al), 5.666-18.51 (Cr), 112.7-1642 (Fe), 11.71-116.2 (Co), 0-5.863 (Ni), 45.69-516.2 (Cu), 370.5-497.1 (Zn), 1.295-7.717 (Cd), 5.352-10.25 (Pb), and 0.302-0.534 (U) [15].

Ngweme stated that *A. viridis* comes from various sources. The contamination of Kinshasa's garden with Cr, Co, Cu, Zn, As, Cd, Pb, and Hg may have a negative impact on consumers. The results showed that *A. viridis* leaf heavy metal concentrations varied significantly between sampling sites (P.05), reaching levels of 2.97 (Cr), 1.73 (Co), 12.30 (Ni), 16.11 (Cu), 652.91 (Zn), 0.10 (As), 1.62 (Cd), 8.91 (Pb), and 0.1 (in mg kg<sup>-1</sup> wet

weight) of metals. The calculated EDI and EWI for Cd in Cecomaf and Lemba-Imbu leaves, as well as Pb in Cecomaf, Rifflaert, and Lemba-Imbu leaves, exceeded the permitted limits. Cu, Zn, As, and Hg had EDI and EWI values that were less than the recommended limits. With the exception of as at the Lemba-Imbu site, the computed THQ values exceeded the suggested values [19].

Ngweme stated that *A. viridis* marketed on the market contained high levels of the majority of the harmful metals studied (Cr, Co, Cu, Zn, As, Cd, Pb, Hg). Hazardous metal concentrations in leafy vegetables varied significantly across sample sites and seasons (p < 0.05). The findings revealed high metal concentrations in edible leaf vegetables during both the dry and wet

DOI: 10.23937/2572-4061.1510060 ISSN: 2572-4061

Table 2: HMs analytical results from foods control.

Food	Year					Heavy	metals co	ncentrati	ions (Mea	Heavy metals concentrations (Means or ranging) in mgkg <sup>-1</sup> or µg g <sup>-1</sup>	ing) in m	gkg-¹ or µ	g g-1				
		Cu	As	Нg	Fe	Mg	Mn	Se	Z	ם	₹	ပိ	Pb	PS	င်	Zn	Sb
Amaranthus	2013	21.9				ı	ı	ı	1		ı	1.2	1.72	1.2		101.33	
	2015	45-516			622.7	22513.3	ı	ı	3.78	0.44	7326.7	68.5	7.71	3.66	10.01	426.3	
	2020	16.11	0.1	0.1	,	ı	ı	ı	12.3		ı	1.73	8.91	1.62	2.97	652.91	
	2021a	7.4-11.3	1.7	0.2	,	ı	ı	ı	ı		1	1.5	18.3	1.5	3.6	348.2	
	2021b	139	ı	ı	ı	ı	15.8- 2606	ı	2.2-119	0.1-3.2	ı	21-2624	1.3-354	0.42	ı	8.3-482	ı
Spinach	2013	24.3	ı			ı	14.14	ı	ı		ı	1.49	0.72	1.49		94.24	ı
Cabbage	2017	3.8	3.33	Q.		ı	5.34	0.64	ı		52.1		2.14	2.93	3.07	27.93	ı
Beans	2017	3.3	1.62	Q.		ı	ı	0.18	ı	1	22.56	ı	1.98	1.13	1.63	19.05	
Manihot	2015	52.1			245.13	4902.7	ı	ı	0	0.2	7128.7	33.46	4.07	0.835	4.58	383.26	
Psidium	2015	102.9			457.33	5789.7	ı	ı	6.39	0.33	5823	35.19	5.074	0.09	3.48	321.13	
Ledermaniella	2020	5.5-78.4	0.08- 0.15	0.02-0.07	ı	ı	ı	ı	0.1-0.6		1	ı	0.4-2.07	0.2-0.48	0.4-0.74	336- 596.7	
Fish/Atlantic	2018	0.02-0.5		1.21		ı	ı	1.05	4.25	-	ı	ı	60.0	0.59	1.00	29.9	60.0
Oysters	2019	4.2-37.2	ı	0.2	457.3	I	1.87	1.89	0	ı	ı	0.52	0.08	0.03	0.12	46.36	0.0-
Shrimp	2019	16-60.5	ı	0.05-0.26	14.3-95	I	1.1-3.6	0.6-2.5	90.0	ı	I	0.04	0.2-0.18	0.03- 0.13	.03-0.08	47-55.9	< LD- 0.01
Fish/market	2017	6.53	3.48	1.53		ı	10.51	0.12	ı		9.1		0.39	0.64	0.37	14.17	
Beef	2017	69.0	1.62	ND	-	ı	7.18	0.29	ı	-	11.93	ı	0.16	0.16	0.58	5.47	
Fishes/Congo River	2019	0.09-2.7	ı	0-0.72	ı	1	ı	0.12- 1.5	ı	1	ı	ı	0.2-4.96	0.01- 0.05	0-1.21	2.8- 59.72	1
Permisible level		73*	1.00**	0.001***	425		500*		₀0∠	0.36	12-71**	20,	0.3*	0.2	1.3*	99.4*	
mg/kg		30e		<del>ر</del>									0.59	<del>م</del>	12°	50 <sup>f</sup>	
													0.2 <sup>h</sup>	0.1			

seasons. Metal concentrations reached (in mg kg<sup>-1</sup>) 3.6 (Cr), 1.5 (Co), 29.7 (Cu), 348.2 (Zn), 1.7 (As), 1.5 (Cd), 18.3 (Pb), and 0.2 (Hg). Except for Cu, the analysed metal concentrations in leafy vegetables exceeded the permissible levels established by the Food and Agriculture Organization/World Health Organisation (FAO/WHO) for human consumption [21].

