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Cardiovascular dysfunction and oxidative stress following human contamination by fluoride along with environmental xenobiotics (Cd & Pb) in the phosphate treatment area of Togo, West Africa

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ABSTRACT

In Togo, the phosphate ore mill discharges waste containing xenobiotics like cadmium, lead and fluoride. If the role of heavy metals in the appearance of pathologies is known, the role of fluoride remains to be studied alongside xenobiotics. This study tested the hypothesis that the toxicity of fluoride contributes, along with heavy metals, to physiological dysfunction. In this process, we have studied the variation in the parameters of cardiovascular functioning, depending on the level of human contamination by fluoride and xenobiotics. The concentration of Cd and Pb in blood samples were determined by AAS and fluoride by titanium-chloride method. Lipid peroxidation, the total antioxidant potential of collected blood samples and the parameters of cardiovascular dysfunction were also measured. Cd, Pb and F contents and lipid peroxidation were found to be significantly elevated in polluted areas than control zone as well as total cholesterol, LDL and triglyceride. HDL and antioxidant potential of blood decreased in the polluted areas. Correlation tests showed that fluoride levels are related to variations in the bio-indicators of high blood pressure and oxidative stress (R varied from 0.354 to 0.907). Togo phosphate treatment leads to human contamination with fluoride, along with Cd and Pb, increasing the risk of cardiovascular dysfunction and oxidative stress.

1. Introduction

Pollution caused by environmental xenobiotics is a major source of contamination of the human body [1–7]. It can also be the cause of fluoride poisoning in endemic areas of fluorosis, resulting in dental and bone fluorosis [7]. Among the anthropogenic activities, industrial activity is decisive in environmental pollution [1,6] and the food chain is the main pathway for the introduction of pollutants into the human organism [3,7]. In Togo, the Société Nouvelle des Phosphates du Togo (SNPT) plant rejects industrial effluents and solid waste containing trace elements such as cadmium (Cd), lead (Pb) and other elements such as fluoride (F) in the sea and on soils at Kpeme, Goumoukope, Agbodrafo and Aneho in the préfecture des Lacs. This pollution causes the bioaccumulation of xenobiotics by marine floral and faunal species. Through the soil, dust and combustion gases, agricultural products are also contaminated with Cd, Pb and F [2,7]. Table 1 summarizes data from studies of Cd, Pb and F content ranges in the soil, phosphate ores, marine sediments, seawater, drinking water, seafood and agricultural products in the studied area. These data express the degree of population exposure through the food chain [9–13]. This same population is exposed through transcutaneous and respiratory contamination by dust and gases that pollute the atmosphere and contain about 49 ppm of Cd and 1500 ppm of F. It is also worth to note that about 5100 tons of sludge is poured daily into the sea. That sludge, when it dries, contains 28 ppm of Cd and 13,500 ppm of F according to the studies. The same studies evaluated 2.3 mg/l of Cd in liquid sludge [10].

The daily feeding of contaminated food in this industrial zone of Togo, is the cause of heavy metals and fluoride bioaccumulation in human [7]. Such bioaccumulation would then justify the frequency of certain pathologies in this zone. The role of heavy metals in

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Table 1
The concentration of Cd, Pb and F in soil, phosphate ores, marine sediments, seawater, drinking water, seafood and agricultural products in the study area (ppm).

Pollutants	Environmental and biological samples						
	Soil	Agricultural products	Phosphate sediments	Marine sediments	Seawater	Seafood	Drinking water
Cadmium Cd)	1.27–42.53 [8]	0.006–2.84 [8]	2.00–109.00 [9]	2.00–44.00 [9,10]	3.50–12.00 [10]	0.10–1.68 [10]	0.00–0.025 [11]
Lead (Pb)	2.58–16.05 [8]	0.04–1.37 [8]	NA*	22.0–176.0 [9,10]	0.33–6.97 [12]	5.99–8.49 [10]	0.06–0.26 [11]
Fluoride (F)	0.05–1.54 [13]	0.16–2.06 [13]	1500.00 [10]	NA*	0.85–3.77 [10]	0.18–4.56 [10]	0.15–0.63 [13]

NA*: Not Available.

