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Assessment of renal and hepatic dysfunction by co-exposure to toxic metals (Cd, Pb) and fluoride in people living nearby an industrial zone

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ABSTRACT

Togo's phosphate processing plant at Kpeme discharges waste, containing Cd, Pb, and fluoride, into the sea and on the soil. Heavy metals toxicity on kidneys and the liver has been studied. However, fluoride toxicity on these organs remains to be investigated. The present study deals with the variation in renal and hepatic functioning parameters due to fluoride, Cd and Pb. Totally, 350 volunteers were recruited from five different localities around this phosphate processing plant for sample collection. Cd and Pb contents in blood samples were determined by spectrophotometry and fluoride by the titanium chloride method. Biochemical parameters were measured using Biolab kits. The pollutant contents were elevated in polluted areas where ASAT, ALAT, creatinine, and urea increased, and total protein decreased. Correlation and multivariate tests showed that fluoride is related to the various pathologies mentioned. PCA revealed that phosphate processing in Togo is a source of renal and hepatic toxicity.

1. Introduction

Human activities are the cause of environmental pollution [1]. Contamination of the human body by pollutants is the cause of several pathologies in polluted environments [2,3] as in the case of industrial areas [4,57,5]. The developing countries are not immune to this situation because of the ever-increasing industrial activities. These industrial activities which are sources of sustainable human development then become a factor of alteration of human health [6,7]. This is the case of the phosphate plant in Togo. Although, the extraction and processing of phosphate remains one of the main sources of revenue for the Togolese State, this industrial activity results in intense and diversified pollution [6,8,9]. In effect this plant discharges solid waste and effluents into the sea and on the soil, and dust and combustion gas into the atmosphere (Fig. 1). The pollutants these wastes include toxic heavy metals such as cadmium (Cd) and lead (Pb) as well as fluoride (F) [10,11,12,13]. These elements then contaminate seafood, agricultural products, and water. Table 1 summarizes the data from studies on Cd, Pb, and F contents ranged in the soil, phosphate ore, marine sediments, seawater, drinking water, seafood, and agricultural products in the study area. These data express the degree of population exposure through the food chain [14,8,15,16]. This same population is exposed through transcutaneous and respiratory contamination by dust and gas that pollute the atmosphere and contain about 49 ppm of Cd and 1500 ppm of F. It is also to be noted that about 5100 tons of sludge are released into the sea every day. The dry sludge contains 28 ppm of Cd and 13500 ppm of F according to earlier studies [14]. The same studies evaluated 2.3 mg/L of Cd in the liquid sludge [14]. Thus, following the anthropogenic contamination by the pollutant, several pathologies are observable in the area surrounding this plant. Among these pathologies, we not only note the renal and hepatic dysfunction, but also dental and bone fluorosis especially [6]. The frequency and intensity of dental and bone fluorosis suggest that

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this is an area of endemic fluorosis. In addition, the prevalence of renal and hepatic dysfunction according to the localities follows that of fluorosis [6]. However, apart from the alteration of the hard parts of the body such as teeth and bones through fluorosis, very few studies have tied the fluoride toxicity to the physiological dysfunction. Yet, some authors have reported probable toxicities of fluoride in the kidneys [17–19], nervous system [20,21], and implications for the severity of diabetes mellitus [22,23]. Fluorides have also been somewhat incriminated in hormone and endocrine dysfunction [17,22]. The toxicity of fluoride is also cited in cardiovascular diseases [24,25] and the occurrence of cancers [26]. Nevertheless, only a few studies have tied the fluoride toxicity and hepatic pathologies. Fluoride is a trace element which is naturally present in the soil, water, and food, and is also known as an important environmental toxicant obtained from industrial sources [27]. It is normally used in low concentrations to reduce the incidence of caries and proper bone mineralization. However, chronic excessive fluoride exposure, results in higher fluoride accumulation in the blood and different tissues due to its metabolism and utilization in the body. This impairs human health and induces damages to the skeletal system and teeth [27] leading to simultaneous increase in osteocalcin and calcitonin levels in the plasma which may cause fluoride-dependent bone damage and dental fluorosis, characterized by hypo-mineralization of enamel and dentine. Because of the high fluoride affinity to calcium ions, calcification of tissues, vascular walls, and synovial capsules and ligaments appears, leading to persistent joint pain and limited joint movement [28,29]. Studies based on animal models have shown oxidative stress, DNA damage, and apoptosis in the liver as an effect of fluorosis [30–32]. However, the mechanism of fluoride-induced hepatic toxicity has not been definitely explained. This study tested the hypothesis that fluoride intoxication may be associated with a renal and hepatic dysfunction in an area polluted by fluoride and heavy metals in Togo.

The objective of this study is to determine the variations of certain biochemical parameters related to renal and hepatic pathologies with the contents of Cd, Pb, and F in human blood. This work has been carried out in the interest of contributing to the resolution of persistent health problems and to check the link between industrial pollution and the frequency of these diseases in the industrial zone of New Phosphate Mining Society of Togo (SNPT).This study also shows the role of the toxicity of fluoride, alongside toxic metals, in the renal and hepatic alteration.

2. Materials and methods

2.1. Location of study area

The study area is described by Melila et al. [25]. It is located in the south side of Lome-Aneho National Road, from the village of Gbodjome (west) to Aneho (east) via Agbodrafo, Kpeme, and Goumoukope, for approximately 20 km (Fig. 2). By its geographical location, this complex is part of the prefecture of the lakes, located at an altitude of 66 m in 06°22'0" N and 01°40'0" E in the maritime region of Togo. The geographical description of the five villages in this study is as follows: Kpeme (06°2'45.9" N and 01°30'16.1" E) is the site of the phosphate treatment plant. After this treatment, sludge and solid waste are released into the sea and on the soil while dust and gas are released into the atmosphere (Fig. 1); Goumoukope (06°12'51, 7" N and 01°31'57.4" E) is a village located about 2 km east of Kpeme. This site is also subject to release of phosphate sludge and solid waste resulting from the treatment of phosphates; Aneho (06°13'41.5" N and 01°36'09.9" E) is located about 9–10 km east of Kpeme where the phosphate waste discharged at Kpeme and Goumoukope comes from the movements of seawater; Agbodrafo (06°12'39.8" N and 01°28'4,7" E) is a village located about 3–4 km west of Kpeme where the solid wastes resulting from the treatment are also rejected. In addition to solid wastes, sludge discharged at Kpeme and Goumoukope is eventually drained to this site due to relative movements of seawater; Gbodjome (06°11'39.6" N and 01°25'03.8" E) is a village located to the west at 10–11 km from Kpeme and 13 km from Goumoukope (upstream) and is not directly affected by the rejection of the waste from the plant. This locality is considered as control zone.

2.2. Methodology

Blood sampling was carried out between April and September 2012 in 350 people, 70 people per locality, (Table 2) under the supervision of a doctor with the help of nurses and laboratory technicians. The age of these persons was between 16 and 52 years. Blood samples were collected from individuals who had been fasting for 12 hours (night-time fasting) in lead-free 5-ml tubes. 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 [25].

The criteria for inclusion and exclusion of people for sampling were mainly concerned by their 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 < 4 years), irregular persons in the localities, pregnant and lactating women, HIV positive, and hepatitis people were excluded. The evaluation of the anthropometric parameters concerned the weight and the height of the respondents.

2.3. Sociological and health data on respondents

Sociological and health data on the respondents were obtained using survey forms. The survey sheets were pre-tested and readjusted before the actual survey.

2.4. Frequency of food consumption

The frequency of food consumption was determined from food consumption surveys using survey cards that were pre-tested and readjusted before the actual survey.

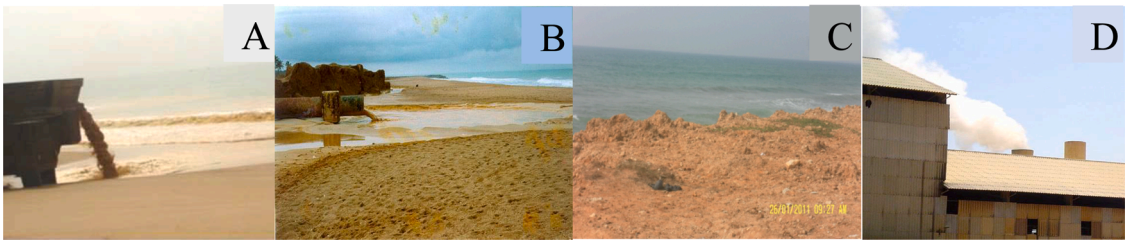

Fig. 1. Reject of phosphate sludge in the sea at Kpeme (A), at Goumoukope (B); of phosphate wastes in the sea at Agbodrafo (C) and factory chimney of the industry of Kpeme spreading gas (and/or dust) of phosphates in the surroundings (D).