According to Ambayeba, *A. hybridus* has been found to be contaminated with Mn, Co, Ni, Cu, Zn, As, Cd, Pb and U. Metal concentrations (in  $\mu$ g/g) ranged from 39.4-128 (Mn), 1.63-16.4 (Co), 4.9-17.9 (Ni), 24.8-166 (Cu), 59.5-324 (Zn), 0.800-2.60 (As), 0.359-2.25 (Cd), 1.11-10.4 (Pb), and 1.47-5.79 (U). The levels of Cd and Pb exceeded international standards. These findings also applied to other vegetables such as pumpkins (*Cucurbita maxima*), cassava (*Manihot esculanta*), sweet potatoes (*Ipomea batatas*), tomatoes (*Lycopersican esculentum*), and commun beans (*Phaseolus vulgaris*) [22].

# b) Other vegetables

Mpumbu stated that *Spinacia oleracea* is contaminated with Cu, Co, Cd, Pb, and Zn. Metal concentrations greater than 24.32 (Cu), 1.49 (Co), 1.49 (Cd), 0.72 (Pb), and 94.24 (Zn) mg kg $^{-1}$  [14].

Kalonda indicated that *Manihot esculanta* and *Psidium guajava* L. are contaminated by Mg, Al, Cr, Fe, Co, Ni, Cu, Zn, Cd, Pb and U. Metal concentration reaching the values (in mg kg<sup>-1</sup>) ranged between 4045-5867(Mg), 6719-7835 (Al), 3.357-6.604 (Cr), 222.1-257.8 (Fe), 11.79-54.87 (Co), 0 (Ni), 30.28-67.24 (Cu), 418.9-717 (Zn), 0.102-1.621 (Cd), 3.331-5.21 (Pb) and 0.169-0.22(U) for *Manihot esculanta* and the values (in mg kg<sup>-1</sup>) ranged between 3958-6929 (Mg), 6580-7524 (Al), 2.620-4.455 (Cr), 218.1-900.2 (Fe), 4.653-76.84 (Co), 0-19.19 (Ni), 200.1 (Cu), 279.3-393.2 (Zn), 0.059-0.139 (Cd), 4.089-5.797 (Pb) and 0.222-0.452(U) for *Psidium guajava* L [15].

Nuapia reports that both beans (*Phaseolus vulgaris*) and cabbage (*Brassica oleracea*) are contaminated with Al, Cd, Cr, Cu, Hg, Mn, Pb, Zn, As, and Se. The mean microelement concentrations in the cabbage and bean samples were in this order: Al > Zn > Mn > Cu > As > Cr > Cd > Pb > Se. The majority of the metals tested in raw foods exceeded the maximum permissible limit set by the Joint FAO/WHO Expert Committee on Food. Each food's estimated daily intake exceeded the WHO/FAO [23] upper tolerable limit (UL); however, As, Cd, Cr, Hg, and Se were all above the UL level [16]. All of the combined THQ values exceeded one.

Mata claimed that the aquatic plant Ledermaniniella schlechteri is contaminated with Cr, Ni, Cu, Zn, As, Cd, Pb, and Hg. LDMSC metal concentrations varied dramatically between sampling sites. The typical values (in mg kg<sup>-1</sup>) range from 0.44 to 9.1 Cr, 0.14 to 4.52 Ni, 5.5 to 78.4 Cu, 336.14 to 1520.91 Zn, 0.08 to 0.9 As, 0.21-0.78 Cd, 0.44 to 11.81 Pb, and 0.02-0.24 Tb (Hg). The

average content of Zn, As, Cd, and Hg in plant samples from all sampling sites exceeded what the FAO and WHO considered safe for human consumption. The impact on human health is expected to occur, according to FAO/WHO regulations and computed values.

# Heavy metals in fish

According to Nuapia, the heavy metals with the highest mean concentrations in fish muscle were Zn, Mn, Al, Cu, As, Hg, Cd, Pb, Cr, and Se. The majority of the metals tested in raw foods exceeded the maximum permissible limit set by the Joint FAO/WHO Expert Committee on Food. The estimated daily intake for each food exceeded the WHO/FAO-set acceptable limit [23]. Al, Cu, Mn, Pb, and Zn had lower estimated daily intakes from raw food, whereas As, Cd, Cr, Hg, and Se had estimates that exceeded the upper tolerable daily limit (UL). Both male and female food samples had total THQ values greater than one overall [16].