cardiovascular dysfunction with oxidative stress is relatively indicated by previous studies [1,3,6]. However, the phosphate treatment area in Togo, polluted by trace metallic elements, is also an area of endemic fluorosis with some cases (4.3%) of detected bone fluorosis [7]. The occurrence of bone fluorosis in this area indicates the degree of fluoride poisoning. Thus, the severity of dental fluorosis follows the cardiovascular pathologies which result from the variation of blood lipid contents. Indeed, blood lipids, in particular LDL-cholesterol, HDL-cholesterol and triglycerides have an impact on cardiovascular function according to their levels. The increase in the LDL-cholesterol and triglyceride content with a decrease in that of HDL-cholesterol indicate cardiovascular system's dysfunction. Hyperlipidemia, as the increase of LDL cholesterol, leads to atherosclerosis which can lead to myocardial infarction, obstruction of an artery, even a stroke [14]. Among the causes of cardiovascular diseases, pollutants such as toxic metals and fluoride occupy an important place, their implication in the damage of bio-molecules such as lipids, with the production of reactive oxygen species (ROS) which are a cause of the oxidative stress, have been studied [1,3]. Fluoride, in particular, inhibits the functioning of the thyroid gland and leads to hypo-thyroidism [15] which can lead to an increase in blood cholesterol with cardiovascular dys-function. However, apart from the alteration of the hard parts of the body such as teeth and bones through fluorosis, very few studies have linked the fluoride toxicity to physiological dysfunction. These studies have shown that the toxicity of fluoride impacts the nervous system by causing a neurological disorder with cardiac convulsions and arrest [16,17]. These convulsions can increase the heart rate and therefore increase the blood pressure with an impact on the antioxidant system. The present study tested the hypothesis that fluoride intoxication may be associated with a physiological dysfunction of the cardiovascular system in Togo phosphate treatment area polluted by fluoride and heavy metals such as Cd and Pb. The objective of this study is to determine the content of cadmium, lead and fluoride in human blood as well as certain biochemical parameters related to cardiovascular pathologies. The main aim of the study is to contribute the resolution of health problems, to check the link between industrial pollution and the frequency of these diseases in the industrial zone of SNPT in Togo. This study also makes is not directly impacted by the industrial activity of the SNPT's plant is selected as a control zone (Figs. 1 and 2).

1.1. Methodology

Blood sampling was carried out between April and September 2012 from 350 persons, 70 people per locality. The age of these persons (men and women) was between 16 and 52 years. Blood samples were taken from individuals who had been fasting for 12 h (nighttime fasting) in lead-free 5 ml tubes, cadmium and fluorine (including stoppers) on anticoagulant (EDTA) or not. For each person, 10 ml of venous blood was collected and divided into two tubes, one without anticoagulant for assay of biochemical parameters; the other with anticoagulant (EDTA) was used for metals (Cd and Pb) and F after mineralization. The sampling criteria for inclusion and exclusion is mainly concern lifetime in the localities, sources of heavy metals and fluorine contamination other than industrial pollution, physiological state and dietary and living habits. Thus, consumers of tobacco (active smoking), alcoholics (chronic alcoholism), newcomers (shelf life time in the locality < 4 years), irregular persons in the localities, people with infections whose health status did not allow to take blood pressure, pregnant and lactating women, HIV positive people were excluded. This exclusion has the advantage of minimizing any socio-nutritional and physiological situations that may interfere with the data and thus, 77 to 100 people per locality participated in the study. However, only the data of 70 individuals per locality, 350 people in total, were definitely analyzed in the study. This adjustment was based on the minimum amount of data available in one of the localities.

In addition, our study area consists of a relatively homogeneous coastal population, living in the same subequatorial climate with the same eating habits and subjects are of the same race in the five localities [7]. Most professional activities are trade, fishing and agriculture with almost identical incomes. People working in the public service were not considered because of their reduced stability compared to the minimum duration set for inclusion in the study. Similarly, employees of the phosphate processing plant were excluded so as not to consider professional exposure.

The blood pressure of each individual was determined twice on the right and left arms in a sitting position using a HEINE® tensiometer (Germany). The evaluation of the anthropometric parameters concerned the weight, the size and the age of the respondents.

1.2. Determination of the contents of Cd, Pb and F in the blood

The levels of Cd and Pb in the blood samples were determined with

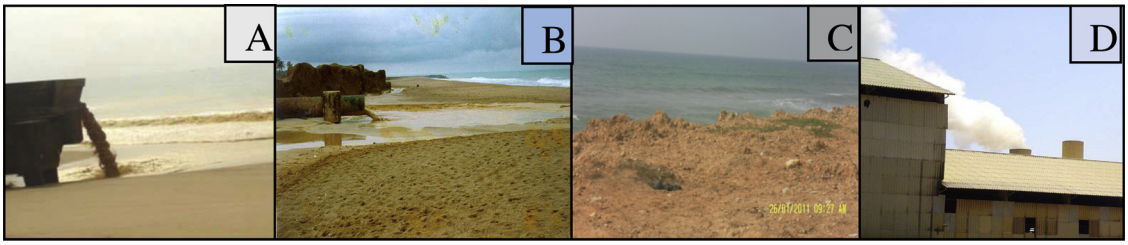

Fig. 1. Reject of phosphate sludge in the sea at Kpeme (A), at Goumoukope (B); of phosphate wastes in the sea at Agbodrafo (C) and factory chimney of the industry of Kpeme spreading gas (and/or dust) of phosphates in the surroundings (D).