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

The content of Cd and Pb in the blood samples was determined with the aid of Bulk Scientific Atomic Absorption Spectrophotometer, AAS, 2000 series. An acetylene–air mixture was used as the flame. The values were obtained by extrapolation from the standard curve [4,34].

Generally, ICPMS is recommended for measuring the metallic trace elements in the blood due to the low concentrations. However, the previous studies in the concerned area have shown that the exposure is high and diverse [14,6,8,15,16]. Given this level of exposure, we were able to use the atomic absorption spectrophotometer to measure Cd and Pb in the blood of individuals. The accuracy of the results obtained was checked each time by standards whose concentration was well known, and the blank made it possible to check whether the samples were contaminated during mineralization or not.

The fluoride was dosed by the method using titanium chloride based on the decolorization of the red $Ti0_2{}^{++}$compound in the presence of the fluoride ion by the formation of colourless $(Ti(F)_6)H^+$ [35,36].

2.6. Determination of biochemical parameters

The samples reserved for this purpose were centrifuged at 3000 rpm for 15 min at room temperature. The plasma was then used for the different dosages. The biochemical parameters (ASAT, ALAT, creatinine, urea, and total protein) were measured using Biolab kit reagents. Our research protocol has been certified by the Bioethics Committee for Health Research (CBRS) from the Ministry of Health of Togo under No. 87/2012/MS/CAB/DGS/DPLET/CBRS.

2.7. Statistical Analysis

All results were expressed in terms of mean ± ESM and analyzed using the GraphPad Prism software version 7.0. Correlation analysis was performed with Pearson correlation and 95% confidence interval. ANOVA was used to compare each experimental sample with the control one. If significant differences were found ($P < 0.05$), the polluted areas were compared with the control using Student's t-test. All the statistics were carried out in SAS (The SAS System for Windows, v8; SAS Institute Inc., Cary, NC). Principal component analysis (PCA), a multivariate statistical method, was used to reduce the dimensionality of the data and predict the influence of fluorine on various renal and hepatic biomarkers with the help of PAST 3.0 package (Windows version).

3. Results

3.1. Variation in the anthropometric parameters of the respondents

The various anthropometric parameters, such as age, weight, height, and body mass index (BMI), of the respondents across the five different localities have been tabulated in Table 2. The minimum and the maximum age of the total respondents was 16 and 52 years, respectively, with a P value of 0.74. The lowest and highest weight was 45 and 84 kg, respectively, with a P value of 0.55. The height of all the respondents fell in the range of 1.52–1.76 m, with a P value of 0.27. The BMI of the respondents was observed in the range of 16.22–32.03, with a P value of 0.91. There was no significant difference ($P > 0.05$) between the anthropometric parameters of the respondents from the localities. The data from this study are, therefore, very little influenced by anthropometry.

3.2. Heavy metals (Cd and Pb) and fluoride contents in blood

The highest mean concentration of Cd in blood was recorded in Kpeme (13.53 ± 1.07 μg/L), and the least mean concentration was in Gbodjome (1.01 ± 0.21 μg/L). The maximum Pb value was in Kpeme (384.09 ± 22.45 μg/L), and the minimum in Gbodjome (87.27 ± 12.57 μg/L). Similarly, the value of F was also highest in Kpeme (540.12 ± 51.07 μg/L) and the lowest in Gbodjome (101.36 ± 17.73 μg/L). The results show that the contents of Cd and Pb in blood are 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). Blood fluoride contents increased at Agbodrafo and Aneho relative to Gbodjome but not significantly ($P > 0.05$); this increase is significant ($P < 0.05$) at Kpeme and Goumoukope compared to Gbodjome (Table 3).

3.3. Variation in biochemical parameters of renal toxicity

The variation in the biochemical bio-indicator parameters for renal toxicity like creatinine, urea, and total protein are shown in Table 4. The lowest mean creatine value (8.480 ± 0.35 mg/L) was from Gbodjome, whereas the highest value (9.982 ± 0.31 mg/L) was reported in Kpeme. Following a similar trend, the mean value of urea was also lowest in Gbodjome (17.03 ± 0.51 mg/dL) and the highest in Kpeme (27.18 ± 0.86 mg/dL). However, in the case of total protein, the lowest mean value was from Kpeme (60.50 ± 1.79 g/L) and the highest in Gbodjome (68.53 ± 2.78 g/L). Significant increases in creatinine ($P < 0.05$) and urea ($P < 0.05$, $P < 0.01$, and $P < 0.001$) were observed with decrease in total proteins contents ($P < 0.05$) in polluted areas compared with the control (Table 4). The correlation test shows that there is a relationship between fluoride concentration in the blood and the bio-indicators of renal dysfunction ($P < 0.0001$). The coefficient of correlation R varied from 0.6102 to 0.8475 for creatinine, from 0.6523 to 0.7835 for urea, and from 0.5402 to 0.7966 for total protein (Figs. 3 to 5, and Tables S1–S5).

3.4. Variation in biochemical parameters of hepatic dysfunction

The variations in the biochemical bio-indicator parameters for hepatic toxicity like aspartate aminotransferase (ASAT) and alanine aminotransferaese (ALAT) are shown in Table 5. The mean values of ASAT across the different localities are as follows: Gbodjome, 39.54 UI/L; Agbodrafo, 42.71 UI/L; Kpeme, 54.39 UI/L; Goumoukope, 53.04 UI/L; and Aneho, 43.29UI/L. The average values of ALAT across the different localities are as follows: Gbodjome, 39.71 UI/L; Agbodrafo, 50.25 UI/L;

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
Cd	1.27–42.53 [55]	0.006–2.84 [55]	2.00–109.00 [10]	2.00–44.00 [10,14]	3.50–12.00 [14]	0.10–1.68 [14]	00.00–0.025 [16]
Pb	2.58–16.05 [55]	0.04–1.37 [55]	NA	22.0–176.0 [10,14]	0.33–6.97 [54]	5.99–8.49 [14]	0.06–0.26 [16]
F	0.05–1.54 [33]	0.16–2.06 [33]	1500.00 [14]	NA	0.85–3.77 [14]	0.18–4.56 [14]	0.15–0.63 [33]

NA : Not Available ; **Cd :** cadmium ; **Pb :** lead ; **F :** fluoride.

