Concentration of Selected Elements in the Hair of Malagasy Girls in Relation to Their Nutritional Status and Environmental Exposure

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Abstract

Although the children malnutrition in Madagascar and the environmental pollution of this country has been widely discussed, there is no research on the susceptibility of malnourished Malagasy to toxic elements. Nine elements concentration (Al, As, Cd, Cr, Hg, Ni, Pb, Sn, Sb) was determined in 103 samples of schoolgirls scalp hair (8–15 years old), and in twenty samples of water and soil, from two areas: Antananarivo (UR) and Berevo (RU). Samples were analysed by an inductively coupled plasma-optical emission spectrometer using a microwave-induced nitrogen plasma atomic emission spectrometer. The nutritional status was evaluated by Cole's Index. The recommended level of Sb was exceeded in the soil samples, while the Al and Ni in the water. Underweight was related to higher accumulation of Al, Cd, Cr, Pb, and Ni in the hair and more common among girls living in RU than UR region (42% vs 28%). . Two-factor analysis of variance showed differences in the Al and Cr content in the girls' hair depending both on their place of residence and nutritional status. This indicate that the malnourished girls may be more susceptible to soil and water pollution with toxic elements than girls with adequate nutritional status.

1. Introduction

Toxic elements, including metals and metalloids, demonstrate an undesirable effect on the environment and living organisms. They inhibit the activity of key metabolic enzymes and may bioaccumulate in living tissues, giving rise to symptoms of toxicity. Thus, they are considered non-biodegradable pollutants of the environment. Some of these metals and metalloids at low concentrations are essential to maintain the physiological and biochemical functions of living organisms, although they become toxic at higher concentrations (e.g., Fe, Cr, Cu, Zn). Moreover, some elements are toxic even in trace amounts (Pb, Hg, Cd, As)[1]. Since these elements (or their excessive amounts) cannot be included in metabolic processes, they build up in selected human tissues and are only partially excreted (in adipose tissue, liver, kidneys, hair and urine). Long term exposure to even low concentrations of these metal ions poses a threat to human health, especially children. The human body is exposed to the adverse effects of toxic elements caused by their natural occurrence in high amounts in some geographical regions of the earth's crust. Furthermore, this problem is increasing as a consequence of industrial development without the application of environmentally friendly technologies. The economies of mainly developing countries such as Madagascar have been struggling with this type of problem. The population of Madagascar is subjected to high concentrations of toxic elements in water, soil and air. The Lancet Commission on Pollution and Health (2017)[2] reported that environmental pollution was responsible for 22.3% of all deaths in Madagascar. The interference of malnutrition and environmental pollution and their impact on young Malagasy may cause even worse health problems for this population.

Malnutrition is a consequence of the country's underdeveloped economy. The source of income for most Malagasy people is agriculture (rice, cassava and sweet potato cultivation). Ninety-two per cent of the Malagasy population lives on less than US $ 1.90 a day. Madagascar is a third-world country where malnutrition and environmental pollution is at the top of the list of factors contributing to death or disability [3] . Lack of nourishment affects the whole country and acute malnutrition afflicts 8% of children under five years of age and in many regions is even higher [4] , accounting for 83% of all deaths in infants and children under five years of age. It is one of the most serious health problems in Madagascar [5] , and many public organizations around the world are involved in feeding programs for Malagasy children. Because of the low industrialisation, for many years Africa was considered safe from heavy metal pollution. However, the beginning of the 21st century showed that pollution had increased drastically, probably as a result of urbanisation processes and intensive and predatory exploitation of natural resources [6] . The problem also affects the urban community of Antananarivo, one of the main industrial centres of Madagascar, especially because of the location (10 km from the city centre) of a huge landfill without water treatment or containment system. [7]

Although there is limited information on the Madagascar pollution with heavy metals and metalloids, it has been confirmed that the high mortality on the island is caused by these factors [2] .

To study the Malagasy girls' exposure to environmental pollution with toxic elements, analyses of the concentration of Al, As, Cd, Cr, Hg, Pb, Ni, Sn, and Sb were conducted. Arsenic, lead, mercury, cadmium and chromium are usually considered the most toxic elements for the human body. However, because of the unique research area, the list of the analysed toxic elements was extended for the purposes of the presented studies. Antimony, tin, nickel and aluminium were included. Their accumulation in the human body causes chronic toxicity. The first of these metals (antimony) demonstrates a chemical behaviour similar to arsenic. It is a natural co-contaminant with arsenic in the drinking water[8]. An increased concentration of these two metal ions has been observed in African soil and water[9] and both of them are considered carcinogenic[10,11]. The next element (tin) is exceptionally hardly biodegradable. Moreover, an increase in the content of this element was noted in both mining areas and agricultural regions as a residue from excessive use of pesticides[12] and also currently as a consequence of the increasing amount of electronic waste[13,14]. Poisoning with tin is responsible for digestive tract irritation and anaemia in the human body[15]. An increase in nickel concentration in African countries, especially in Madagascar, has also been noted along with intensive and improper exploitation of natural resources. Nickel might also be present in the environment due to landfilling used nickel/cadmium-based batteries and other waste such as printed circuit boards[16,17] or as another product of the industrial application of this metal[6]. This metal is carcinogenic and irritates the skin, the respiratory and digestive systems[18]. The latter of the studied elements (aluminium) does not belong to the group of heavy metals. In Europe, its excessive accumulation in food or water has not been recorded. It should be mentioned that many aluminium manufacturers were found in the studied region, which suggests possible environmental pollution. Moreover, locals process aluminium using homemade methods (especially close to the studied rural area), but also in factories (close to the capital) for the production and sale of dishes. Additionally, food has been commonly consumed from aluminium vessels and with aluminium kitchen utensils[19]. The Malagasy population habitually drink water boiled in aluminium pots with burnt rice stuck to the bottom. Aluminium is implicated in many health problems. It is necessary to underline that humans ingest aluminium through the respiratory and digestive systems and skin, which can affect health and impair the central nervous system. The literature reports cognitive impairment in Al-exposed subjects, Alzheimer's disease and other neurodegenerative disorders related to aluminium exposure[20].

Samples of soil, water and hair of girls living in two-regions of Madagascar – urban (close to the capital city of Antananarivo) and rural (from Berevo) were analysed. Soil and water samples were collected to study the environmental pollution of these two areas.