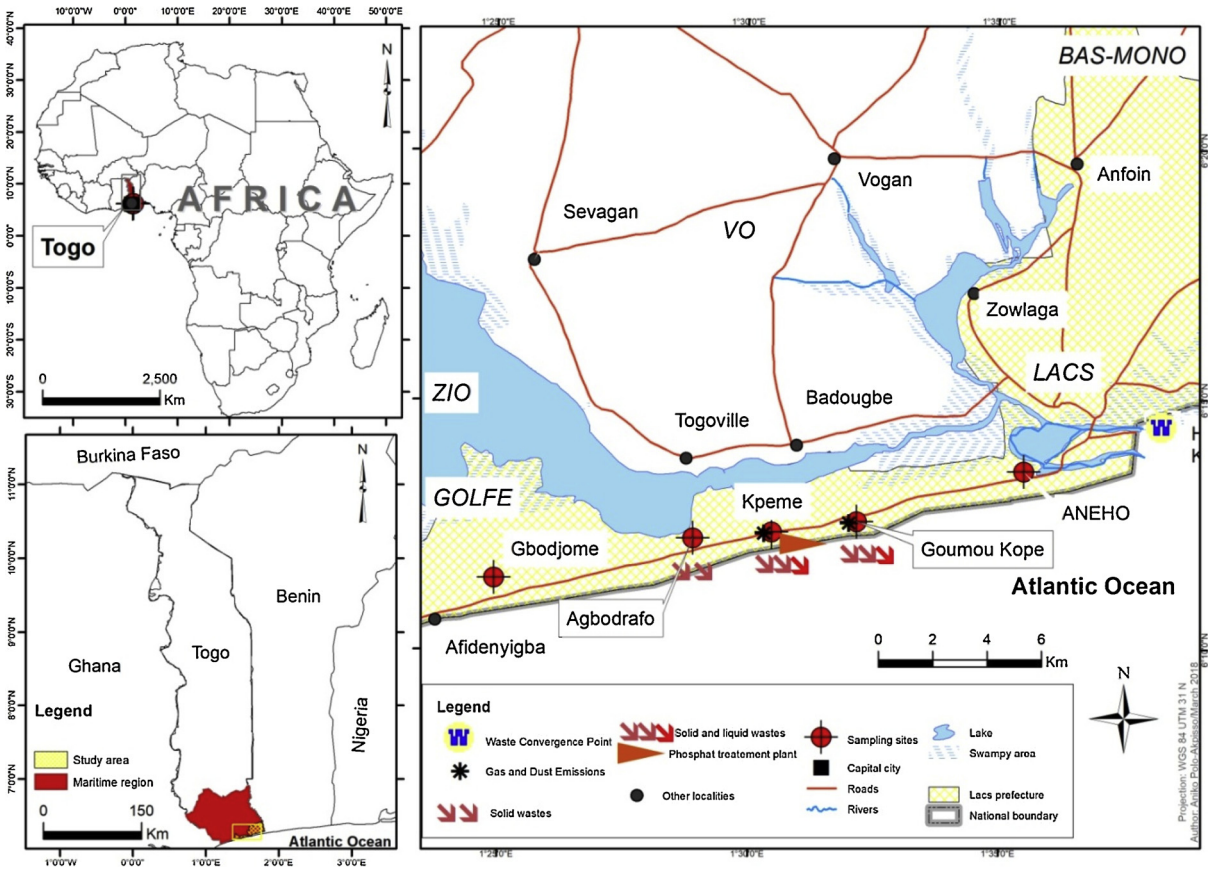

Fig. 2. Map of study area with the prospected localities.

the aid of Bulk Scientific Atomic Absorption Spectrophotometer, AES, 2000 series. An acetylene-air mixture was used as the source of flame. The working standard for each of the metals has aspired to the flame in the order of 0.0 ppm, 0.8 ppm and 1.6 ppm. The values were used to plot a standard curve [1]. The fluoride was dosed by the method of Epars and Chim [18] using titanium chloride based on the decolorization of the red TiO_2^{++} compound in the presence of the fluoride ion by the formation of colorless $(Ti(F)_6)H^+$.

1.3. Determination of biochemical parameters, total protein and bio-indicators of oxidative stress in the blood

The samples reserved for this purpose were centrifuged at 3000 rpm for 15 min at room temperature. The plasma is then used for the different dosages [19]. The biochemical parameters (triglyceride, LDL, HDL and total cholesterol) were measured using Biolab kit reagents [19,20]. The total protein content of the samples was estimated using the method of Lowry et al. [21] using bovine serum albumin (BSA) as the standard protein. Lipid peroxidation, expressed by malondialdehyde (MDA), was determined by measuring the thiobarbituric acid reacting substances (TBARS) as described previously [22,23] and then, malondialdehyde (MDA) was quantified by using the following formula $\Sigma = 1.56 \times 105\ M^{-1}\,cm^{-1}$ described by Farombi et al [22].

The antioxidant power of blood plasma - Ferric Reducing Antioxidant Power (FRAP) - was determined by measuring the total antioxidant potential of the plasma [24].

Our research protocol has been certified by the Bioethics Committee for Health Research (BCRS) of the Ministry of Health of Togo under N° 87/2012/MS/CAB/DGS/DPLET/CBRS.

1.4. Statistical analysis

All results were expressed in terms of mean ± ESM and analyzed using the GraphPad Prism software version 7.0. The correlation analysis was performed with Pearson correlation coefficiency with 95% confident interval. One-way ANOVA was used to compare the anthropometric parameters of the people from the five localities while *t*-test was used to compare each experimental sample with the control one. The threshold of significance was set at 5% ($P < 0.05$). All the statistics were carried out in SAS (The SAS System for windows, v8; SAS Institute Inc., Cary, NC).

2. Results

2.1. Variation of the anthropometric parameters of the respondents

The average age, weight and height of individuals who participated in the study in each locality and their BMI were not significantly different ($P > 0.05$). In addition, the proportions of women and men who participated in the study are also not significantly different. The data from this study are therefore not influenced by anthropometry (Table 2).