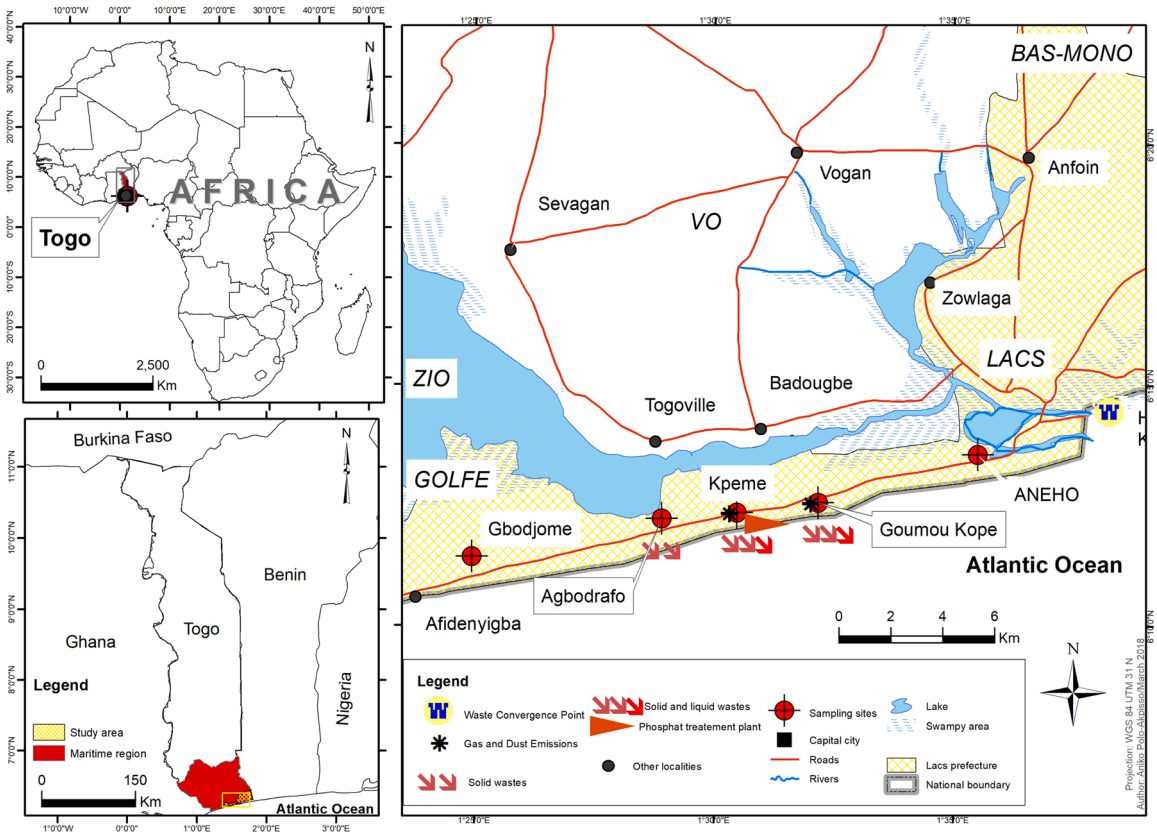

Fig. 2. Map of study area with the prospected localities.

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

Localities & respondents		Anthropometric parameters			
		Age (year)	Weight (kg)	Height (m)	BMI
Gbodjome	Range	16–52	47–78	1.52–1.74	18.36–32.03
Men = 51 (72.86%)	Median	34.50	69.00	1.69	23.62
Women = 19 (27.14%)	Average	34.66 ± 0.97	67.39 ± 0.75	1.69 ± 0.01	23.59 ± 0.26
Agbodrafo	Range	18–51	45–84	1.59–1.75	16.22–29.05
Men = 48 (68.57%)	Median	36.50	68.00	1.70	23.81
Women = 22 (31.43%)	Average	35.87 ± 1.09	67.99 ± 0.91	1.69 ± 0.004	23.79 ± 0.30
Kpeme	Range	16–49	54–82	1.52–1.74	18.83–32.03
Men = 47 (67.14%)	Median	34.50	67.00	1.69	23.40
Women = 23 (32.86%)	Average	34.66 ± 1.004	66.13 ± 0.68	1.68 ± 0.005	23.46 ± 0.24
Goumoukope	Range	19–51	55–84	1.57–1.76	18.83–28.39
Men = 49 (70.00%)	Median	34.00	67.5	1.69	23.57
Women = 21 (30.00%)	Average	33.97 ± 0.96	67.34 ± 0.77	1.68 ± 0.004	23.62 ± 0.24
Aneho	Range	19–51	54–84	1.57–1.74	19.94–28.39
Men = 52 (74.29%)	Median	34.50	68.00	1.70	23.29
Women = 18 (25.71%)	Average	35.24 ± 1.02	67.09 ± 0.77	1.69 ± 0.005	23.52 ± 0.25
P value		*P* = 0.74	*P* = 0.55	*P* = 0.27	*P* = 0.91

The results are expressed as mean of 70 samples ± ESM. The analysis of the variables showed insignificant differences between the localities surveyed (*P* values > 0.05).

Kpeme, 58.75 UI/L; Goumoukope, 56.58 UI/L; and Aneho, 52.33 UI/L. Significant increases in ASAT ($P < 0.001$) and ALAT ($P < 0.05$ and $P < 0.01$) were observed in polluted areas as compared with control (Table 5). There is a relationship between the fluoride concentrations in blood the bio-indicators of hepatic toxicity. The coefficient of correlation R varied from 0.5240 to 0.7907 for ASAT and from 0.6528 to 0.7938 for ALAT (Figs. 6 and 7, and Tables S1–S6).

3.5. Multivariate analysis

In all the five study areas, including the control site, first principal component clearly explains the strong positive correlation of fluorine with urea and creatinine followed by either ALAT or ASAT (Table 5). Among the total variables observed, the above four increased in samples taken from participants in localities close to the phosphate processing plant. Likely, the total protein level was decreased with the evidence of negative correlation in all the polluted and control sites. Decreased protein concentration is indicative of reduction in kidney function due to the loss of capable reabsorption of protein. This shows the strong influence of fluorine concentration towards an increasing trend of renal biomarkers. Furthermore, in terms of fluorine concentration on hepatic biomarkers, a strong association was found in ALAT and ASAT in Gbodjome (control site), Goumoukope, and Aneho. Even though Gbodjomeis is far away from the polluted sites, there was evident renal and hepatic toxicity. Based on the PCA analysis, it was confirmed that fluorine influences either renal or hepatic or both toxicities.

3.6. Frequency of food consumption

The frequency of food consumption like the agricultural products from the locality, seafood, and the usage of well water are provided in Table 6. The *P* values for the consumption of agricultural products, consumption of seafood, and the usage of well water across the localities

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

Localities		Pollutants Cd	Pb	F
Gbodjome (control)	Range	0.00 (ND)–7.87	10.72–305.03	39.57–359.8
	Average	1.01 ± 0.21	87.27 ± 12.57	101.36 ± 17.73
Agbodrafo	Range	0.00 (ND)–17.21	21.33–476.74	57.72–688.70
	Average	7.73 ± 1.23***	165.66 ± 19.92***	320.24 ± 39.11*
	% of difference	665.34	89.82	215.94
Kpeme	Range	0.00 (ND)–20.09	18.97–637.23	69.79–865.50
	Average	13.53 ± 1.07***	384.09 ± 22.45***	540.12 ± 51.07***
	% of difference	1239.60	340.11	432.87
Goumoukope	Range	0.00 (ND)–19.13	54.67–621.29	58.79–861.37
	Average	11.68 ± 2.38***	327.88 ± 32.13***	504.01 ± 47.98***
	% of difference	1056.43	275.70	397.24
Aneho	Range	0.00 (ND)–17.37	71.49–574.28	19.79–846.30
	Average	3.59 ± 1.08***	167.31 ± 21.57***	340.08 ± 31.87*
	% of difference	255.44	91.71	235.51
Limit standard		< 5.00 [56]	< 100.00 [56]	100.00–200.00 [17,23]

The results are expressed as mean of 70 samples ± ESM.
Significantly different in relation to the control: * $P < 0.05$; *** $P < 0.001$. **ND:** not detected.

are $P = 0.4516$, $P > 0.999$, and $P > 0.999$, respectively. The results show that there is no significant difference ($P > 0.05$) between the frequencies of food consumption (agricultural or market garden products and seafood) between the localities surveyed. The same is true for the use of well water (Table 6). Additionally, all these food products are frequently consumed in these localities (Table 6).

3.7. Sociological and health data on respondents

The average lifespan of the participants in each of the localities surveyed is greater than 10 years with a non-significant difference between the localities (Table 7). The distribution of ethnic groups was generally similar with a predominance of ethnicities from Southern Togo. This is explained by the fact that the study area is in the south of Togo. However, there were more ethnic groups from the north at Kpeme compared with other localities due to the fact that it is a cosmopolitan locality with the presence of the phosphate processing plant. However, this difference in the distribution of ethnic groups was not significant (Table 7).

All the participants in this study have socio-professional and economic characteristics that are not significantly different from one locality to another, with a predominance of the level of secondary education (Table 7). In addition, the level of knowledge about pathologies linked to metallic trace elements and fluorine is low in all the localities surveyed because of the low level of education. The diseases declared or observed were mainly cardiovascular diseases and fluorosis with higher proportions at Kpeme and Goumoukope. However, renal and hepatic dysfunctions have also been reported (Table 7).

4. Discussion

4.1. Fluoride, Cd, and Pb contents

The fluoride, Cd, and Pb contents were generally high compared to the concentration of these elements in the environment and food products (Tables 1 and 3). This observation confirms a true bioaccumulation of these xenobiotics by humans, which remains the final receptacle of these pollutants. Given the intensity and diversity of the pollution induced by industrial activities in the study area, human exposure to pollutants can be through trophic, transcutaneous and respiratory routes. This justifies the higher levels of these toxic elements in human blood, especially in the areas close to the factory [25,38].