Hair is perceived as an attractive biological material for the analysis of human health and human exposure to toxic metals. It is convenient for sampling, transport and storage compared to physiological fluids and other tissues (e.g., blood, urine, or saliva). Hair samples offer a long period of stability, constant chemical composition, can bind metal ions and the elements stored in them are not used for metabolic processes. Thus, hair is considered in biomedical sciences as a tissue which reflects the metabolism of certain microelements in the human body[21,22]. Moreover, some studies have also suggested that an analysis of soil and air delivers worthless data for assessing the health hazard of environmental contamination, because of the high variability of the metal concentration over time[22,23]. The Environmental Protection Agency noted hair analysis as being an important marker of the long term exposure of the human body to the action of heavy metal ions[24]. The data on the occurrence of metals and metalloids in water and soil of Madagascar and its reflection in their concentration in girls' hair has not been widely reported in scientific papers. This study analysed the above-mentioned relation for two differently economically developed (industrial and rural) regions. The scientific validity of the study was supported by an analysis of the nutritional status of girls in relation to environmental pollution in the studied areas.

2. Study Sample And Methods

2.1. Study design

The study was conducted in Madagascar in autumn 2018 and involved a total of 103 girls aged 8–15 years from two regions of the country (study design Fig. 1, Table 1). The recruitment was carried out in elementary schools and a college, located in urban - Antananarivo (UR) and rural - Berevo (Menabe) (RU) regions. In the UR region, 50 girls from a halfway house for girls and college were enrolled while in the RU region, 53 girls from a school run by a Catholic mission were selected for the study. At schools in the UR region, girls participated in a feeding program financed by a non-governmental organisation, whereas girls from the halfway house received breakfast and dinner. There was no additional form of feeding programme in the RU region. Table 1 presents the characteristics of the study.

Table 1
Sample characteristic by sociodemographic and lifestyle variables of RU and UR groups.

Parameter (Mean ± SD) [1]	Total sample (n = 103)		Urban (Antananarivo) (n = 50)		Rural (Menabe) (n = 53)	
	x ± SD	Min-Max	x ± SD	Min-Max	x ± SD	Min-Max
Age (years)	12 ± 2	8–15	12 ± 2[a*]	8–15	11 ± 2[b]	8–15
Weight (kg)	34.0 ± 11.0	17.0–60.4	39.2 ± 11.3[a]	18.3–60.4	29.2 ± 8.1[b]	17.0–55.2
Height (cm)	138 ± 13	112–163	143 ± 12[a]	115–163	133.6 ± 11.8[b]	112.0–162.0
BMI (kg/m^2)	17.3 ± 2.9	11.9–24.6	18.7 ± 3.1[a]	13.3–24.6	16.0 ± 2.1[b]	11.9–23.6
Cole index (-)	96 ± 11	71–124	101 ± 12[a]	79–124	92 ± 9[b]	71–116
Cole index distribution n (%) ($p < 0,001$)[2]:						
Underweight	36 (35)		14 (28)		22 (42)	
Recommended values	52 (50)		23 (46)		29 (55)	
First degree	12 (12)		10 (20)		2 (4)	
Second degree	3 (3)		3 (6)		0 (0)	
The frequency of meals n (%):						
Everyday breakfast	15 (15)		15 (30)		0 (0)	
Regular lunch [3]	90 (87)		50 (100)		0 (0)	
Random meals	12 (12)		5 (10)		45 (85)	
Economic situation of the family[4]						
Below average	87 (85)		37 (75)		50 (94)	
Average	16 (15)		13 (25)		3 (6)	
Above average:	0 (0)		0 (0)		0 (0)	

[1]Statistically significant differences between UR and RU children were marked with different letter inscriptions ab as $p < 0.001$ [2]Statistical significance (Person's chi-squared test): $p < 0.0001$ [3] Participation in the feeding programme at school (1 meal at school) [4] The financial situation was assessed using the question: 'How would you describe your household's overall situation?'; The 'modest' category consisted of two answers: 'we have to be very careful with our daily budget' and 'we have enough money for our daily needs, but we need to budget for bigger purchases'; The 'comfortably' category consisted of one answer: 'we have enough money for our needs without particular budgeting'; The 'wealthy' category consisted of one answer: 'we can afford some luxury'.

Parameter (Mean ± SD) [1]	Total sample (n = 103)		Urban (Antananarivo) (n = 50)		Rural (Menabe) (n = 53)	
	x ± SD	Min-Max	x ± SD	Min-Max	x ± SD	Min-Max
Caregiver completed education level: Primary or lower Secondary Upper secondary	61 (59) 42 (41) 0 (0)		13 (25) 37 (75) 0 (0)		48 (90) 5 (10) 0 (0)	
Sources of drinking water: Water supply network Surface water (rivers, lakes, wells)	15 (40) 45 (55)		0 (0) 50 (100)		53 (100) 0 (0)	
Toilets availability at home: Yes No	50 (49) 53 (51)		50 (100) 0 (0)		0 (0) 53(100)	

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The research was approved by Bioethics Committee of the Faculty of Medical Sciences, Poznan University of Medical Sciences (No. 1273/18). Informed consent was obtained from parents of all participants and/or their legal guardians. Research were performed in accordance with the Declaration of Helsinki.

2.2. Anthropometrics

The measurements of body weight (kg) and height (cm) were taken and recorded with a precision of 0.1 kg or 0.1 cm, respectively, using professional devices and a measuring tape. All measurements were taken in light clothing and without shoes according to the guidelines[25]. Body mass index (BMI, kg/m^2) was then calculated. The above parameters were used to calculate the Cole's Index [26].

$$CI\% = \frac{Weight\ [kg] x\ height\ for\ age\ and\ sex\ (50th\ percentile)^2}{Standard\ body\ weight\ [kg] x\ height^2} x100\%$$

Based on the criteria, the results were interpreted as follows: < 90 underweight, 90–109 - recommended values, 110–119 - first-degree obesity (overweight), 120–139 - second-degree obesity, 140 and above - third-degree obesity [9,25,27]. The data were presented in Table 1.

2.3. Hair sample collection

Approximately 1 g hair samples from the occiput area were collected from subjects who did not have coloured or treated hair (dreadlocks etc.) in autumn. The samples were stored in encoded, sealed polyethylene bags at room temperature until analysis.