2.2. Heavy metals (Cd and Pb) and Fluoride (F) levels in blood

The result shows that the averages of Cd and Pb concentrations in blood samples were significantly high at Agbodrafo, Kpeme, Goumoukope and Aneho ($P < 0.001$) compared to Gbodjome. Values decline as one moves away from Kpeme and Goumoukope which represent the areas closest to the factory (Table 3). The average of Cd and Pb contents exceeds the standard limits set by WHO (< 5 µg/L) at Agbodrafo, Kpeme and Goumoukope. However, the average of Cd

Table 2
Anthropometric parameters of people whose blood has been taken.

Variables	Aneho	Goumoukope	Kpeme	Agbodrafo	Gbodjome
Men (%)	52 (74.29)	49 (70.00)	47 (67.14)	48 (68.57)	51 (72.86)
Women (%)	18 (25.71)	21 (30.00)	23 (32.86)	22 (31.43)	19 (27.14)
Mean Age	35.24 ± 1.32	33.97 ± 0.81	34.66 ± 0.84	35.87 ± 1.02	34.66 ± 0.84
Mean Weight (Kg)	68.71 ± 2.71	67.35 ± 2.59	66.10 ± 1.70	68.40 ± 3.15	67.53 ± 2.85
Mean Height (m)	1.69 ± 0.04	1.69 ± 0.19	1.68 ± 0.08	1.69 ± 0.27	1.69 ± 1.01
BMI	24.67 ± 0.97	23.79 ± 1.09	23.44 ± 0.78	24.01 ± 0.67	23.97 ± 1.03

The results are expressed as mean of 70 samples ± ESM.

contents is lower than the limit value (< 5 µg/L) at Gbodjome and Aneho whereas for Pb, it is only at Gbodjome that an average of the contents below the limit value (< 100 µg/L) has been obtained [25].

Blood fluoride levels increased significantly ($P < 0.05$) at Agbodrafo and Aneho relative to Gbodjome but, this increase is very significant ($P < 0.001$) at Kpeme and Goumoukope compared to Gbodjome (Table 3). These fluoride contents exceed the WHO standard limits (< 100–200 µg/L) in all surveyed localities [15].

2.3. Variation of biochemical parameters

Significant increases in LDL, Total Cholesterol (CT), Triglyceride and LDL/HDL were observed with decreases in HDL levels ($P < 0.01$ and $P < 0.05$) in polluted areas compared to control (Table 4). All values are broadly in line with the standards recommended by WHO as follows LDL - < 1.6 g/L; CT - 0.9–2 g/L; HDL - > 0.45 g/L; LDL/HDL - < 3.0 [26,27]. However, at Kpeme, the average of LDL/HDL ratios is above the limit value. In addition, at Kpeme and Goumoukope, the average of HDL contents is below the recommended limit value (HDL - > 0.45 g/L).

The correlation test shows that there is a relationship between the Cd and Pb concentrations in the blood and that of the bio-indicators of hypertension. The coefficient of correlation R ranged, considering all biochemical parameters, between 0.216 to 0.675 for Cd and 0.137 to 0.786 for Pb. However, this correlation is important with the fluoride contents and, in the details, the coefficient of correlation R ranged between 0.354 to 0.589 for TC, 0.437 to 0.824 for LDL, 0.529 to 0.727 for HDL and 0.475 to 0.822 for Triglycerides (Fig. 3).

2.4. Variation of arterial pressure of people

The mean of systolic blood pressure in the left arm at Gbodjome is 134.09 ± 3.68 mmHg and therefore less than 140 mmHg, from which point hypertension can be defined for a subject [19]. At Agbodrafo and Aneho, the values are significantly higher than those obtained at Gbodjome ($P < 0.01$); but at Kpeme and Goumoukope the values are more significantly higher than those of Gbodjome ($P < 0.001$) with values exceeding 140 mmHg (Fig. 4).

Considering diastolic blood pressure, there was no significant difference between Gbodjome and Agbodrafo ($P > 0.05$). However, diastolic pressures at the two localities reach 90 mmHg, the limit from which subjects can be considered hypertensive [19]. Moreover, the values obtained at Kpeme, Goumoukope and Aneho are significantly higher than those of Gbodjome ($P < 0.05$, $P < 0.01$ and $P < 0.001$) (Fig. 4).

2.5. Variation of bio-indicators of oxidative stress in blood plasma

2.5.1. Malondialdehyde (MDA)

MDA levels were significantly increased in the blood of subjects at Agbodrafo, Kpeme, Goumoukope and Aneho ($P < 0.001$) compared to Gbodjome. The increase percent in lipid peroxidation is 24.05% at Agbodrafo; 63.46% at Kpeme; 77.09% at Goumoukope and 21.18% at Aneho (Fig. 5).

2.5.2. The total antioxidant potential of blood plasma

FRAP (Ferric Reducing Antioxidant Power) values significantly decreased in the blood of subjects at Agbodrafo, Kpeme, Goumoukope and Aneho ($P < 0.001$) in relative to Gbodjome, with a more significant decrease in Goumoukope. The percentage decrease in the total antioxidant potential of the blood plasma is 50.18% at Agbodrafo; 65.85% at Kpeme; 74.57% at Goumoukope and 35.39% at Aneho (Fig. 6).