Suami discovered significant differences in heavy metal concentrations in fish muscle tissues across sites and species (P < 0.05), with maximum values (in mg  $kg^{-1}$  wet weight) of 1.00 (Cr), 2.69 (Cu), 29.90 (Zn), 4.25 (Ni), 0.09 (Sb), 0.59 (Cd), 0.09 (Pb), 1.05 (Se), and 1.21 (in mg  $kg^{-1}$  wet weight) (Hg). The Food and Agriculture Organisation, European Union, and World Health Organisation all determined that the levels of metals in fish were safe for human consumption, with the exception of mercury [17].

Mata found that the concentrations of hazardous metals (measured in mg kg¹ ww) in fish muscle tissues ranged from 0.00 to 1.21 for Cr, 2.80 to 59.72 for Zn, 0.12 to 1.53 for Se, 0.01 to 0.05 for Cd, 0.22 to 4.96 for Pb, 0.00 to 0.72 for Hg, and 0.09 to 2.65 for Cu. *M. moorii* and *D. fasciolatus* had the highest concentrations of Pb (4.96 mg kg⁻¹) and Zn (59.71 mg kg⁻¹) of any of the fish species studied. Similarly, *B. ubangensis* (0.72 mg kg⁻¹), *D. fasciolatus* (0.53 mg kg⁻¹), and *M. moorii* had the highest levels of Hg (0.70 mg kg⁻¹). These concentrations exceeded the FAO/WHO-set reference limit limits for human consumption (0.53 mg kg⁻¹(w:w)). In general, all fish species had Cd, Cr, and Cu concentrations that were acceptable [24].

# Heavy metals in invertebrates

This study provides the first metal measurements in four major seafood species from Muanda's Atlantic Coast: oysters (*Egeria congica*) and prawns (*Macrobrachium* spp., *Parapenaeus* spp., and *Penaeus* spp.). Suami discovered significant differences in metal accumulation between oyster and sand shrimp species, including Hg, Cr, Cu, Sb, Mn, Co, and Fe, but not Ni, Zn, Se, Cd, or Pb. Except for the levels of Cu and Pb in a few samples of *Macrobrachium* spp. and *Egeria Congica* spp., all tested samples (both oyster and prawn species) contained metal amounts below those that would raise concerns about seafood safety [18].

# Heavy metals in beef

According to Nuapia, cabbage and beans are contaminated with Al, Cd, Cr, Cu, Hg, Mn, Pb, Zn, As, and Se. Metal levels in beef samples were quantified in the following sequence: Al, Mn, Zn, As, Cu, Cr, Se, Cd, and Pb. Most of the metals studied in raw foods exceeded the Joint FAO/WHO Expert Committee on Food's recommended maximum acceptable limit. WHO/FAO [23] established upper tolerable limits for estimated daily intake for each food. The estimated daily intake of raw food was lower for Al, Cu, Mn, Pb, and Zn, whereas As, Cd, Cr, Hg, and Se exceeded the upper tolerable daily limit (UL). All of the food samples had combined THQ values greater than one, both male and female [16].

# **Discussion**

10 retrieved studies indicated that food samples are contaminated by Cu, Sb, As, Hg, Fe, Mg, Mn, Se, Ni, U, Al, Co, Pb, Cd, Cr and Zn. 4 trace elements (Cu, Pb, Cd and Zn) (25%) were the most determinated in all recolted samples. Among the most prevalent heavy metals are lead and cadmium, both of which are extremely hazardous [10,25]. Other metals, including copper and zinc, are necessary for vital biochemical and physiological processes and for preserving health over the course of a person's life [26-28].

The Pb concentration ranged from 0.08 to 354 mg Kg<sup>-1</sup>, with Amarathus hybridis from the former Katanga having the highest value. Pb contents in vegetable samples were higher than those allowed by the Food and Agriculture Organization for human consumption (FAO). Others food (Fishes, ocean invertebrates and beef) were below the permissible value. We want to point out that the concentration of Pb in fishes marketed in Kinshasa was 4.33 times higher than in fishes from Atlantic coast. The Cd concentration was ranged from 0.03 to 3.66 mg. Kg<sup>-1</sup> with the highest concentration in Amaranthus hybridus from former Katanga. Except for Psidium guajava from the former Katanga, the Cd contents in vegetable samples were higher than those allowed by the Food and Agriculture Organization (FAO) for human consumption. Other items (fish, marine invertebrates, and cattle) had amounts that were below the allowable limit. An excessive amount of these metals in diet is linked to a variety of illnesses, including those that affect the neurological, skeletal, cardiovascular, and renal systems [29-32]. Additionally, these heavy metals are linked to teratogenesis, mutagenesis, and carcinogenesis [10].