The variations in contents and those in parameters linked to renal and hepatic functioning are not linked to a difference in the eating habits of individuals in the localities surveyed. The level of contamination of individuals by fluoride and metals would then be linked to the degree of pollution of the living environment, concomitantly with the contamination of food and water used by these xenobiotics [39]. The intensity of the pollution induced by the phosphate treatment plant in Togo is felt greater at Kpeme and Goumoukope and decreases the further when one moves away from this plant. The impact of this pollution on man will be felt more in these two localities, as the results of this study indicate. Moreover, the results of the present study are also not linked to the socio-demographic characteristics of the participants (Tables 6 and 7).

4.2. Pollutants and renal toxicity

Data from the previous work [6] have shown that renal diseases are among the prevalent pathologies in the phosphate treatment area of Togo, especially at Goumoukope and Kpeme. This is also justified by the results of this study, especially in these two localities with an increase in creatinine levels and uremia and a decrease in total protein (Table 4) not

Table 4
Variation of biochemical bio-indicators parameters of renal toxicity.

Plasma parameters		Localities				
		Gbodjome	Agbodrafo	Kpeme	Goumoukope	Aneho
Creatinine (mg/L) Accepted value: 8–12 [37]	Range	2.31–14.25	5.08–12.73	4.09–15.45	2.37–15.98	5.12–14.57
	Median	9.08	9.10	10.56	10.58	8.88
	Average	8.480 ± 0.35	8.991 ± 0.23	9.982 ± 0.31*	9.787 ± 0.37*	8.912 ± 0.24
Urea (mg/dL) Accepted value: 10–40 [37]	Range	5.23–25.65	15.21–31.79	6.09–43.46	11.65–29.98	16.01–28.57
	Median	18.05	22.16	27.89	24.32	22.89
	Average	17.03 ± 0.51	22.83 ± 0.41*	27.18 ± 0.86***	23.50 ± 0.43**	22.86 ± 0.27*
Total protein (g/L) Accepted value: 60–76 [37]	Range	34.34–91.89	37.55–87.45	24.38–88.25	20.87–91.89	31.26–87.56
	Median	68.58	64.33	60.48	57.98	64.20
	Average	68.53 ± 2.78	62.52 ± 1.49*	60.50 ± 1.79*	60.70 ± 2.22*	65.50 ± 1.77

Values are expressed in terms of average ± ESM of 70 samples.
Significantly different from control: * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$.

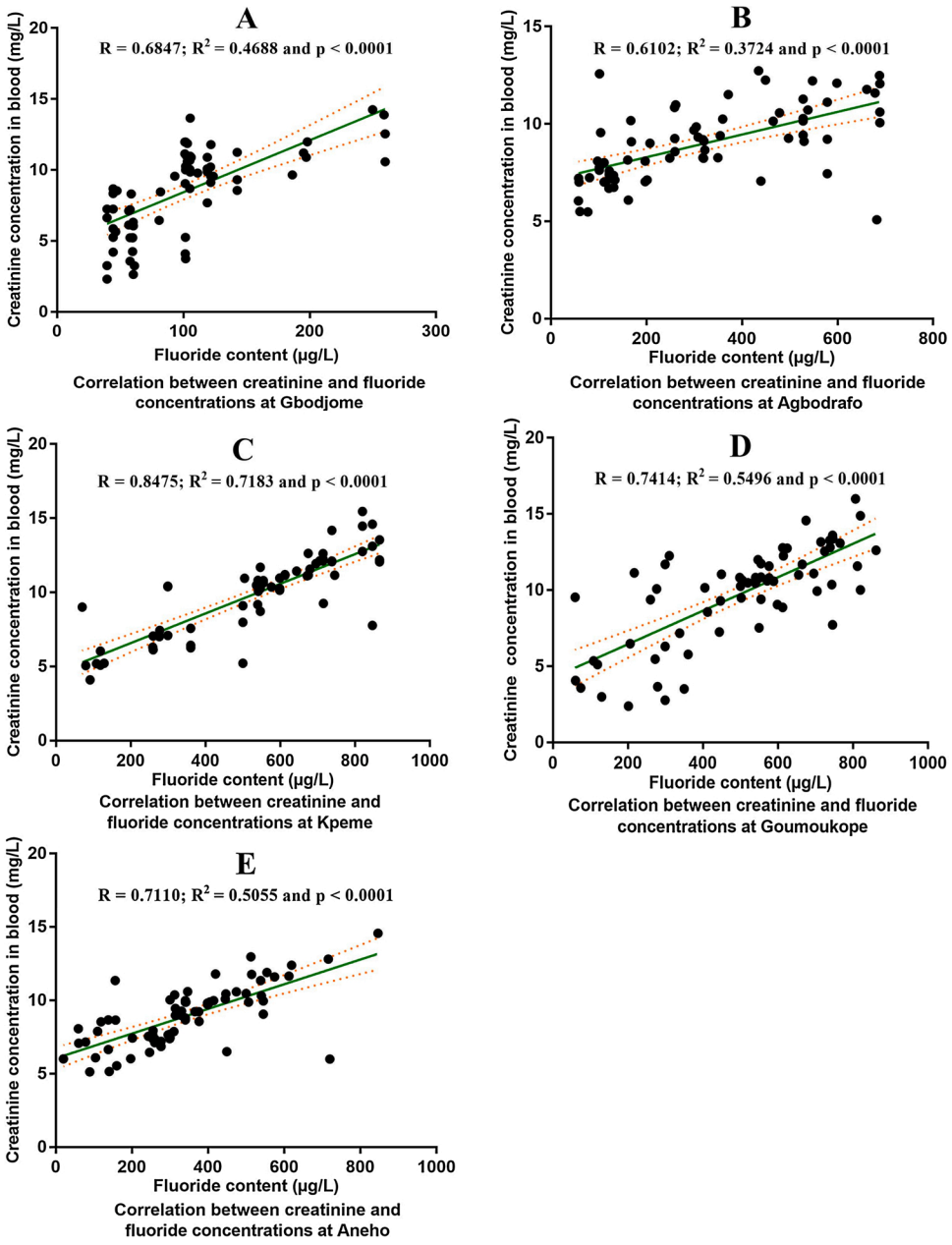

Fig. 3. Correlation between creatinine and fluoride concentrations in blood. The correlations are established by considering the five localities and in relation to the values of 70 samples corresponding to the number of individuals recruited per locality; A: at Gbodjome, B: at Agbodrafo, C: at Kpeme, D: at Goumoukope and E: at Aneho.

only in these polluted areas (Goumoukope and Kpeme) in relation to control, but also in Aneho and Agbodrafo ($P < 0.05$,$P < 0.01$, and $P < 0.001$). The kidneys are not only the main organs responsible for excretion of xenobiotics, but also intervene in their metabolism [4]. These organs will therefore be vulnerable to the toxic effects of pollutants [4,40,41]. The damage often caused to the kidneys can lead to an increase in the serum creatinine levels and uremia, as confirmed by our results, and also a decrease in other biochemical parameters such as total protein [42,43]. Indeed, the variation in these parameters as observed through our results at Agbodrafo and Aneho, but especially at Kpeme and Goumoukope, is an indicator of renal damage [42–44] which was consistent with the prevalence of renal disease in previous studies [6]. Moreover, the variation in cadmium, lead, and fluoride levels in the blood and biochemical parameters confirms the role of the SNPT's industrial activities in the occurrence of renal pathologies. The increased serum creatinine indicates a decrease in glomerular filtration rate and hence kidney failure. This is the inability of the kidney to eliminate the products of nitrogen metabolism. It also indicates the acid–base, hydro-electrolytic and hormonal unbalance [45].