The correlation test between the blood contents of Cd, Pb and fluoride and the variation of the bio-indicators of oxidative stress shows a significant ($P < 0.01$) coefficient of correlation. R varied, for MDA contents, from 0.304 to 0.812 with Cd; from 0.473 to 0.720 with Pb and from 0.599 to 0.907 with fluoride (Fig. 3). R varied, for FRAP (F_e^{2+} ions contents), from 0.327 to 0.693 with Cd; from 0.413 to 0.653 with Pb and from 0.635 to 0.860 with fluoride (Fig. 3).

3. Discussion

3.1. Heavy metals and fluorine contents in blood and cardiovascular toxicity

The arterial pressure was higher ($P < 0.05$, $P < 0.01$ and $P < 0.001$) at Kpeme and Goumoukope followed by Agbodrafo and Aneho ($P < 0.05$, $P < 0.01$) in comparison to values obtained at Gbodjome (Fig. 4). This observation and the increase in LDL, total cholesterol, triglyceride, LDL/HDL ratio and in the CT/HDL ratio at

Table 3
Heavy metals (Cd and Pb) and Fluoride contents in blood according to the localities (µg/L).

Pollutant	Gbodjome (control)	Agbodrafo		Kpeme		Goumoukope		Aneho	
	Average	Average	% D	Average	% D	Average	% D	Average	% D
Cd	1.01 ± 0.21	7.73 ± 1.23 ***	665.34	13.53 ± 1.07 ***	1239.6	11.68 ± 2.38 ***	1056.43	3.59 ± 1.08 ***	255.44
Pb	87.27 ± 12.57	165.66 ± 19.92 ***	89.82	384.09 ± 22.45 ***	340.11	327.88 ± 32.13 ***	275.7	167.31 ± 21.57 ***	91.71
F	101.36 ± 17.73	320.24 ± 39.11*	215.94	540.12 ± 51.07 ***	432.87	504.01 ± 47.98***	397.24	340.08 ± 31.87*	235.51

The results are expressed as mean of 70 samples; % D = % of difference.
Significantly different in relation to the control: * $P < 0.05$; *** $P < 0.001$.

Table 4
Variation of biochemical parameters and bio-indicators of arterial hypertension.

Plasma parameters		Localities				
		Gbodjome	Agbodrafo	Kpeme	Goumoukope	Aneho
CT (g/L)	Range	0.17-5.12	0.87-4.61	0.69-4.77	0.70-3.57	0.73-2.79
	Mean	1.65 ± 0.78	1.76 ± 0.78	1.84 ± 0.70	1.89 ± 0.61	1.67 ± 0.61
LDL (g/L)	Range	0.13-2.11	0.11-3.12	0.2-3.45	0.07-3.57	0.01-2.99
	Mean	0.789 ± 0.42	0.858 ± 0.44	0.912 ± 0.58*	1.122 ± 0.44**	0.873 ± 0.57
HDL (g/L)	Range	0.12-1.6	0.01-1.57	0.03-2.09	0.10-2.68	0.05-2.91
	Mean	0.566 ± 0.57	0.494 ± 0.52	0.397 ± 0.29	0.443 ± 0.48	0.487 ± 0.40
Coef : LDL/HDL	Range	0.16-4.21	0.21-3.91	0.18-4.67	0.27-4.94	0.28-3.81
	Mean	2.003 ± 0.51	2.810 ± 0.34	3.173 ± 0.28*	2.990 ± 0.30	2.850 ± 0.38
CT/HDL	Range	0.23-3.56	0.17-5.32	0.12-6.01	0.23-5.86	0.10-5.04
	Mean	3.021 ± 0.27	3.551 ± 0.30	4.304 ± 0.42*	4.141 ± 0.33*	3.207 ± 0.26
Triglyceride (g/L)	Range	0.21-2.98	0.28-4.27	0.20-4.47	0.31-3.56	0.32-2.87
	Mean	1.152 ± 0.12	1.352 ± 0.17	1.407 ± 0.10	1.385 ± 0.14	1.302 ± 0.10