The copper content of vegetables in the research we looked into ranged from 3.3 to 3416 mg Kg<sup>-1</sup>. The spinach from the old Katanga region had the greatest quantity of copper (3416 mg Kg<sup>-1</sup>), followed by *Amaranthus viridis* from a garden in Kinshasa (516.2 mg Kg<sup>-1</sup>), and *Ledermaniella* from Kinshasa (102.9 mg. Kg<sup>-1</sup>). These values were higher than the 73 mg Kg<sup>-1</sup> FAO permitted

limit. The highest concentration in invertebrates from the Atlantic coast was found in the shrimp species Macrobracium spp. (60.46 mg kg<sup>-1</sup>), exceeding the FAO allowed limit of 30 mg kg<sup>-1</sup> (wet weight). In Fish samples, the concentrations of Cu were far below the permissible value of 30 mg kg<sup>-1</sup> (wet weight). Samples from fishes marketed in South Africa showed an average higher than Kinshasa's fishes [16] and Fishes marketed samples from Kinshasa was 12.74 times above than fish samples from Atlantic Coast of Muanda. The Zn concentration was ranged from 5.47 to 652.91 mg. Kg<sup>-1</sup> with the highest concentration in Amarathus viridis from former Kinshasa's gardens. Except for spinach and cabbage, Zn concentrations in vegetable samples were higher than those allowed by the Food and Agriculture Organization (FAO) for human consumption. Other items (fish, marine invertebrates, and cattle) had amounts that were below the allowable limit. The Zn concentrations in the fish samples from Kinshasa were less than the CFIA's [33] 50 mg kg<sup>-1</sup> limit guideline. The samples from Johannesburg and Nigeria, however, were above the upper limit [16,34]. In rare situations, various disorders can lead to a buildup of zinc and copper in bodily tissues. Zn and Cu rarely cause toxicity in the human body, but they can do so at greater concentrations [35,36]. Zn can lower levels of high-density lipoproteins and immunological response [37]. Numerous negative health effects, such as liver and kidney damage, anemia, immunotoxicity, and developmental toxicity, can be brought on by prolonged exposure to high copper levels. Numerous of these outcomes are consistent with membrane or macromolecule oxidative damage. Several enzymes, including glutathione reductase and glucose-6phosphatase, can have their sulfhydryl groups bound by copper, preventing them from protecting cells from free radical damage. Cu can cause gastrointestinal distress, liver damage, immune system damage, neurological system damage, and impairment of the ability to reproduce if it is accumulated excessively [38,39]. One of the most frequently reported negative effects of copper on health is gastrointestinal distress [40].

Only samples from Kongo Central were used to determine Sb, while samples only from the old Katanga region were used to determine Mg and U. The biggest issue with uranium is the release of radon, one of its gaseous decay products, in confined areas (such poorly ventilated homes or mines) [41,42]. However, uranium is also nephrotoxic and may have an impact on other organs [43]. Fe was not identified in all vegetable samples from Kinshasa whereas As, Hg, and Se were not present in samples from the old Katanga.

This review's arsenic content ranged from 0.1 to 3.48 mg Kg<sup>-1</sup>. The fish sold in Kinshasa had the greatest levels of As (3.48 mg/kg<sup>-1</sup>), followed by the cabbage from Kinshasa (3.33 mg/kg<sup>-1</sup>), the *Amaranthus viridis* sold in Kinshasa (1.7 mg/kg<sup>-1</sup>), and the beans and beef sold in Kinshasa (1.62 mg. Kg<sup>-1</sup> each). The FAO permitted

limit of 1 mg Kg<sup>-1</sup> was exceeded by these amounts. As reported by Oliveira, et al. [44] in the fish (Tilapia) sold in the Indonesian market, the concentration of arsenic in *Amaranthus viridis* samples marketed in Kinshasa was higher than the level of *Amaranthus viridis* samples from Kinshasa's garden by at least 17 times. We also want to draw attention to the fact that the concentration of arsenic in fish marketed in Kinshasa was higher than the level. An increasing body of research suggests that long-term exposure to inorganic arsenic (iAs) may raise the risk of keratosis, hyperpigmentation, and cardiometabolic (CM) illnesses, such as diabetic mellitus (DM) and cardiovascular diseases (CVD) [45-49].

The range of the Hg content was 0.02 to 1.53 mg Kg<sup>-1</sup>. Fish samples from the Atlantic Coast (1.21 mg. Kg<sup>-1</sup>), amaranthus viridis sold in Kinshasa (0.2 mg. Kg<sup>-1</sup> 1), Amaranthus viridis from Kinshasa's garden (0.1 mg. Kg<sup>-1</sup>), and Ledermanielle from the Congo River all had lower levels of mercury than the fish marketed in Kinshasa (1.53 mg. Kg<sup>-1</sup>) did (0.02 mg. Kg<sup>-1</sup>). The Food and Agriculture Organization (FAO), the European Union (EU), and the World Health Organization (WHO) have established permitted thresholds for vegetables (0.001 mg.kg<sup>-1</sup>) and fish for human consumption (1 mg kg<sup>-1</sup>). The fish samples from Kinshasa had a lower Hg concentration than those from Johannesburg and were greater than the levels found in fish marketed in Palestine, according to Hossan, et al. [16,50]. Mohamed, et al. [51] reported contamination of cabbage (0.011 mg. Kg<sup>-1</sup>) and of beans (0.024 mg. Kg<sup>-1</sup>) in Saudi Arabia, and Zvjezdana, et al. [52] reported contamination of cabbage (0.0097 mg. Kg<sup>-1</sup>) and of beans (0.013 mg. Kg<sup>-1</sup>) in Croatia when the cabbage and beans from Kinshasa were not contaminated by Hg. The toxicity of methyl mercury (MeHg) is higher than that of inorganic mercury, and the effects of mercury on human health are intimately tied to the form in which it exists. The primary stable organic form of mercury that is absorbed by the body from food and is well recognized to be neurotoxic is methylmercury [18,53,54].