2.4. Environmental samples

Samples of water and soil were collected from the areas presented in Fig. 2. Water samples in the UR area were collected from the water supply network, and in the RU area from rivers, lakes and wells. Water samples from the river (RU), were collected in several places along the river, before the water flows into Berevo. However, since children originated from approximately a 30-square km area, samples were also collected from sites located at a distance from the school. The samples of water were taken at the place of daily activity of the studied groups, i.e. place of use of drinking water, and water intended for hygienic purposes or vegetable and crops cultivation (Fig. 2). The soil samples were obtained from the same places. The water was filtered through a 0.45 μm filter into 40 mL tubes and stored at room temperature until analysis. Soil samples collected from the surface (100 cm^2), from the same places as water and were mixed and stored in Eppendorf-type tubes at room temperature until analysis. Two soil types were collected: red ferruginous (dominant among sampling points 1–10) and ferralitic soil, generally characterised by high Fe and Al levels with a deficiency of N and P.

2.5. Determination of elemental composition by Inductively Coupled Plasma - Optical Emission Spectrometers (ICP-OES)

2.5.1. Sample preparation

Hair samples were washed with acetone and water and dried at the ambient temperature. The samples (0.100 ± 0.001 g) were then digested in 5 mL of 65 % nitric acid (Merck, Darmstadt, Germany) in closed Teflon containers in the Mars 6 Xpress microwave digestion system (CEM, Matthews, USA) and were filtered through paper filters and diluted with water to a final volume of 15.0 mL. Extraction of the acid leachable fraction from the hair samples was carried out using hydrochloric acid following the previously developed procedure[28]. Extraction of the acid leachable fraction from the environmental samples (1.000 ± 0.001 g of soil or 1.000 ± 0.001 mL of water) was performed with 20 mL 2 M HCL (Merck, Darmstadt, Germany) at 80°C. Samples were then filtered through paper filters and diluted with water to a final volume of 20.0 mL. Each of the samples was processed in three replicates.

2.5.2. Sample analysis and quality control

An inductively-coupled plasma spectrometer with optical emission detection (Agilent 5110 ICP-OES, Agilent USA) was used to analyse the samples. The following conditions were maintained for 62 measurements in total: plasma gas flow of 12.0 L min^{-1}, nebuliser gas flow of 0.7 L min^{-1}, auxiliary gas flow of 1.0 L min^{-1} and Radio Frequency (RF) power of 1.2 kW. The most sensitive analytical wavelengths were used to measure all elements. Potential spectral interferences were recognised in the method validation and the background correction methods (fitted or off-peak) were selected. Commercial ICP analytical standards (Romil, England) and demineralised water (Direct-Q system, Millipore, USA) were used for the calibration. The detection limits were estimated in the range of 0.01–0.09 mg kg^{-1} dry weight (DW) using the criteria of 3-sigma. The uncertainty level was estimated for the procedure, including sample preparation at the level of 20%. Both certified reference material analyses (soils: CRM and CRM S-1; sediments: CRM 667 and CRM 405; CRM NCSDC (73349) – bush branches and leaves) and standard addition methods were used in quality control with acceptable recovery (80–120%). Results of an analysis of nine toxic elements (aluminium, arsenic, cadmium, chromium, mercury, nickel, lead, tine and antimony) in the samples of soil, water and hair obtained from the studied girls are presented.

2.6. Statistical analysis

The Clinical Calculator (ClinCalc, LLC) was used to calculate the hair sample size[29]. The calculation was based on means and standard deviations of As in soil. The minimum number of studied subjects for adequate study power was calculated as 43 for each independent group with the enrolment ratio set at 1, type I error at 0.05 and 80 % power. The data were presented as means (standard deviations) or medians (lower and upper quartiles) as appropriate. A Shapiro–Wilk test evaluated the normality of the distribution of continuous variables in the total sample, rural and urban areas. Skewed variables were logarithmically transformed. Differences between the concentrations of metals and metalloids in hair in urban vs rural area, water and soil samples were examined using independent t-tests ANOVA. The significance of the main effects and interaction of experimental factors was determined with a two-way analysis of variance.

For exploratory analysis, a Spearman rank correlation of Cole's Index with the concentration of metal and metalloid in hair and age as well as the correlation between the element concentrations in hair were analysed. For these analyses, a significance level of < 0.05 was adopted and correlation levels were interpreted as from 0.00 to 0.19–very weak correlation, 0.20 to 0.39–weak correlation, 0.40 to 0.69–moderate correlation, 0.70 to 0.89–strong correlation and 0.90 to 1.00–very strong correlation[30]. All statistical analyses were performed using STATISTICA software (version 12.0 PL; StatSoft Inc., Tulsa, OK, USA; StatSoft, Cracow, Poland). The threshold for statistical significance was $p \leq 0.05$.

3. Results And Discussion

In the current study, samples of hair, soil and water were collected. They were divided according to the area of collection: urban or rural. The urban area (UR) represented the province of the capital Antananarivo, located on the east coast of the island, the most industrialised region of East Madagascar. The rural area (RU) was Berevo, a town situated 360 km south-west of Antananarivo. The region of the Tsiribihina River is the only drinking water supplier and transport route to the Berevo and is oil-polluted from barges and sewage from local farms. The studied environmental samples were taken from the sites of the girls' everyday life, where they may have been exposed to toxic metals. According to collected data, in the UR area, all girls participated in the feeding program. In the halfway house, the diet was composed of locally produced food crops. There were limited (maximum two times per week) animal protein sources (mainly chicken, pork, fish) and a limited amount of fruit and other vegetables in the daily diet. Children in the RU region did not participate in any feeding program. Foodstuffs, mainly rice, were supplied there only periodically by Catholic missions. It should be mentioned that the girls of both groups consumed unprocessed food, which was prepared from raw materials obtained from the areas in which they lived (from the surrounding gardens and fields), thus growing on the tested soil. The subjects drank the tested water.

3.1. WATER SAMPLES

Table 2 shows metal and metalloid concentrations in water samples from the RU and UR areas. The analysed samples were water drunk in the given region by the examined girls, i.e. tap water in the case of the urban region of Antananarivo (taps in the 'Home for girls', canteen, schools and municipal water intakes) and water from a river, lakes and wells receiving shallow groundwater in the rural region. The differences in the average metal and metalloid concentrations between the RU and UR areas were not found. There was no trend observed for an association between the water intake type and increased contents of metals or metalloids. Such associations were reported only for both the tap water and water from the river samples. However, the median concentration of arsenic in drinking water in the UR area was twice higher than the maximum permitted level (Table 3) and the median concentration of Sn was also higher in the UR water. The arsenic level was exceeded in eight of the tested water samples (including three from the Menabe –RU region), chromium level in one, mercury level in two, lead level in eleven, antimony level in four and tin level in two samples (from the 20 studied water samples). A higher median of the aluminium concentration was detected in the samples collected from the rural region, which could be connected with the manufacturing of aluminium articles in this area. It should be emphasised that samples of water in the RU region (from the lake 0.76 mg/L and well 0.99 mg/L) significantly exceeded the reference level (compare Table 3). The amount of nickel was far above the recommended value in each of the studied samples (which confirms the problem of nickel pollution in African countries)[6], while cadmium was below LOD (limit of detection). The increased content of the studied elements in water samples may suggest an increased supply of these metals and metalloids in a bioavailable form to people living in these areas. The problem of water quality in north-western cities of Madagascar was discussed previously by Akers et al.[20] who showed that 67% of analysed samples exceeded the World Health Organization's provisional guidelines for drinking water. It should be noted that water is one of the essential utilities necessary for life and its quality should be particularly monitored and protected.