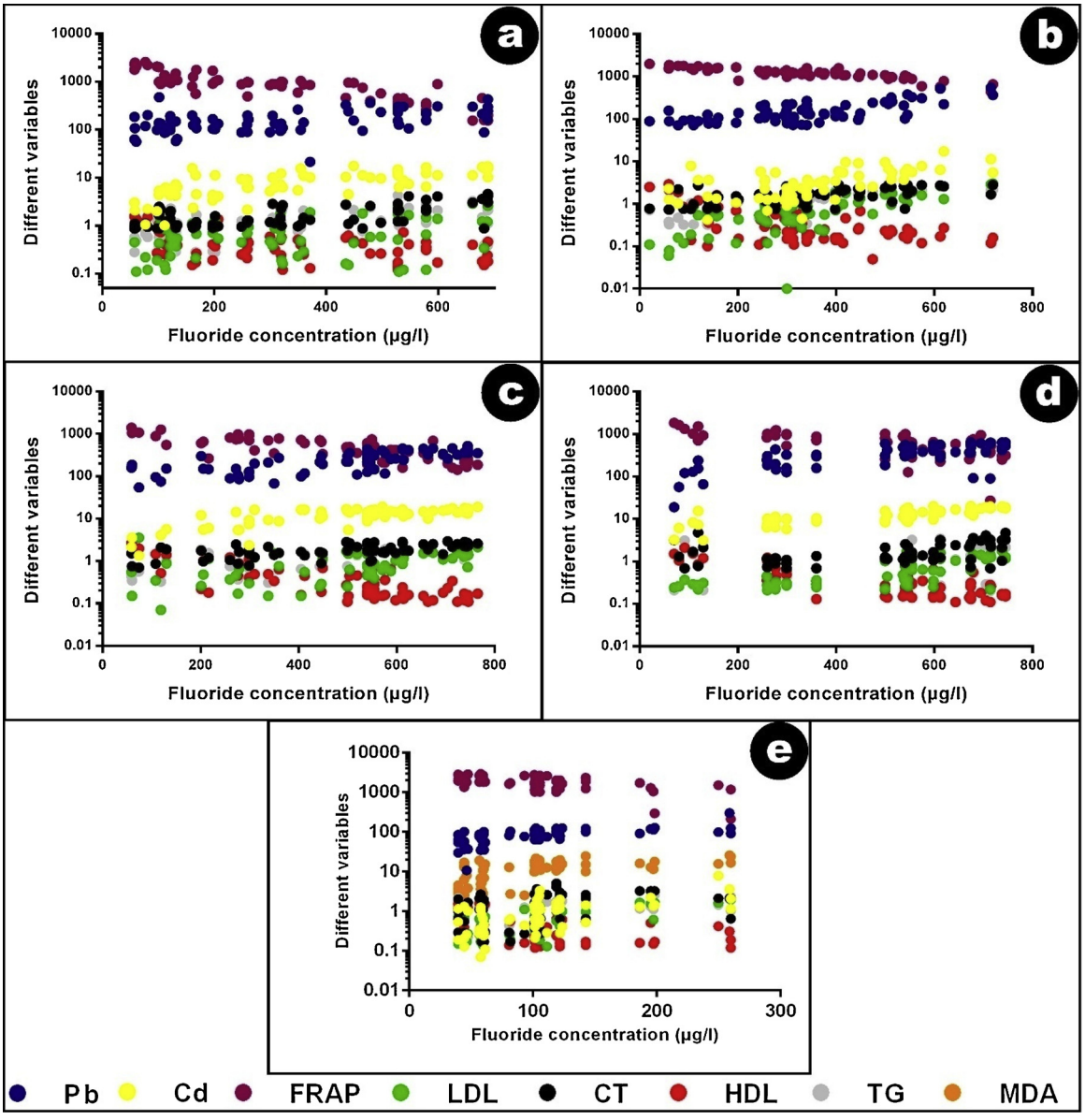

Fig. 3. Correlation between different variables (Cd, Pb,LDL, HDL, CT, TG, FRAP, MDA) and fluoride concentration at **A:**Gbodjome, **B:**Agbodrafo, **C:**Kpeme, **D:**Goumoukope and **E:**Aneho. The correlation is established by considering the five localities and in relation to the values of 70 samples corresponding to the number of individuals recruited per locality. Correlation is significant at $P < 0.05$and $P < 0.01$ (2-tailed).

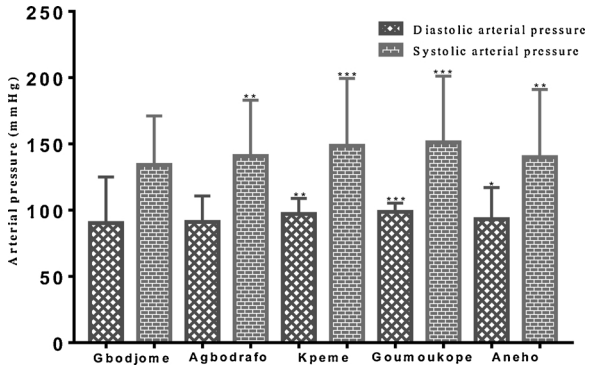

Fig. 4. Histogram of diastolic and systolic blood pressures of left arms of respondents by location Values are expressed as mean ± SD of 70 subjects. Significantly different from control (Gbodjome): * P < 0.05; ** P < 0.01 and *** P < 0.001.

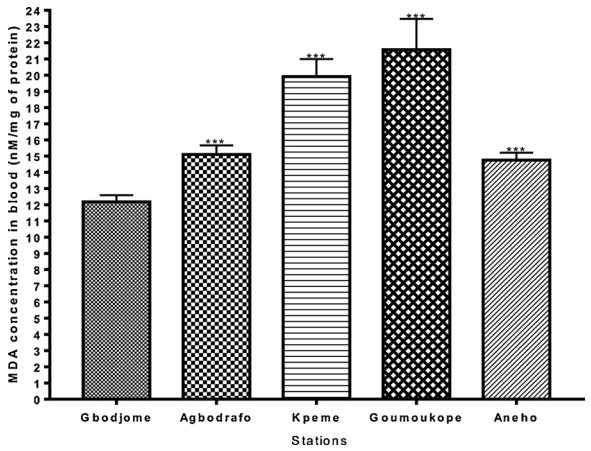

Fig. 5. Concentration of malondialdehyde (MDA) in human blood according to the localities. Values are expressed as mean of 70 samples ± ESM. Significantly different from control: *** $P < 0.001$.