The range of the Al content was 9.1 to 7326.7 mg Kg<sup>-1</sup>. The veggies from the old Katanga region had the highest levels of Al. (7326.7 mg. Kg-1 in Amaranthus hybridis and 7128.7 mg. Kg-1 in Manihot esculanta and 5823 mg. Kg<sup>-1</sup> in *Psidium guajava*). These concentrations were significantly higher than those found in veggies from Kinshasa and far over the Food and Agriculture Organization's (FAO) permitted levels for human consumption. For instance, Amaranthus hybridis had a concentration that was 140.6 times higher than cabbage and 324.7 times higher than beans. One of the potentially harmful elements with no biological use in the body is aluminum (AI). Although AI can be hazardous at greater concentrations, it is significantly less toxic than Hg or Pb. Numerous health issues, including osteomalacia, Parkinson's disease, Alzheimer's disease, autism, and autoimmune, could be brought on by high levels of Al [6,55].

The range of Cr concentrations was 0.03 to 10.1 mg Kg<sup>-1</sup>, with Amarathus hybridis from the former Katanga having the highest concentration. Except for Ledermaniella, the levels of Cr in the vegetable samples were higher than those recommended by the Food and Agriculture Organization (FAO) as safe for human consumption. Other items (fish, marine invertebrates, and cattle) had amounts that were below the allowable limit. Suami and colleagues [18] found a significant difference (p < 0.05) between oyster and shrimp samples, even though the Cr concentrations were below the permitted limit (12 mg. Kg-1) established by the Food and Drug Administration Guidance Document for Chromium in Shellfish [56]. The Oysters had more Cr accumulated than the Shrimp, according to the average Cr concentrations in the Oysters and Shirip. Regarding the metabolism of glucose, lipids, and proteins in both humans and animals, Cr (III) is a necessary and nourishing element that makes it easier for insulin to bind with its receptor site [57]. Although it may inhibit some enzyme systems or interact with organic molecules at high levels, it is less harmful. Cr (VI) is a powerful oxidant that damages cells, and exposure to it in the general population is mostly caused by dietary consumption and home emissions [58,59]. It causes allergies and is regarded as a lung cancer risk for people who breathe it in [60].

If all studies focused on heavy metal contamination of food, only 5 (50%) of them were interested in evaluating the potential health risk assessment for consumption of some foods by determining the Estimated Daily Intake (EDI) [14,16,19,24] or the Target Hazard Quotient (THQ) [16,24]. The EDI was estimated based on the daily gram intake of food as well as the consumption of each heavy metal in diet. Consuming Amaranthus sp. leafy vegetables can be connected with dangers to human health, according to Mpumbu and Ngweme's analysis of EDI values. The estimated daily intake of raw food from Kinshasa was shown to be low for Al, Cu, Mn, Pb, and Zn by Nuapia. Although the estimated daily intake of As, Cd, Cr, Hg, and Se surpassed the UL level established by WHO/FAO [23]. The EDI of Mata 2019 results showed that estimated values for examined hazardous metals were within acceptable limits, indicating that there are no negative effects on human health.

The complex measure known as the target hazard quotient (THQ) is used to calculate the possible health risk from exposure to chemical contaminants over an extended period of time (US EPA, 2006; Hague et al., 2008, Petroczi et al., 2009). Except for Zn and As, the obtained values of THQ for each metal in Ledermaniella were less than 1, according to Mata, et al. [20], indicating low risks to human health from intake through consumption of this vegetable; Mata, et al. [24], found that, with the exception of Hg in *M. moorii* and *S. mystys*, obtained THQ values for individual metal in different fishe sample were less than 1, indicating

negligible risks to human health for intake through consumption of them. Nuapia, et al. [16], found that consumption of an average quantity of beans, cabbage, beef, and fish results in high combined THQ values. The local consumers are potentially exposed to health risks.