This study confirms the toxicity of heavy metals on the kidneys, as the highest levels are observed in the areas surrounding the plant, where there are more reports of renal dysfunction [6]. Moreover, the concentration of the pollutants decreases with the renal pathologies as one move away from the plant. Beyond the role of the phosphate treatment plant in Togo in renal dysfunction, our results reveal a likely toxicity of fluorides on the kidneys. In fact, a significant ($P < 0.0001$) correlation was observed between the fluoride concentration in blood and renal alteration parameters values with a correlation coefficient (R) varied from 0.6102 to 0.8475 for creatinine, from 0.6523 to 0.7835 for urea, and from 0.5402 to 0.7966 for total proteins (Figs. 3–5, and Tables S1–S6). The multivariate analysis clearly indicates the high impact of fluoride in the variation of biochemical parameters with

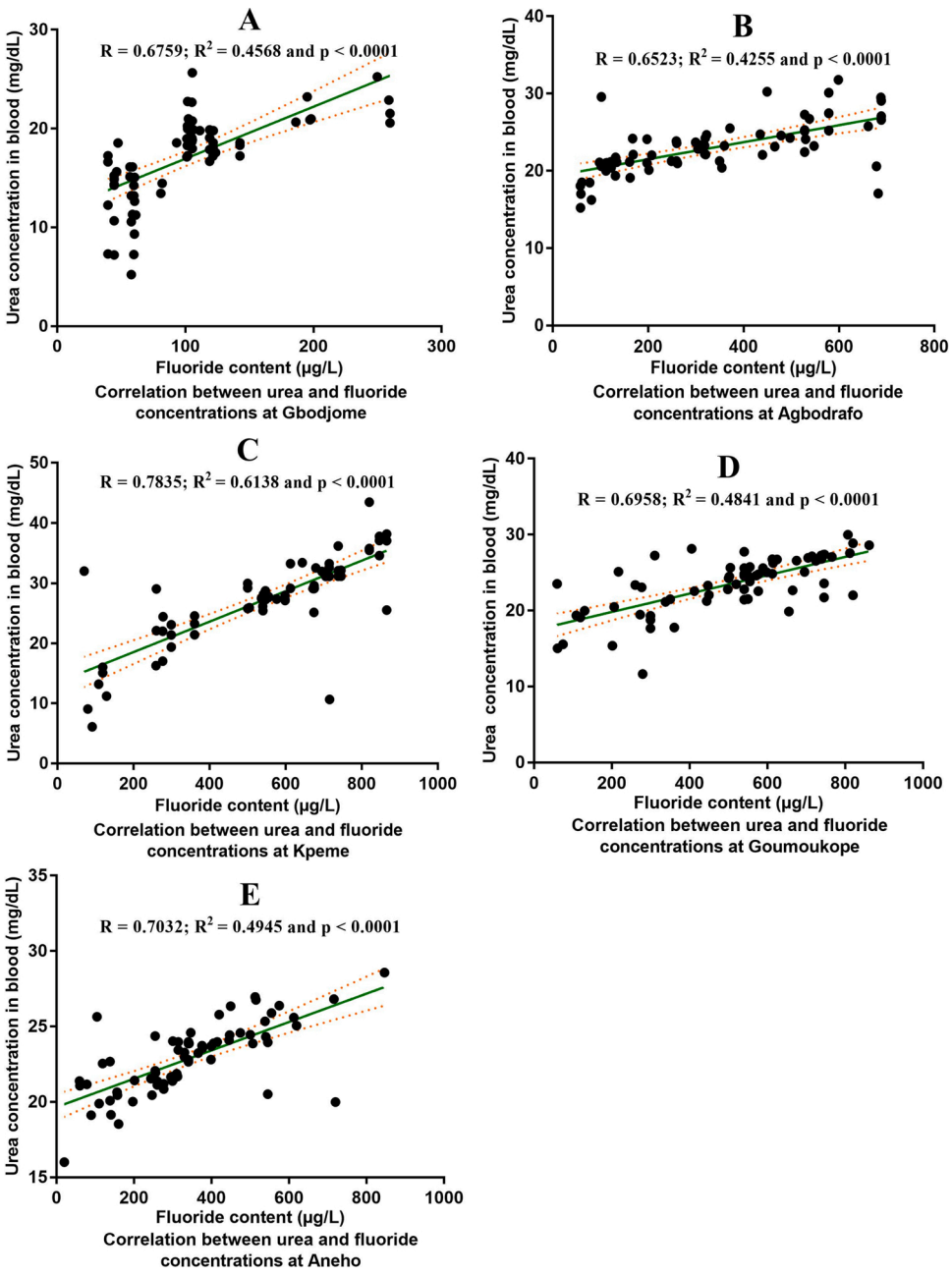

Fig. 4. Correlation between urea and fluoride concentrations in blood. The correlations are established by considering the five localities and in relation to the values of 70 samples corresponding to the number of individuals recruited per locality; A: at Gbodjome, B: at Agbodrafo, C: at Kpeme, D: at Goumoukope and E: at Aneho.

respect to Cd and Pb (Tables S1–S5). This indicates that in areas of endemic fluorosis, the impact of fluoride toxicity may exceed that of toxic metals. This confirms with the few studies that have indicated the toxicity of fluoride on the kidney in endemic fluorosis zone, with an increase in the intensity of diabetes mellitus expressed by a decrease in insulin secretion [44]. This toxicity could be explained by the fact that in the case of fluoride poisoning, the kidneys constitute the main excretory organ and can therefore be impacted by the toxicity of this element [41, 46,47]. It is then true that fluoride exhibits renal toxicity in an endemic zone of fluorosis, such as that of the SNPT's plant in Togo.

4.3. Pollutants and hepatic toxicity

Significant variations ($P < 0.05$, $P < 0.01$, and $P < 0.001$) in the ASAT and ALAT enzymes observed in our results (Table 4) indicate an alteration of liver functions [27], especially at Kpeme and Goumoukope. Indeed, the liver is the first site of biotransformation and detoxification of xenobiotics; and thus, this organ may be vulnerable when overwhelmed by high concentrations of cadmium and lead [48–51], and fluoride [27]. This shows that toxic metals and fluoride cause liver damage. The liver is the center of metabolism and has a high ability to concentrate xenobiotics because of the proteins that facilitate their agglutination [4,50]. Here, our data showed again a significant correlation ($P < 0.0001$) between the fluoride content and the hepatic dysfunction parameters with a correlation coefficient which varied from 0.5240 to 0.7907 for ASAT and from 0.6528 to 0.7938 for ALAT (Figs. 6 and 7, and Tables S1–S5). Multivariate analysis showed that Cd and Pb impact liver function. However, this analysis reveals the critical role of fluoride toxicity in relation to toxic metals (Tables S1–S5). It can therefore be concluded that fluoride toxicity is effective in organ dysfunction as shown in this study. This may be justified by the role of the liver in fluoride metabolism as in the case of xenobiotics [27,50].