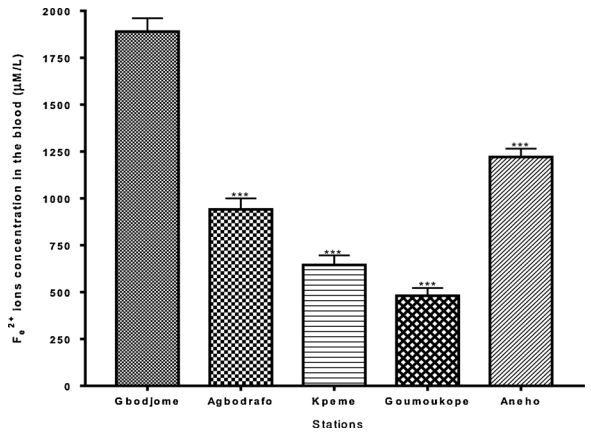

Fig. 6. Concentration of F_e^{2+} ions (FRAP value) in human blood according to the localities. Values are expressed as mean of 70 samples ± ESM. Significantly different from control: *** $P < 0.001$.

Kpeme and Goumoukope, as well as the decrease in HDL in these localities (Table 4) are predictive factors for the risk of cardiovascular diseases in the population. The present results were justifying the higher arterial pressure at Kpeme and Goumoukope followed by Agbodrafo and Aneho (Fig. 4). It does agree with the variation in Cd and Pb concentrations (Table 3) [24,25]. Our results on this aspect have been consistent with those of other authors [19,28] who have shown the same variations in the risk of cardiovascular disease. The ratio of low-density lipoprotein cholesterol and high-density lipoprotein cholesterol (LDL-C/HDL-C) is a reliable predictor of cardiovascular risk. Low HDL-C levels with the coronary artery disease are associated with a high risk for cardiovascular events. Reduction of HDL-C is generally considered pro-atherogenic [28,29]. This may explain why the presence of Cd and Pb in subjects at Agbodrafo, Kpeme, Goumoukope and Aneho may have led to an increased risk of heart disease by increasing LDL-C/HDL-C ratio and CT/HDL-C ratio (Table 4). The present study shows that the risk of cardiovascular disease increases with blood levels in Cd and Pb (Tables 3 and 4). The prevalence of this pathology can therefore be linked to the contamination of organisms by industrial pollutants [30,31]. These results and the correlation's tests are consistent with those of other studies [3,32] which have indicated that, toxic metals such as cadmium and lead can induce cardiovascular disease. Thus, this study confirms well on the toxicology of heavy metals such as Cd and Pb when the levels in the human blood are above the norms as observed through our data [25,33–37].

However, in the area of the Togo phosphate plant, the frequency of cardiovascular pathologies is proportional to the endemicity of fluorosis [7]. Indeed, this endemicity has been revealed by previous studies with dental fluorosis (12.71–37.22%) and bone fluorosis (4.30%) [7]. The hypothesis that a probable relation between the toxicity of fluorides and these pathologies was therefore to be verified. Considering the increasing toxicity of fluoride at high levels, the correlation tests between the presence of fluorides in the human blood and the variation in the predictive parameters of cardiovascular disease have shown possible fluoride toxicity on the cardiovascular system (Fig. 3). There are very few studies concerning fluoride toxicity on cardiovascular system. Indeed, the toxicity of fluorides has been proven on the kidneys functioning [15–17,38–44] and on the endocrine system especially on the decline in the thyroid gland activity [15,17] and the insulin secretion inhibition [42]. Similarly, fluoride has been incriminated at very high levels in the occurrence of cancers [17,43] and paralysis [43]. In addition, the neurotoxicity of fluoride has also been demonstrated [16,39] with migraines, depressions and extreme fatigue [17], in muscle toxicity by convulsions followed by coma and cardiac arrest [45]. Beyond that, studies have shown the role of fluorides in increasing the severity of diabetes [17]. Thus, it is clear that fluorides have neurotoxic effects, can lead to heart convulsions and depressions and aggravate diabetes mellitus. This data confirms a strong link between various pathologies already proven and the cardiovascular diseases. In endemic fluorosis, the toxicity of fluoride can therefore affect the cardiovascular system as demonstrated by the correlation tests performed (Fig. 3). Indeed, the correlation test between bio-indicators parameters of arterial hypertension and fluoride levels in the blood showed a significant ($P < 0.05$ and $P < 0.01$) coefficients of correlation R varied from 0.3545 to 0.5903 for total cholesterol, 0.4352 to 0.8305 for LDL-C, 0.5298 to 0.7267 for HDL-C and 0.4747 to 0.8275 for triglycerides. This data of correlation test, which are important than those obtained with Cd and Pb, indicates some implication of fluoride toxicity in the cardiovascular diseases in endemic fluorosis zone. The results we report in this study do not agree with those of Sezgin et al. [5] who reported that fluorides have no impact on the cardiovascular system in their study. However, it can be justified by the fact that these authors worked on children aged between 7 and 13 years exposed to fluoride through drinking water. Thus, exposure and duration of impacts are low compared to our study, which concerns people who have been exposed for a long time with severe intoxication expressed through cases of bone fluorosis in the study area [7]. In addition, our study has the merit of being more representative by the large sample size compared to that of Sezgin et al. [5].