According to Nuapia, et al. [16], all of the food samples for both male and female consumers had HI values greater than 1, indicating a substantial potential risk associated with consuming the food available in open markets in Kinshasa. The health of the consumer may be impacted in a synergistic way by exposure to multiple contaminants. According to Mata, et al. [24], the HI values for fish species are listed below in decreasing order: A. occidentalis (0.565) > D. fasciolatus (0.751) > C. gibbosus (0.565) > B. ubangensis (1.021) > M. moorii (1.202) > S. mystus (1.166) > B. ubangensis (0.410). The USEPA (2015) found that M. moori, S. mystus, and B. Ubangensis gave higher HI values than the acceptable limit compared to other species; according to HI values, regular consumption of these fish species could have a negative impact on human health if the HI value is greater than 1.

MPI identifies the buildup of harmful metals in food, providing detailed information about the level of contamination. The MPI values for fish species died in the following order, according to Mata, et al.'s [24] research: *D. fasciolatus* > *B. ubangensis* > *M. moorii* > *A. occidentalis* > *C. gibbosus* > *S. mystus*. The findings show that among the examined fish species, *D. fasciolatus* recorded the highest MPI value.

# **Conclusion**

The current study is the first review of heavy metals in food in the DRC, and it found that overall, the level of trace metal contamination in the environment was higher in the former Katanga region than in Kinshasa. Some heavy metal concentrations in food samples above the permissible levels for human consumption established by the Food and Agriculture Organization (FAO), the European Union (EU), JECFA, and the World Health Organization (WHO). Regular eating of these foods may seriously endanger people's health. Vegetable production on polluted soil, wastewater irrigation, pesticides used to treat and prevent vegetable diseases, and atmospheric deposition in areas with contaminated air are the main sources of heavy metal contamination. There have been few studies on food, primarily in vegetables (especially Amaranthus sp.) and only in 3 provinces (Kinshasa, former Katanga and Kongo Central). The authors concluded that in order to accurately estimate the hazards to human health, more thorough periodic studies should be conducted to monitor the levels of heavy metals in various foods from all regions of the Democratic Republic of the Congo.

# **Conflict of Interest**

The authors report no conflict of interest.

# **Funding**

This research received no external funding.

# References

- Luo W, Zhang N, Li Z, Xu Z, Wang D, et al. (2021) Increasement of Cd adsorption capacity of rice stubble from being alive until death in a modified rice-fish system. Ecotoxicology and Environmental Safety 208: 111441.
- 2. Rizwan MS, Imtiaz M, Zhu J, Yousaf B, Hussain M, et al. (2021) Immobilization of Pb and Cu by organic and inorganic amendments in contaminated soil. Geoderma 385: 114803.
- 3. Huff J, Lunn RM, Waalkes MP, Tomatis L, Infante PF, et al. (2007) Cadmium-induced Cancers in Animals and in Humans. Int J Occup Environ Health 13: 202-212.
- Andujar P, Bensefa-Colas L, A Descatha A (2010) Acute and chronic cadmium poisoning. Rev Med Interne 31: 107-115.
- 5. Tang S, Yu X, Wu C (2016) Comparison of the levels of five heavy metals in human urine and sweat after strenuous exercise by ICP-MS. Journal of Applied Mathematics and Physics 4: 183-188.
- Ekhator OC, Udowelle NA, Igbiri S, Asomugha RN, Igweze ZN, et al. (2017) Safety evaluation of potential toxic metals exposure from street foods consumed in Mid-West Nigeria. J Environ Public Health 2017: 8458057.
- 7. Gollenberg AL, Hediger ML, Lee PL, Himes JH, Buck Louis GM (2010) Association between lead and cadmium and reproductive hormones in peripubertal U.S. girls. Environ Health Perspect 118: 1782-1787.
- 8. Chang S-H, Cheng B-H, Lee S-L, Chuang H-Y, Yang C-Y, et al (2006) Low blood lead concentration in association with infertility in women. Environ Res 101: 380-386.
- 9. Tang N, Zhu ZQ (2003) Adverse reproductive effects in female workers of lead battery plants. Int J Occup Med Environ Health 16: 359-361.
- Radwan MA, Salama AK (2006) Market basket survey for some heavy metals in Egyptian fruits and vegetables. Food Chem Toxicol 44: 1273-1278.
- Oyebode O, Gordon-Dseagu V, Walker A, Mindell JS (2014)
   Fruit and vegetable consumption and all-cause, cancer and CVD mortality: Analysis of Health Survey for England data.
   J Epidemiol Community Health 68: 856-862.
- Shahid M, Xiong T, Castrec-Rouelle M, Leveque T, Dumat X (2013) Water extraction kinetics of metals, arsenic and dissolved organic carbon from industrial contaminated poplar leaves. J Environ Sci (China) 25: 2451-2459.
- 13. Mombo S, Schreck E, Dumat C, Laplanche C, Pierart A, et al. (2015) Bioaccessibility of selenium after human ingestion in relation to its chemical species and compartmentalization in maize. Environ Geochem Health 38: 869-883.
- 14. Michel MMM, Yannick US, François NN, Emmanuel MM, Prisca KK, et al. (2013) Évaluation des teneurs en éléments traces métalliques dans les légumes feuilles vendus dans les différents marchés de la zone minière de Lubumbashi. J Appl Biosci 66: 5106-5113.
- 15. Kalonda DM, Tshikongo AK, Koto FKK, Busambwa CK, Bwalya YK, et al. (2015) Profil des métaux lourds contenus dans les plantes vivrières consommées couramment dans quelques zones minières de la province du Katanga. J Appl Biosci 96: 9049-9054.
- 16. Nuapia Y, Chimuka L, Cukrowska E (2017) Assessment of