Table 2
Comparison of the studied elements content in the water samples [mg/L][1].

Element	Place	Median	Min	Max	25th	75th	Mean	SD	p
Al	Tana	<LOD*	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	p = 0.11
	Berevo	0.0186	0.0005	0.9882	0.0116	0.0885	0.1960[a]	0.3640	
As	Tana	0.0200	<LOD*	0.0680	<LOD	0.0380	0.0250[a]	0.0270	p = 0.54
	Berevo	0.0030	0.0010	0.0580	0.0010	0.0340	0.0170[a]	0.0250	
Cd	Tana	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	p = 0.14
	Berevo	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	
Cr	Tana	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	p = 0.40
	Berevo	0.0008	<LOD	0.0026	0.0005	0.0016	0.0011[a]	0.0010	
Hg	Tana	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	p = 0.27
	Berevo	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	
Pb	Tana	0.0162	<LOD	0.0444	0.0144	0.0304	0.0200[a]	0.0150	p = 0.76
	Berevo	0.0122	<LOD	0.0605	0.0011	0.0284	0.0170[a]	0.0200	
Ni	Tana	0.0300	0.0100	0.0400	0.0200	0.0300	0.0200[a]	0.0100	p = 0.99
	Berevo	0.0200	0.0100	0.0400	0.0200	0.0300	0.0200[a]	0.0100	
Sb	Tana	0.0133	<LOD	0.0417	0.0093	0.0208	0.0160[a]	0.0120	p = 0.26
	Berevo	0.0077	<LOD	0.0279	0.0011	0.0174	0.0100[a]	0.0100	
Sn	Tana	0.0059	<LOD	0.0225	0.0005	0.0104	0.0070[a]	0.0070	p = 0.82
	Berevo	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	

* Values of LOD (level of determination) was 0.0005 mg/L

[1] Data were analysed after their logarithmic transformation, but in the table, elements concentration was presented as raw data.

Table 3
Values of the studied metal concentrations recommended for soil and water

	Al	As	Cd	Cr	Hg	Pb	Ni	Sb	Sn
Soils [mg/kg](FAO 2006; Nicholson and Chambers 2008)	X	10	5	250	1.0	150	100	0.2–0.3 (Tschan et al. 2009)	2–3
Water [mg/l](WHO 2017)	0.2	0.01	0.003	0.005	0.001	0.01	0.007	0.005	0.002
X – the natural level of aluminum is connected with the mineralogical composition of soils (the level of aluminosilicates)									

3.2. SOIL

Table 4 presents the levels of metals and metalloids in collected soil samples from the UR and RU regions. A significantly high (above five times higher) concentration of aluminium was observed in the urban area. Surprisingly, such a concentration of aluminium was detected in UR soil samples, although in the water from the urban area it was below LOD, while aluminium usually dissolves well in water. This may suggest the presence of a non-soluble form of this metal in the soil, while the content of Al-phosphates in the laterite was reported in the studied area[31,32]. The toxicity of aluminium to plants grown in these areas is difficult to predict because the total content does not reflect its bioavailability, which varies depending on soil pH (soil of Madagascar is considered to be acidic[33]). Aluminium is usually considered an agent inhibiting the growth of plants, but it is also accumulated in crops[34] and, consequently, enters the food chain. Moreover, it was observed that lead was significantly more concentrated in the soil from the urban area. Such an outcome may result from industry, transport or runoff from landfills. The concentrations of cadmium and tin in soil samples were below LOD.

Table 4
Comparison of studied elements content in the soil samples[1] [mg/kg].

Element	Place	Median	Min	Max	25th	75th	Mean	SD	p
Al	Tana	15643	6363	31761	13151	26282	18593[b]	10045	p < 0.001
	Berevo	2830	1283	6561	1458	3018	2804[a]	1835	
As	Tana	0.761	<LOD*	4.178	0.008	3.131	1.560[a]	1.926	p = 0.82
	Berevo	1.705	<LOD	2.420	0.624	1.993	1.340[a]	0.917	
Cd	Tana	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	p = 0.57
	Berevo	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	
Cr	Tana	8.45	5.71	19.02	6.34	14.40	10.61[a]	5.59	p = 0.36
	Berevo	6.19	3.16	16.33	5.63	9.86	8.09[a]	4.43	
Hg	Tana	0.016	0.005	0.166	0.005	0.098	0.060[a]	0.071	p = 0.32
	Berevo	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	
Pb	Tana	22.4	5.1	49.6	9.0	38.9	24.7[b]	18.8	p < 0.001
	Berevo	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	
Ni	Tana	6.1	2.8	20.4	4.3	9.1	8.2[a]	6.5	p = 0.070
	Berevo	2.3	2.1	10.6	2.2	3.4	3.8[a]	3.1	
Sb	Tana	3.8	2.2	8.9	2.7	5.8	4.6 [a]	2.6	p = 0.64
	Berevo	3.5	2.8	5.5	3.0	4.5	3.8[a]	1.1	
Sn	Tana	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	p = 0.77
	Berevo	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	

* Values of LOD was 0.01 mg/kg

[1] Data were analysed after their logarithmic transformation, but in the table, elements concentration was presented as raw data.