Other studies have shown possible consequences for amelogenesis in a fetus [46] with a relationship between fluoride levels and age, sex, ethnicity, race, and source of intoxication [46,47]. These parameters cannot be applied in our case, since there is no significant difference

between the different groups ($P > 0.05$) (Table 2). These authors also reported in their studies low levels of fluoride compared to our values. However, these results can be justified by the source of intoxication not important compared to our study.

Moreover, the variation in biochemical parameters confirms the role of the SNPT's industrial activities in the occurrence of the various pathologies. The role of fluorides in the appearance of migraines, depressions and convulsions reveals, among other things, an induction of oxidative stress at high levels as in the case of heavy metals [32–35,37].

3.2. Heavy metals and fluorine contents in blood and oxidative stress

The rate of MDA increased significantly in the blood of subjects at Agbodrafo, Kpeme, Goumoukope and Aneho ($P < 0.001$) compared to the values in Gbodjome (Fig. 5). This variation is due to the oxidative stress induced by the presence of xenobiotics in the organism [1,12,48,49]. The higher lipid peroxidation at Kpeme and Goumoukope are justified by the higher contents of Cd, Pb and F in these localities. Indeed, these pollutants generate reactive oxygen species capable of damaging macromolecules such as DNA, proteins and lipids [50,51]. The increase in the MDA levels in the blood observed at Agbodrafo, Kpeme, Goumoukope and Aneho compared with those in Gbodjome shows a struggle of the body against oxidative stress caused by xenobiotics [1,52,53]. The values of the Ferric Reducing Antioxidant Power (FRAP) decreased significantly in the blood of subjects in Agbodrafo, Kpeme, Goumoukope and Aneho ($P < 0.001$) compared with those in Gbodjome, with a more significant decrease at Goumoukope (Fig. 6). These results confirm an alteration of the antioxidant system in the pollution zones with respect to Gbodjome [20,54]. Our data are consistent with previous studies [24] which observed a significant decrease in the total antioxidant potential of plasma with an increase in the level of MDA in the blood of Wistar rats stressed by CCl_4 intoxication.

The correlation tests between bio-indicators of oxidative stress and fluoride levels in the blood have also shown a significant ($P < 0.01$) coefficient of correlation R varied from 0.5987 to 0.9072 with the MDA contents (Fig. 3) and R varied from 0.6366 to 0.8599 with the F_e^{2+} ions contents (Fig. 3). This data of correlation test are also important than those obtained with Cd and Pb. It confirms previous data which stipulate that fluoride toxicity leads to convulsions, depressions and migraines [17,45]. In addition, the parameters of oxidative stress were more significant in the localities where the fluoride levels were higher. It can therefore be confirmed that fluoride can induce oxidative stress in an area of endemic fluorosis.

These results justify the pollutants levels that are higher at Kpeme and Goumoukope compared to other localities, thus confirming the role of the plant in the genesis of the different pathologies observed in the population in this zone. Food contamination with pollutants is therefore a factor that makes the man at the end of the food chain vulnerable in a polluted environment. It proved that fluoride can induce oxidative stress and cardiovascular dysfunction in an area of endemic fluorosis. The toxicity of fluoride, on cardiovascular and antioxidant systems, could be through convulsions leading to an increase in heart rate and therefore an increase in blood pressure and oxidative stress. This toxicity would result in dysfunction of the thyroid gland [15] which could lead to an increase in blood cholesterol levels. Heart convulsions are probably due to neurological disorders [16,17].

4. Conclusion

The high concentrations of Cd, Pb and F in the blood of people at Agbodrafo, Kpeme, Goumoukope and Aneho in relation to Gbodjome are due, on the one hand, to the consumption of contaminated food through waste discharged into the sea, on soil and in the atmosphere and on the other hand to direct contamination by the respiratory and transcutaneous routes. The bioaccumulation of pollutants by exposed individuals is more important at Kpeme and Goumoukope than Agbodrafo and Aneho. Such a variation shows that the contamination of human by pollutants is due to the industrial activities in the phosphate ore processing plant. The correlation tests and the variation of the fluoride concentrations show that fluoride toxicity impacts cardiovascular system and induce oxidative stress in the human. Industrial activity in the phosphate treatment area of Togo leads to human contamination by fluoride along with xenobiotics, leading to increases the risk of cardiovascular dysfunction and oxidative stress. It is then necessary to continue the study to understand the mode of action of fluoride toxicity on the cardiovascular system.

Authors' Contributions

Conceived and designed the experiments: MM KA SA. Performed the experiments: MM SA RR GL. Analyzed the data: MM AG GL KLA. Contributed reagents/materials/analysis tools: MM SA RR AG GL KLA KA. Wrote the paper: MM RR AG GL MK.

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Ethical consideration

The ethical consideration was completely observed by the authors.

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