- heavy metals in raw food samples from open markets in two African cities. Chemosphere 196: 339-346.
- 17. Suami RB, Periyasamy Sivalingam P, Kabala CD, Otamonga J-P, Mulaji CK, et al. (2018) Concentration of heavy metals in edible fishes from Atlantic Coast of Muanda, Democratic Republic of the Congo. Journal of Food Composition and Analysis 73: 1-9.
- Suami RB, Al Salah DMM, Kabala CD, Otamonga J-P, Mulaji CK, et al. (2019) Assessment of metal concentrations in oysters and shrimp from Atlantic Coast of the Democratic Republic of the Congo. Heliyon 5: e03049.
- 19. Ngweme GN, Atibu EK, Al Salah DMM, Muanamoki PM, Kiyombo GM, et al. (2020) Heavy metal concentration in irrigation water, soil and dietary risk assessment of Amaranthus viridis grown in peri-urban areas in Kinshasa, Democratic Republic of the Congo. Watershed Ecology and the Environment 2: 16-24.
- 20. Mata HK, Al Salah DMM, Konde JN, Kiyombo GN, Mulaji CK, et al. (2020) Level of toxic metals in consumable aquatic plant ledermanniella schlechteri from Congo river and potential risk assessment through plant consumption. J Food Sci Nutr 6: 074.
- 21. Ngweme GN, Konde JNN, Laffite A, Kiyombo GM, Mulaji CK, et al. (2021) Contamination levels of toxic metals in marketed vegetable (*Amaranthus Viridis*) at Kinshasa, Democratic Republic of the Congo. J Food Sci Nutr 7: 087.
- 22. Muimba-Kankolongo A, Nkulu CBL, Mwitwa J, Kampemba FM, Nabuyanda MM, et al. (2021) Contamination of water and food crops by trace elements in the African Copperbelt: A collaborative cross-border study in Zambia and the Democratic Republic of Congo. Environmental Advances 6: 100103.
- 23. FAO/WHO (2000) Evaluation of certain food additives and contaminants. World Health Organization Technical Report Series. vol. 859. World Health Organization. Joint FAO/WHO Expert Committee on Food Additives, Geneva 859: 29-35.
- 24. Mata HK, Sivalingam P, Konde J, Otamonga J-P, Niane B, et al. (2019) Concentration of toxic metals and potential risk assessment in edible fishes from Congo River in urbanized area of Kinshasa, DR Congo. Human and Ecological Risk Assessment: An International Journal 26: 1676-1692.
- 25. Maleki A, Zarasvand MA (2008) Heavy metals in selected edible vegetables and estimation of their daily intake In Sanandaj, Iran. Southeast Asian J Trop Med Public Health 39: 335-340.
- 26. Prentice A (1993) Does mild zinc deficiency contribute to poor growth performance? Nutr Rev 51: 268-270.
- 27. Agency for Toxic Substances and Disease Registry (ATSDR) (1994) Toxicological profile for zinc. Atlanta, GA: US Department of Health and Human Services, Public Health Service.
- 28. Linder MC, Hazegh-Azam M (1996) Copper biochemistry and molecular biology. Am J Clin Nutr 63: 797S-811S.
- 29. WHO (1992) Cadmium environmental health criteria. Geneva: World Health Organization: 134.
- 30. WHO (1995) Lead environmental health criteria. Geneva: World Health Organization: 165.
- 31. Steenland K, Boffetta P (2000) Lead and cancer in humans: where are we now? Am J Ind Med 38: 295-299.
- 32. Jarup L (2003) Hazards of heavy metal contamination. Br Med Bull 68: 167-182.