The problem of different concentrations of metals in the soils of Madagascar was discussed by Herve et. al[35], who reported higher levels of heavy metal concentrations in coastal sediments in the north-western cities. Generally, the metals and metalloids in the soil, except for antimony, did not exceed the acceptable levels presented in Table 3. As regards antimony, its concentration was comparable to those in heavy traffic cities[36,37]. It is hypothesised that antimony levels in Madagascar could be affected by rock remineralisation and binding by iron oxides[38] present in laterite. Simultaneously, the level of total iron in the studied samples did not exceed the typical concentrations (in Berevo, RU region, it was on average 4242.2 ± 2973.11, while in Tana-UR it was 11025.55 ± 12570 mg/kg). The levels of antimony in soil should be considered harmful to the Malagasy population[10,11]. It is necessary to underline that the mean concentration of antimony in both soil and water samples exceeded acceptable levels: approximately 3.2-fold in water from the UR area and 2-fold in the RU. In turn, in soil, its concentration was 15.3-fold higher and 12.7-fold higher than the acceptable level in the UR and RU areas, respectively. It should also be noted that soil and water are crucial media which affect the accumulation of metal ions in the body. Malagasy children are food-insecure, at a level necessary to develop, and the basis of their nutrition is rice grown in residential areas, from which the tested soil and water samples were collected. It should also be emphasized that the bioavailability of the studied elements differs depending on their origin. The correlations between the content of individual elements in water and soil were not significant (for Al: $R=-0.405$, $p = 0.170$; As: $R = 0.431$, $p = 0.142$; Ni: $R=-0.504$, $p = 0.079$; Cr: $R = 0.200$, $p = 0.512$, Hg: $R = 0.343$, $p = 0.251$, Sb: $R = 0.265$, $p = 0.382$, Sn: $R = 0.300$, $p = 0.320$; Cd: $R=-0.164$, $p = 0.593$; and Pb: $R = 0.110$, $p = 0.971$).

3.3. HAIR

The hair samples of girls from the UR region were collected in schools and a care centre for girls in the capital and the surrounding area. The samples collected in the RU region originated from Berevo town. This place was practically isolated from the industrialised area. The locals travel a long distance to the nearest small town (about 98 km) by waterway to reach the nearest hospital. The isolation of the village and gaps in education contribute to the development of extreme poverty. Collected outcomes were compared to the data from Poland[39], Japan[40], France[41], Turkey[42], Sweden[43], Italy[44], Brazil[45], Spain[46], India[47], China[22] and Egypt[24,48]. The results in Table 5 revealed a significantly higher average concentration of aluminium, chromium and mercury in the hair of girls from the RU area. According to the literature, the average bioavailability of aluminium from water is ~ 0.3%, and that from food and other beverages is ~ 0.1%[49]. Despite the significantly higher aluminium concentration in soil of the UR region, the accumulation of this metal in the hair of RU children was higher than in the UR region (median 1950 vs 338 mg/kg, $p < 0.001$). The current study found a much higher aluminium content in Malagasy girls' hair than in the hair of Japanese children, whose content of aluminium in the hair was 5.35–20.6 mg/kg (three-fold higher than in adults' hair)[40]. The other authors reported data from healthy Egyptian children (16.78 ± 17.3 mg/kg) and autistic children (59.219 ± 38.00 mg/kg)[24,48].

Table 5

Comparison of studied elements content in the hair samples[1] [mg/kg].

Element	Place	Median	Min	Max	25th	75th	Mean	SD	p
Al	Tana	338.308	40.711	4527.591	124.650	1330.133	913.010[a]	1119	p < 0.001
	Berevo	1950.913	61.221	6336.527	1414.119	3165.736	2383.211[b]	16807	
As	Tana	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	p = 0.80
	Berevo	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	
Cd	Tana	0.228	<LOD	1.488	0.141	0.380	0.293[a]	0.253	p = 0.24
	Berevo	0.200	<LOD	1.128	0.124	0.345	0.241[a]	0.192	
Cr	Tana	0.520	0.040	3.710	0.220	1.110	0.770[a]	0.790	p < 0.001
	Berevo	2.780	0.130	8.250	1.910	4.250	3.050[b]	1.940	
Hg	Tana	0.148	<LOD	0.387	0.106	0.213	0.164[a]	0.101	p < 0.001
	Berevo	0.277	<LOD	1.478	0.203	0.471	0.350[b]	0.243	
Pb	Tana	6.082	<LOD	27.473	0.327	10.719	6.922[a]	6.723	p = 0.26
	Berevo	5.379	<LOD	67.675	2.889	8.579	8.241[a]	11.188	
Ni	Tana	0.991	<LOD	5.289	0.207	2.274	1.392[a]	1.380	p = 0.51
	Berevo	1.807	<LOD	7.187	0.723	2.579	1.853[a]	1.458	
Sb	Tana	1.554	<LOD	4.814	0.772	2.132	1.594[a]	1.145	p = 0.22
	Berevo	0.958	<LOD	3.888	0.557	2.135	1.313[a]	1.051	
Sn	Tana	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	p = 0.10
	Berevo	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD[a]	<LOD	

*LOD = 0.005

[1]Data were analysed after their logarithmic transformation, but in the table, elements concentration was presented as raw data.

The water most frequently drunk in the RU area (Berevo) was lake water, where the content of Al exceeded the recommended value several times (0.76 mg/L) (Table 3). The level was also high in samples of water collected at a short distance from the school (1.00 mg/L). However, because of such an extremely high concentration of aluminium in the hair, it is suspected that laterite dust could have penetrated the hair structure. The dust was constantly floating in the air and it was especially observed in the RU area (with unpaved roads). However, this only confirms the high exposure of the Berevo children to aluminium concentration and future studies are necessary to explain such a high concentration of Al in the girls' hair.

In the studied hair samples, the concentration of arsenic was noted to be below LOD (which was 0.005 mg/kg). The arsenic concentration was detected only in the samples of four girls, two from each region, and the determined concentration ranged from 0.124 to 1.821 mg/100 g. The bioavailability of this metalloid from water and food varies widely (45–95%)[50] and depends on the element species, with higher toxicity for an inorganic form[51]. The level of arsenic in hair noted in the literature ranges from 0.011 ± 0.007 in Spain up to 0.127 ± 0.078 mg/kg in Beijing [22,46]. A high content of cadmium was reported in the hair of the girls in both regions, although its levels in soil and water samples were below LOD. The literature suggests cadmium concentration in hair ranges from approximately 0.011 mg/kg (in France) to 0.23 ± 0.55 (in Italy)[41,44]. The median level of cadmium in the hair of the studied group from both regions was 0.228 mg/kg (UR) and 0.200 mg/kg (RU). This is surprising because of the low industrialisation of Madagascar, the lack of tobacco smoking as primary sources of cadmium and the high isolation of the rural area. Such high exposure to Cd was observed in other African countries and is explained by the use of leaded gasoline[52], which may also be the case in the present study.