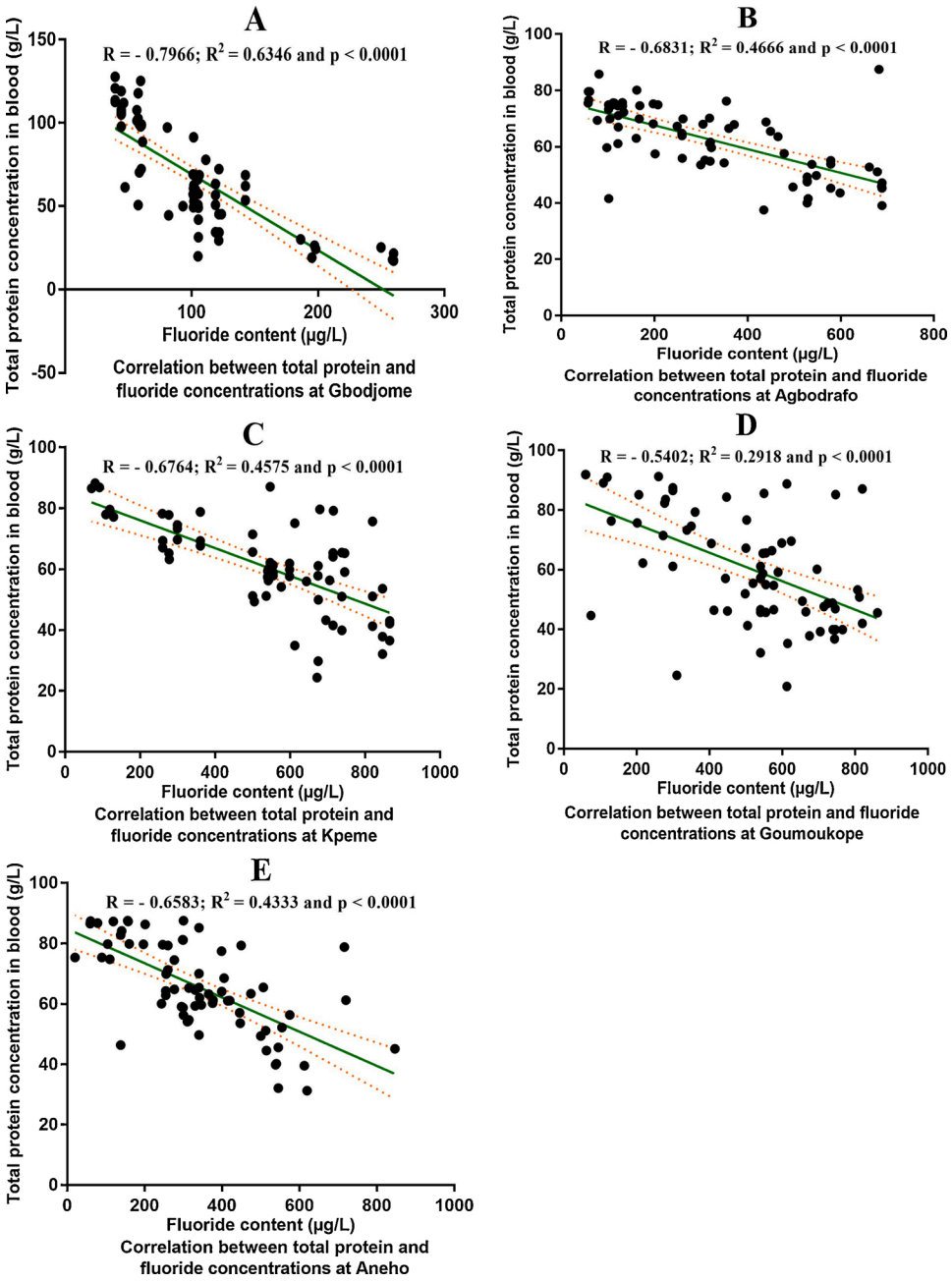

Fig. 5. Correlation between total protein and fluoride concentrations in blood. The correlations are established by considering the five localities and in relation to the values of 70 samples corresponding to the number of individuals recruited per locality; A: at Gbodjome, B: at Agbodrafo, C: at Kpeme, D: at Goumoukope and E: at Aneho.

Table 5
Variation of biochemical parameters, bio-indicators of hepatic toxicity.

Plasma parameters		Localities				
		Gbodjome	Agbodrafo	Kpeme	Goumoukope	Aneho
ASAT (UI/L)	Range	7.65–78.89	20.69–67.06	18.12–69.77	23.37–69.86	20.65–88.57
Accepted value:	Median	44.86	42.71	55.71	54.48	41.78
10–45 [37]	Average	39.54 ± 12.81	42.71 ± 1.34	54.39 ± 1.05***	53.04 ± 0.81***	43.29 ± 1.69
ALAT (UI/L) Accepted value:	Range	5.23–77.89	16.02–73.07	20.01–85.59	33.35–71.87	29.65–85.81
10–65 [37]	Median	42.95	51.10	55.87	55.61	51.33
	Average	39.71 ± 2.15	50.25 ± 1.52	58.75 ± 1.65**	56.58 ± 0.94*	52.33 ± 1.62

Values are expressed as mean ± ESM of 70 samples.
Significantly different from control: * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$.

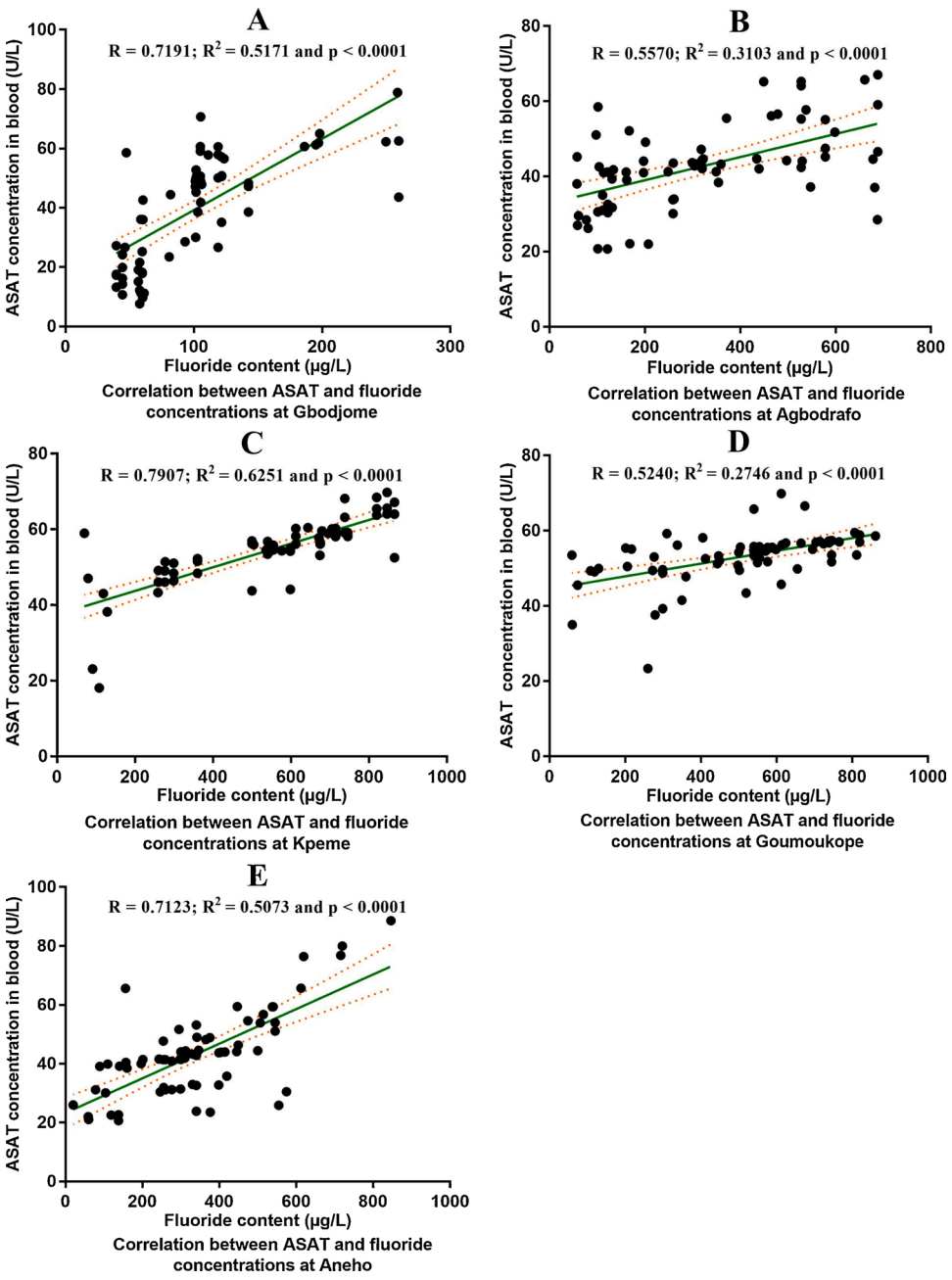

Fig. 6. Correlation between ASAT and fluoride concentrations in blood. The correlations is established by considering the five localities and in relation to the values of 70 samples corresponding to the number of individuals recruited per locality; A: at Gbodjome, B: at Agbodrafo, C: at Kpeme, D: at Goumoukope and E: at Aneho.

Our results clearly indicate that the toxicity of fluoride could also impact on the liver system. Indeed, it suggests that fluoride-induced apoptosis and autophagy in liver were caused by activating the IL-17 signalling pathway [52]. In addition, Girardi and Merler [53] indicated that a high cumulative internal dose of perfluorooctanoic acid showed a statistically significant increase in mortality from liver cancer and liver cirrhosis in male employees. However, further research is needed to explain the mechanism of action of fluoride in liver toxicity. This work shows once again the role of SNPT's industrial activity in the ever-increasing alteration of human health.