- 33. WHO/FAO (2011) Joint FAO/WHO Food Standards Programme Codex committee on Contaminants in Foods. 21-25.
- 34. Wangboje OM, Ekome PC, Efendu UI (2017) Heavy metal concentrations in selected fishes, and water from Orogodo River, Agbor, Delta State in Nigeria. Asian Journal of Environment & Ecology 3: 1-10.
- 35. Anderson HA, Hanrahan LP, Smith A, Draheim L, Kanarek M, et al. (2004) The role of sport-fish consumption advisories in mercury risk communication: A 1998-1999 12-state survey of women age 18-45. Environ Res 95: 315-324.
- 36. Chan HM, Egeland GM (2004) Fish consumption, mercury exposure, and heart diseases. Nutr Rev 62: 68-72.
- 37. Harmanescu M, Alda LM, Bordean DM, Gogoasa I, Gergen I (2011) Heavy metals health risk assessment for population via consumption of vegetables grown in old mining area, a case study: Banat County, Romania. Chem Cent J 5: 64-73.
- 38. Schümann K, Classen HG, Dieter HH, König J, Multhaup G, et al. (2002) Hohenhem consensus workshorp: copper. Eur J Clin Nutr 56: 469-483.
- 39. ATSDR (United States Agency for Toxic Substances and Disease Registry) (2022) Toxicological Profile for Copper. U.S. Department of Health and Human Services, 362.
- 40. Yalc-ın Tepe A (2014) Toxic Metals: Trace Metals Chromium, Nickel, Copper, and Aluminum Encyclopedia of Food Safety, Volume 2.
- 41. Keith LS, Faroon OM, Fowler BA (2015) Uranium. In: Handbook on the Toxicology of Metals (Fourth Edition). Elsevier Inc., 2015: 1307-1345.
- 42. WHO (2010) WHO guidelines for indoor air quality: selected pollutants. The WHO European Centre for Environment and Health, Bonn Office. WHO Regional Office for Europe, Copenhagen, Denmark, 484.
- 43. Ma M, Wang R, Xu L, Xu M, Liu S (2020) Emerging health risks and underlying toxicological mechanisms of uranium contamination: Lessons from the past two decades. Environ Int 145: 106107.
- 44. Oliveira LHB, Ferreira NS, Oliveira A, Nogueira ARA, Gonzalez MH (2017) Evaluation of distribution and bioaccumulation of arsenic by ICP-MS in tilapia (Oreochromis niloticus) cultivated in different environments. J Braz Chem Soc 28: 2455-2463.
- 45. EPA (2002) Region 9 preliminary remediation goals. Washington, D.C.: U.S. 2002. Environmental Protection Agency.
- 46. Kuo CC, Moon K, Thayer KA, Navas-Acien A (2013) Environmental chemicals and type 2 diabetes: An updated systematic review of the epidemiologic evidence. Curr Diab 13: 831-849.
- 47. Maull EA, Ahsan H, Edwards J, Longnecker MP, Navas-Acien A, et al. (2012) Evaluation of the association between arsenic and diabetes: a National Toxicology Program workshop review. Environ Health Perspect 120: 1658-1670.
- 48. Moon K, Gualler E, Navas-Acien A (2012) Arsenic exposure and cardiovascular disease: an updated systematic review. Curr Atheroscler Rep 14: 542-555.
- 49. Hossain MS, Ahmed F, Abdullah ATM, Akbor MA, Ahsan MA (2015) Public health risk assessment of heavy metal uptake by vegetables grown at a waste-water-irrigated site in Dhaka, Bangladesh. J Health Pollution 5: 78-85.

- 50. Zaqoot HA, Aish AM, Wafi HN (2017) Baseline concentration of heavy metals in fish collected from Gaza fishing harbor in the Mediterranean Sea along Gaza Coast, Palestine. Turk J Fish Aquat Sci 17: 101-109.
- 51. Ali MHH, Al-Qahtani KM (2012) Assessment of some heavy metals in vegetables, cereals and fruits in Saudi Arabian markets. Egyptian Journal of Aquatic Research 38: 31-37.
- 52. Stančić Z, Vujević D, Gomaz A, Bogdan S, Vincek D (2016) Detection of heavy metals in common vegetables at Varaždin City Market, Croatia. Arh Hig Rada Toksikol 67: 340-350.
- 53. Garcia-Bravo A, Loizeau J-L, Bouchet S, Richard A, Rubin JF, et al. (2010) Mercury human exposure through fish consumption in a reservoir contaminated by a chlor-alkali plant: babeni reservoir (Romania). Environ Sci Pollut Res 17: 1422-1432.
- 54. Garcia-Bravo A, Bouchet S, Amouroux D, Potéa J, Dominika J (2011) Distribution of mercury and organic matter in particle-size classes in sediments contaminated by a waste water treatment plant: Vidy Bay, Lake Geneva, Switzerland. J Environ Monit 13: 974-982.

- 55. Shaw CA, Tomljenovic L (2013) Aluminum in the central nervous system (CNS): toxicity in humans and animals, vaccine adjuvants, and autoimmunity. Immunol Res 56: 304-316.
- 56. USFDA (1993) Food and Drug Administration, Guidance Document for Chromium in Shellfish. DHHS/PHS/FDA/CFSAN/Office of Seafood, Washington, DC.
- 57. Anderson RA (1997) Chromium as an essential nutrient for humans. Regul Toxicol Pharmacol 26: 35S-341S.
- 58. Merzenich H, Hartwig A, Ahrens W, Beyersmann D, Schlepegrell R, et al. (2001) Biomonitoring on carcinogenic metals and oxidative DNA damage in a cross-sectional study. Cancer Epidemiol Biomark Prev 10: 515-522.
- Costa M, Klein CB (2006) Toxicity and carcinogenicity of chromium compounds in humans. Crit Rev Toxicol 36: 155-163.
- 60. International Agency Research of Cancer (IARC) (1990) Monographs on the evaluation of carcinogenic risk to humans. Chromium, Nickel and Welding, 49. WHO, Lyon, France, 49-256.