The content of chromium in the studied samples differed significantly between the RU and UR areas. The current study reported ~ 5-fold higher concentration in the samples from the RU region (Berevo) than from UR region (Antananarivo) (median 2.78 mg/kg vs 0.52 mg/kg). Such a concentration was much higher than reported in Sweden (0.167 mg/kg ± 0.118) and Italy 0.99 mg/kg ± 2.17 [43,44]. The high content of chromium in the studied samples was not expected because the mean content of Cr in soil and water samples did not exceed the recommended values (Tables 2 and 4). Thus, the reason why girls were exposed to this element is difficult to explain on the basis of the presented research. It is necessary to emphasise that the bioavailability of chromium in human studies ranges between 0.69-10% and depends on its chemical form[53]. A key aspect worth noting in interpreting these results might be related to differences in nutritional status between UR and RU children, discussed below.

A high content of mercury in hair is usually correlated with high Al content and this trend was observed in the studied samples: hair from RU samples contained more Hg, i.e. the median was 0.278 mg/kg, while in UR girls it was 0.15 mg/kg. Fortunately, the content of Hg in the studied hair was lower than noted in the literature, where ranges from 0.26 ± 0.14 mg/kg (in Sweden) up to 3.18 ± 3.40 (in Japan) were quoted[43,54]. This concentration might result from the low contamination of water and soil since Hg is highly absorbed in the digestive tract (from food and water) even up to 80%[55]. Lead content in the hair (median in UR 6.08 and RU 5.38 mg/kg) corresponded to levels reported in the literature (from 0.96 ± 0.84 mg/kg in Sweden up to 14.11 ± 4.64 mg/kg in Turkey)[42,43]. Although the nickel pollution in analysed water samples was higher than admissible in both regions (Tables 2 and 3), the nickel concentrations in hair were 0.99 in UR and 1.81 mg/kg in RU. These levels were lower than in the samples obtained from university students from an urban area in Poland (median from 1.761 for men up to 2.14 mg/kg for women)[56]. This confirms that hair contamination with Ni is often connected with the use of cosmetics and exposure of humans to processed food[56]. Exposure to Sb is usually accompanied by pollution with As. Nevertheless, gastrointestinal absorption of antimony (5–20%) is far lower [8]. The correlation between the content of Sb in hair and soil was previously confirmed and Sb can be detected in hair even one year after exposure[57]. The median of Sb content in the hair of residents of a German mining area was 0.028–0.044 mg/kg [8]. In contrast, the mean concentration in a Chinese mining region was 5.15–15.9 mg/kg[22,58]. The Sb content in the samples collected in the current study depended on the region: a median of 0.958 mg/kg (RU) and 1.55 mg/kg (UR). It is hypothesised that this level could be affected by an increased content of Sb in the soil. Despite the increased content of tin in the water of UR region, this element was not detected in the studied hair samples.

3.4. NUTRITIONAL STATUS AND ENVIRONMENTAL EXPOSURE RELATIONS

Although Cole's index distribution indicated that the majority of studied Malagasy girls were characterised by normal body weight (46% vs 55%, UR vs RU sample), the mean values of body weight, body height and Cole's index were higher in the girls from the UR area (Table 1). Underweight was more common among girls living in RU than in UR settings (42% vs 28%), where food and nutrition support programmes were unavailable (Table 1). Malnutrition of Malagasy children, especially in the poorest parts of the country, such as the studied RU region, was also confirmed and discussed by other studies[59–63]. It was shown that nutritional deficiencies in Madagascar, but also generally, are widely conditioned by many factors, mainly by inadequate food intake and infectious diseases, socioeconomic factors, nutritional education and family influence [62,64,65]. Therefore, the authors decided to include relations between nutritional status (as Cole's index) and concentration of metals and metalloids in hair samples in the discussion concerning environmental exposure in the group of 8-15-year-old Malagasy girls. As demonstrated by other authors, some elements might negatively influence children's development. Lead exposure limits or delays the growth, development and concentration of preschool children[66–68]. There is also research presenting the influence of environmental exposure on metal concentrations in hair from areas adjacent to mines, landfills and contaminated regions where an increased concentration of metals in the hair was observed[69–72]. To the authors' best knowledge, there are no studies reporting data on these factors in Malagasy girls.

Although the research has reached its aims, it may have some limitations, primarily the retrospective setting of the study. It should be emphasised that the obtained results could be supported by air samples and an analysis of the diet composition, which are the missing elements of intoxication sources. Missing hair samples and unreliable anthropometrical data or date of birth were removed from the analyses. Moreover, even though the present study determined Cole's index (commonly used as a nutritional status marker), it was difficult to evaluate the dietary intake by a food consumption frequency questionnaire or dietary recording due to limited language communication skills. Therefore, the photos of main dishes delivered at school and the halfway house were collected and the data will be analysed by the Wellnavi[73] method and published in the next manuscript.

3.5. CORRELATIONS

The current paper analysed the relations between the Cole Indexes of the UR and RU group vs the accumulation of elements in the hair (Table 6). This analysis indicated the significant influence of combined effects of Cole's index and place of residence on the accumulation of Al ($p = 0.0103$) and Cr ($p = 0.028$) in the girls' hair (higher in UR area). Neither nutritional status level expressed by Cole index (Factor A) nor place of residence (Factor B) affected the As, Ni, Pb, Sb or Sn concentration in hair samples (Table 6). The current results indicate that the concentration of metals and metalloids in hair should be considered multifactorial. Although the relations between dietary intake and element concentrations in hair were previously studied in older populations[74], information on the nutritional status of children is scarce.