5. Conclusion

The high concentrations of cadmium, lead, and fluoride in the blood of people at Agbodrafo, Kpeme, Goumoukope, and Aneho in relation to Gbodjome are due to the environmental pollution following the industrial activity of the SNPT plant. This is explained by the fact that bioaccumulation of pollutants by exposed individuals is more important at Kpeme and Goumoukope, which are closer to the factory, than at Agbodrafo and Aneho. The correlation tests, multivariate analysis, and the variation of the fluoride concentrations show that fluoride toxicity impacts renal and hepatic systems already affected by toxic metals. Industrial activity in the phosphate treatment area of Togo leads to human contamination with fluoride along with cadmium and lead which increases the risk of renal and hepatic dysfunction. It is necessary to continue the study to know the mode of action of fluoride toxicity on these organs. Furthermore, it is necessary to consider the present data and those of previous studies on human health impacts of this industrial activity in Togo in order to improve the management of the waste produced in order to prevent or limit these impacts.

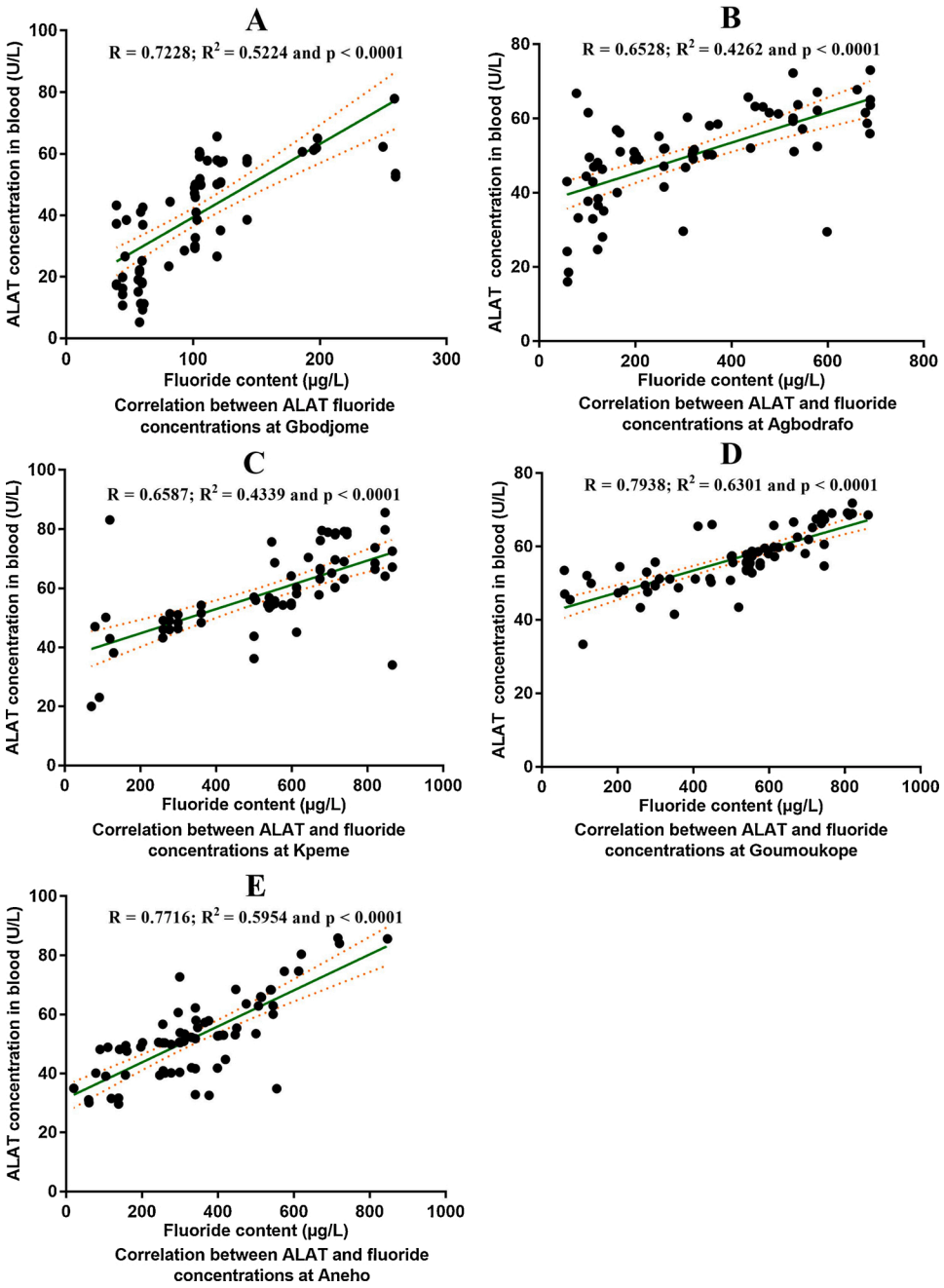

Fig. 7. Correlation between ALAT and fluoride concentrations in blood. The correlations are established by considering the five localities and in relation to the values of 70 samples corresponding to the number of individuals recruited per locality; A: at Gbodjome, B: at Agbodrafo, C: at Kpeme, D: at Goumoukope and E: at Aneho.

CRediT authorship contribution statement

Mamatchi Melila, **Kou'santa Amouzou**, **Sadikou Agbere**: Conceived and designed the experiments. **Mamatchi Melila**, **Sadikou Agbere**, **Iuliana Mihaela Lazar**, **Arumugam Ganeshkumar**, **Tcha Pakoussi**, **Mabozou Kpemissi**, **Gabriel Lazar**: Performed the experiments. **Mamatchi Melila**, **Arumugam Ganeshkumar**, **Rajendran Rajaram**, **Iuliana Mihaela Lazar**, **Gabriel Lazar**: Analyzed the data. **Rajendran Rajaram**, **Iuliana Mihaela Lazar**, **Gabriel Lazar**, **Kou'-santa Amouzou**: Contributed reagents/materials/analysis tools. **Bilal Ahamad Paray**, **Aneela Gulnaz**: Statistical data analysis and language editing. **Mamatchi Melila**, **Rajendran Rajaram**, **Arumugam Ganeshkumar**, **Gabriel Lazar**: Wrote the paper.

Ethical consideration

The authors state that they have respected all ethical considerations in this study and have obtained an agreement from the international Bioethics Committee for Health Research (CBRS) of the Ministry of Health of Togo under No. 87/2012/MS/CAB/DGS/DPLET/CBRS.

Authors' Contributions

Conceived and designed the experiments: MMKA and SA. Performed the experiments: MM, SA, IML, AG, TP, MK, and GL. Analyzed the data: MM, AG, RR, IML, and GL. Contributed reagents/materials/analysis tools: RR, IML, GL, and KA. Statistical data analysis and language editing: B.AP and A Gulnaz. Wrote the paper: MM, RR, AG, and GL.

Table 6
Frequency of food consumption.

Localities	Frequency of consumption per week	Consumption of agricultural products and/or market gardeners from the locality		Consumption of seafood		Use of well water	
		n(%)	*P value*	n(%)	*P value*	n(%)	*P value*
Gbodjome	Two or more times	57 (81.43)	$P = 0.4516$ ($R^2 = 0.000$)	54 (77.14)	$P > 0.999$ ($R^2 = 0.000$)	56 (80.00)	$P > 0.999$ ($R^2 = 0.000$)
	Once	05 (07.14)		06 (08.57)		00 (00.00)	
	Rarely/never	08 (11.43)		10 (14.29)		14 (20.00)	
Agbodrafo	Two or more times	55 (78.57)		59 (84.29)		70 (100.00)	
	Once	03 (04.29)		01 (01.43)		00 (00.00)	
	Rarely/never	12 (17.14)		10 (14.28)		00 (00.00)	
Kpeme	Two or more times	58 (82.86)		56 (80.00)		53 (75.71)	
	Once	02 (02.86)		02 (02.86)		04 (05.71)	
	Rarely/never	10 (14.28)		12 (17.14)		13 (18.57)	
Goumoukope	Two or more times	61 (87.14)		59 (84.29)		70 (100.00)	
	Once	00 (00.00)		02 (02.86)		00 (00.00)	
	Rarely/never	09 (12.86)		09 (12.86)		00 (00.00)	
Aneho	Two or more times	49 (70.00)		51 (72.86)		52 (74.29)	
	Once	02 (02.86)		03 (04.28)		00 (00.00)	
	Rarely/never	19 (27.14)		16 (22.86)		18 (25.71)	

Table 7
Sociological and health data on respondents.