Table 6
ANOVA of the elements accumulation in girls' hair, nutritional status and place of residence.

element	Main effects						
	Factor A Cole's Index (A *vs.* B *vs.* C *vs.* D *vs.* E *vs.* F)			Factor B Place of residence (rural *vs.* urban)			Factor A *x* Factor B
	group	**concentration in the hair [mg/kg]** **Mean ± SD**	**p value**	**group**	**concentration in the hair [mg/kg]** **Mean ± SD**	**p value**	**p value**
Al	A (n = 36) B (n = 52) C (n = 12) D (n = 3)	1965.26 ± 1482.44 1769.86 ± 1754.73 611.62 ± 943.40 609.12 ± 830.11	0.145	Rural Urban	2382.65 ± 1663.64 913.37 ± 1108.26	0.053	**0.010**
As	A (n = 36) B (n = 52) C (n = 12) D (n = 3)	<LOD <LOD <LOD <LOD	0.785	Rural Urban	<LOD <LOD	0.924	0.413
Cd	A (n = 36) B (n = 52) C (n = 12) D (n = 3)	0.35 ± 0.28 0.21 ± 0.14 0.23 ± 0.11 0.41 ± 0.49	**0.023**	Rural Urban	0.24 ± 0.25 0.29 ± 0.19	0.503	0.779

A – underweight; B – recommended values; C – first degree obesity, D – second degree of obesity; n – group size; Data were analysed after their logarithmic transformation, but in the table arepresented as raw data.

element	Main effects						
	Factor A Cole's Index (A *vs.* B *vs.* C *vs.* D *vs.* E *vs.* F)			Factor B Place of residence (rural *vs.* urban)			Factor A *x* Factor B
	group	concentration in the hair [mg/kg] Mean ± SD	p value	group	concentration in the hair [mg/kg] Mean ± SD	p value	p value
Cr	A (n = 36) B (n = 52) C (n = 12) D (n = 3)	2.20 ± 1.66 2.16 ± 2.11 0.66 ± 0.74 0.37 ± 0.38	0.192	Rural Urban	3.05 ± 1.92 0.77 ± 0.77	**< 0.001**	**0.028**
Hg	A (n = 36) B (n = 52) C (n = 12) D (n = 3)	0.29 ± 0.28 0.27 ± 0.16 0.12 ± 0.09 0.16 ± 0.04	0.773	Rural Urban	0.35 ± 0.24 0.16 ± 0.10	**0.002**	0.846
Pb	A (n = 36) B (n = 52) C (n = 12) D (n = 3)	9.57 ± 8.55 6.87 ± 10.31 4.83 ± 4.43 7.67 ± 12.21	0.463	Rural Urban	8.24 ± 11.08 6.92 ± 6.79	0.841	0.784

A – underweight; B – recommended values; C – first degree obesity, D – second degree of obesity; n – group size; Data were analysed after their logarithmic transformation, but in the table arepresented as raw data.

element	Main effects						
	Factor A Cole's Index (A *vs.* B *vs.* C *vs.* D *vs.* E *vs.* F)			Factor B Place of residence (rural *vs.* urban)			Factor A *x* Factor B
	group	concentration in the hair [mg/kg] Mean ± SD	p value	group	concentration in the hair [mg/kg] Mean ± SD	p value	p value
Ni	A (n = 36) B (n = 52) C (n = 12) D (n = 3)	1.94 ± 1.40 1.66 ± 1.51 0.67 ± 0.72 1.94 ± 1.34	0.206	Rural Urban	1.85 ± 1.44 1.39 ± 1.39	0.604	0.083
Sb	A (n = 36) B (n = 52) C (n = 12) D (n = 3)	1.74 ± 1.25 1.28 ± 1.02 1.50 ± 0.76 0.74 ± 1.04	0.085	Rural Urban	1.31 ± 1.15 1.59 ± 1.04	0.088	0.669
Sn	A (n = 36) B (n = 52) C (n = 12) D (n = 3)	<LOD <LOD <LOD <LOD	0.627	Rural Urban	<LOD <LOD	0.888	0.824

A – underweight; B – recommended values; C – first degree obesity, D – second degree of obesity; n – group size; Data were analysed after their logarithmic transformation, but in the table arepresented as raw data.

Analysis of the relationship only between Cole' index and the accumulation of the elements in hair demonstrated that with decreased nutritional status, girls were more exposed to the accumulation of Al (R=-0.384, p = 0.024), Cd (R=-0.204, p = 0.042), Cr (R=-0.379, p = 0.022), Pb (R=-0.251, p = 0.046) and Ni (R=-0.300, p = 0.038). This can be explained by the decreased level of protein and desirable macro- and micronutrients in the diets of malnourished girls [75,76]. Previous studies suggest[76] that the accumulation of toxic elements depends on the age and sex of individuals.

This study presents studies of girls' hair, while the correlation with age was only controlled. It was confirmed that younger girls accumulate more Al (R=-0.502, p = 0.038), Cr (R=-0.494, p = 0.044), Ni (R=-0.348, p = 0.047) and slightly more Hg (R=-0.234, p = 0.048) and Pb (R=-0.231, p = 0.0423).

Nutritional status was negatively correlated with the age of the surveyed girls (R=-0.400, p = 0.026). A negative correlation was also observed between the age and total accumulation of studied elements (R=-0.423, p = 0.029) and the age of the girls and their nutritional status (R=-0.238, p = 0.036). The content of Al in the girl's hair was positively correlated with the content of Cr (R = 0.945, p = 0.023), Ni (R = 0.737, p = 0.031), Pb (R = 0.473, p = 0.043) and Hg (R = 0.379, p = 0.08) and slightly with Sb (R = 0.270, p = 0.049). The content of Cd was correlated with the content of Pb (R = 0.416, p = 0.031) and Ni (R = 0.345, p = 0.029). The concentration of Cr was the most strongly positively correlated with Al but also with Ni (R = 0.661, p = 0.019), Hg (R = 0.435, p = 0.034) and Pb (0.384, p = 0.032). The content of Hg (besides Al and Pb) was slightly correlated with Ni (R = 0.216, p = 0.048), while the concentration of Pb (besides Al, Cd and Cr) was also correlated with Ni (R = 0.426, p = 0.037) and slightly with Sb (R = 0.229, p = 0.048). Therefore, no competition was observed between the absorption of toxic elements in the studied individuals, although such competitiveness is proven in the absorption of elements desired and toxic for human health (e.g. Cd, Pb, Fe, Zn, Cu)[77–79].

4. Conclusions

An adequate dietary intake is one of the conditions for the proper functioning of the body, while the absorption of toxic elements can develop many health problems. Extremely high contents of Al were detected in the hair of the studied girls, which suggests an increased accumulation of this metal in girls' bodies and requires further studies. Very high contents of Cd and Cr were also detected in the hair. This suggests the possibility of adverse effects of the accumulation of these elements, especially in malnourished children. The contents of other toxic elements in hair were found to be within the ranges reported in the literature. It was also confirmed that younger girls were more exposed, especially to Al, Cr, Ni and slightly more to Hg and Pb. However, malnutrition of children resulted in greater accumulations of Al, Cd, Cr, Pb, and Ni in their bodies. The place of residence and malnutrition significantly differentiated the accumulation of Al and Cr in the girls' hair. The presented research suggests that in the case of environmental pollution, malnutrition of children may be a factor which significantly increases the accumulation of harmful elements in their bodies. Accumulation of toxic elements results from the element absorption from the environment, although such absorption was found to be significantly higher for children with lower nutritional status.

In conclusion, the current results highlighted the role of multidisciplinary studies, including environmental research, in connection with the health and nutritional status of society, especially children. It is believed that the presented data provide useful information on the current health risk for Malagasy girls and support the need to develop feeding programmes and environmental protection projects in Madagascar.

Declarations

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COMPETING INTERESTS

The author(s) declare no competing interests.

AUTHORS CONTRIBUTIONS

MZD: conceptualization, formal analysis, resources, writing - original draft, visualization; MCzM: conceptualization, methodology, formal analysis, resources, writing - review & editing, visualization; ZW: sample analysis, formal analysis; JP: sample analysis; PN: conceptualization, methodology, resources, validation

AVAILABILITY OF SUPPORTING DATA

The datasets used and or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

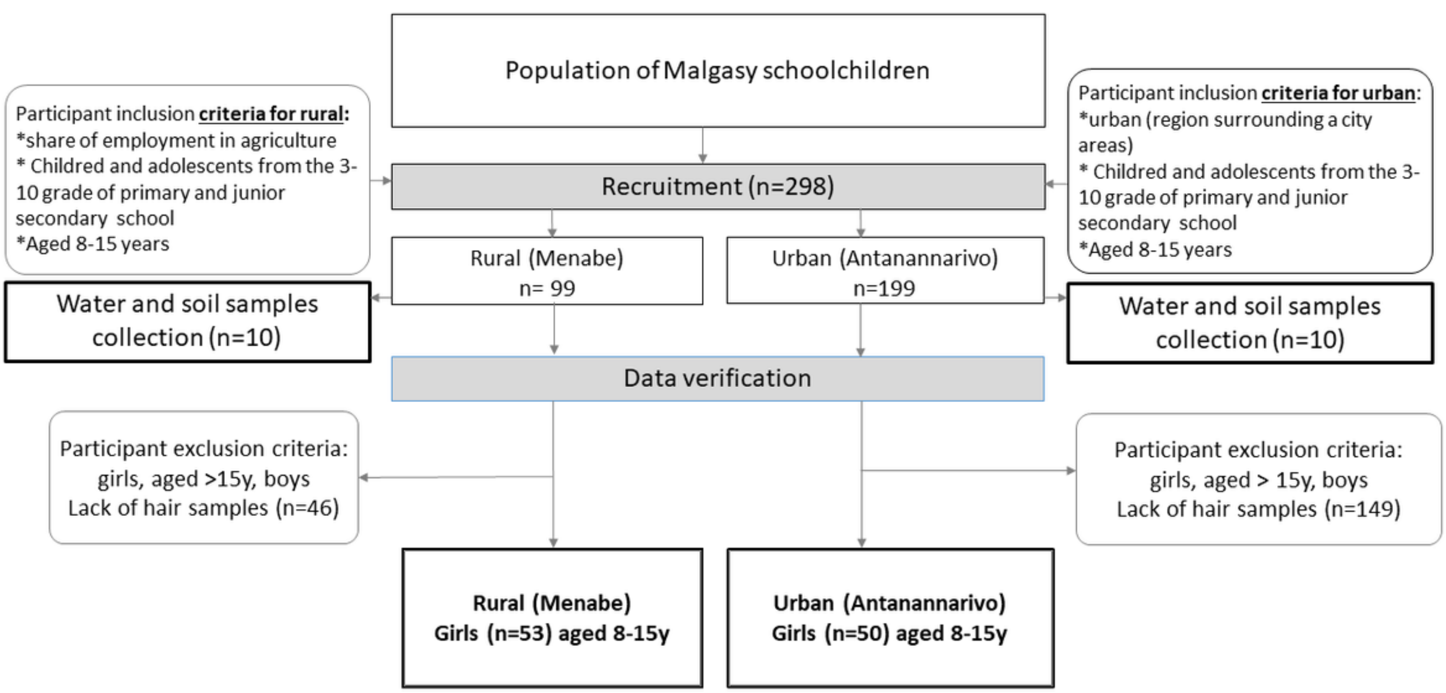

Figure 1

Study design and sample collection

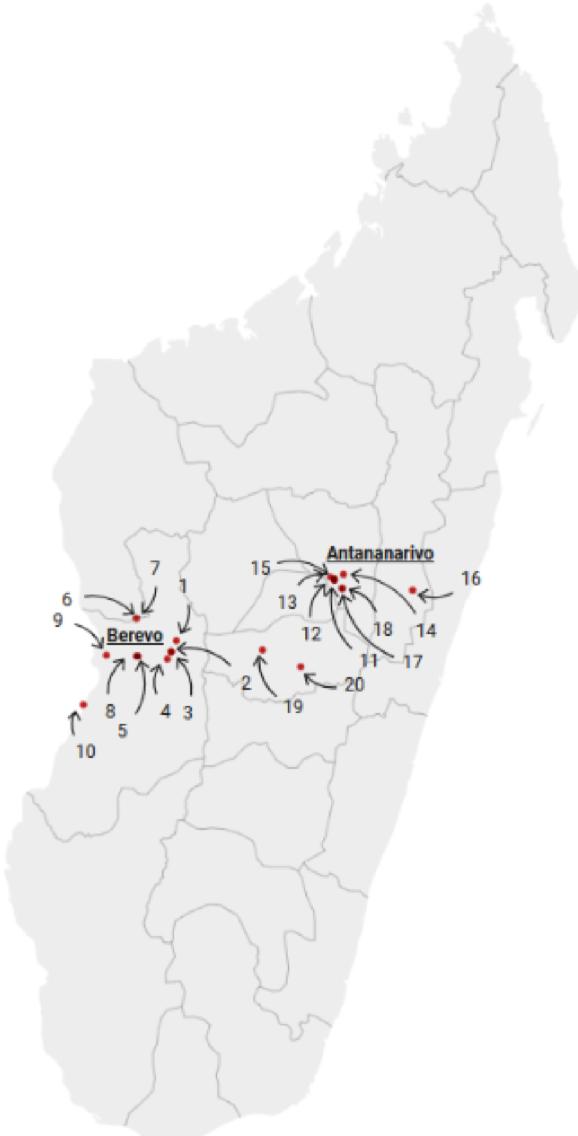

Figure 2

Areas in Madagascar where samples of water and soil were collected Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.