Parameters		Localities				
		Gbodjome	Agbodrafo	Kpeme	Goumoukope	Aneho
Lifespan in the locality	[4–5] years n(%)	16 (22.86)	23 (32.86)	09 (12.86)	07 (10.00)	18 (25.71)
	[6–10] years n(%)	34 (48.57)	32 (45.71)	43 (61.43)	39 (55.71)	27 (38.57)
	> 10 years n(%)	20 (28.57)	15 (21.43)	18 (25.71)	24 (34.29)	25 (35.71)
	Average (years)	14.22 ± 1.89	12.38 ± 1.59	10.14 ± 0.89	15.68 ± 1.84	12.83 ± 1.41
		$P = 0.1359$ ($R^2 = 0.02003$)				
Ethnic group	Southern n(%)	57 (81.43)	49 (70.00)	37 (52.86)	52 (74.29)	47 (67.14)
	Northern n(%)	13 (18.57)	21 (30.00)	33 (47.14)	18 (25.71)	23 (32.86)
		$P = 0.4857$ ($R^2 = 0.00000$)				
Level of knowledge and perception on pathologies linked to the ETM and fluorine	Low n(%)	29 (41.43)	23 (32.86)	18 (25.71)	27 (38.57)	17 (24.29)
	Medium n(%)	26 (37.14)	31 (44.28)	39 (55.71)	29 (41.43)	32 (45.71)
	Good n(%)	15 (21.43)	16 (22.86)	13 (18.57)	14 (20.00)	21 (30.00)
		$P > 0.9999$ ($R^2 = 0.00000$)				
Socio-professional characteristics	Student n(%)	07 (10.00)	09 (12.86)	05 (07.14)	03 (04.29)	08 (11.43)
	Unemployed n(%)	13 (18.57)	12 (17.14)	07 (10.00)	13 (18.57)	09 (12.86)
	Farmer/Market gardener n(%)	11 (15.71)	07 (10.00)	15 (21.43)	18 (25.71)	08 (11.43)
	Fisherman n(%)	12 (17.14)	13 (18.57)	13 (18.57)	17 (24.29)	12 (17.14)
	Trader n(%)	13 (18.57)	11 (15.71)	14 (20.00)	08 (11.43)	14 (20.00)
	Artisan n(%)	06 (08.57)	07 (10.00)	03 (04.29)	05 (07.14)	04 (05.71)
	Functionary n(%)	08 (11.43)	11 (15.71)	13 (18.57)	06 (08.57)	15 (21.43)
		$P > 0.9999$ ($R^2 = 0.00000$)				
School level	Unschooled n(%)	11 (15.71)	08 (11.43)	06 (08.57)	12 (17.14)	05 (07.14)
	Primary n(%)	21 (30.00)	17 (24.29)	11 (15.71)	23 (32.86)	22 (31.43)
	Secondary n(%)	29 (41.43)	34 (48.57)	38 (54.29)	31 (44.28)	27 (38.57)
	University n(%)	09 (12.86)	11 (15.71)	15 (21.43)	04 (05.71)	16 (22.86)
		$P = 0.4380$ ($R^2 = 0.00000$)				
Monthly income (dollars)	< 100 n(%)	12 (17.14)	13 (18.57)	11 (15.71)	14 (20.00)	11 (15.71)
	[100 – 300[n(%)	29 (41.43)	34 (48.57)	26 (37.14)	33 (47.14)	26 (37.14)
	[300 – 500[n(%)	19 (27.14)	17 (24.29)	22 (31.43)	16 (22.86)	23 (32.86)
	≥ 500 n(%)	10 (14.29)	06 (08.57)	11 (15.71)	07 (10.00)	10 (14.29)
		$P > 0.9999$ ($R^2 = 0.00000$)				
State of health declared in a health document and/or observed	Cardiovascular diseases n(%)	18 (25.71)	17 (24.29)	22 (31.43)	21 (30.00)	16 (22.86)
	Diabetes n(%)	02 (02.86)	04 (05.71)	04 (05.71)	02 (02.86)	03 (04.29)
	Kidney dysfunction n(%)	03 (04.29)	02 (02.86)	05 (07.14)	04 (05.71)	03 (04.29)
	Hepatic dysfunction n (%)	01 (01.43)	00 (00.00)	02 (02.86)	01 (01.43)	02 (02.86)
	Dental fluorosis[a] n(%)	15 (21,43)	18 (25.71)	27 (38.57)	30 (42.86)	16 (22.86)
	Bone fluorosis n(%)	01 (01.43)	02 (02.86)	09 (12.86)	11 (15.71)	01 (01.43)
		$P = 0.7684$ ($R^2 = 0.06775$)				

[a] Mild, temperate, moderate and severe dental fluorosis.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jtemb.2021.126890.

References

[1] J. De Valck, J. Rolfe, Linking water quality impacts and benefits of ecosystem services in the Great Barrier, Reef. 130 (2018) 55–66.
[2] W.A. Omar, K.H. Zaghloul, A.A. Abdel-Khalek, S. Abo-Hegab, Risk Assessment and toxic effects of metal pollution in two cultured and wild fish species from highly degraded aquatic habitats, Arch Environ Contam Toxicol. 65 (2013) 753–764.
[3] P.N. Manavi, A. Mazumder, Potential risk of mercury to human health in three species of fish from the southern Caspian Sea, Mar Pollut Bull. 130 (2018) 1–5.
[4] E.O. Farombi, O.A. Adelowo, Y.R. Ajimoko, Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat Fish (*Clariasgariepinus*) from Nigeria Ogun River, Int J Env Res Pub Health. 4 (2007) 158–165.
[5] G.H. Lu, X.C. Jiao, H.T. Piao, X.C. Wang, S. Chen, K.Y. Tan, N. Gai, X.C. Yin, Y. L. Yang, J. Pan, The extent of the impact of a fluorochemical industrial park in eastern china on adjacent rural areas, Arch Environ Contam Toxicol. 74 (2018) 484–491.
[6] M. Melila, I. Bilabina, K.L. Awaga, et al., A high risk of contamination to the toxic heavy metals (cadmium and lead) and to fluorine diet in an inshore industrial zone of south of Togo, Int J Cur Res. 5 (2013) 3278–3283.
[7] S. Li, L. Zhou, J. Chang, Z. Yang, J. Hu, W. Hongjun, The impact of impoundment on mercury bioaccumulation in fish downstream from a newly constructed reservoir, Wujiang river, Southwest China, Arch Environ Contam Toxicol. 73 (2017) 570–577.
[8] M. Melila, I. Bilabina, B.V.S. Houndji, et al., Bioaccumulation of Several Heavy Metals (Cadmium and Lead) and Oxidative Stress in *Sphyraena Barracuda* (marine fish) in Kpeme and Gbodjome (South Togo), Cam J Biol Biochem Sci. 21 (2013) 1–9 (in French with English abstract).
[9] M. Melila, W. Poutouli, B. Houndji, et al., Oxidative stress in human due to metallic traces elements bioconcentration in three coastal villages near phosphate treatment factory in Togo, Afr J Food Sci Technol. 4 (2013) 141–147.
[10] K. Gnandi, H.J. Tobschall, The pollution of marine sediments by trace elements in the coastal region of Togo caused by dumping cadmium-rich phosphorite tailing in to the sea, Environ Geol. 38 (1999) 13–24.
[11] K. Gnandi, G. Tchangbédji, K. Kili, G. Baba, A.I. Ouro-Salim, Processing of Phosphate Mine Tailings by Coagulation Floculation to Reduce Marine Pollution in Togo: Laboratory Tests, Mine Water Environ. 24 (2005) 215–221.
[12] K. Gnandi, G. Tchangbédji, K. Kili, G. Baba, K. Abbe, The impact of Phosphate mine tailing on the bioaccumulation of heavy metals in marine fish and crustaceans from the coastal zone of Togo, Mine Water Environ. 25 (2006) 56–62.
[13] K. Gnandi, B. Rezaie, A.P. Edorh, The geochemical characterization of mine effluents from the phosphorite processing plant of Kpeme (Southern Togo), Mine Water Environ. 28 (2009) 65–73.
[14] North-South Environment (NSE) - IW: LEARN/ONUDI, Reduction of waste from phosphate mines in the GCLME; Togo demonstration project. Final Report, 2007, 144p, available on the site: https://www.google.fr/url, (Accessed 16 June 2017) (in French).
[15] A.A. Aduayi-Akue, K. Gnandi, Assessment of soils and local variety of maize *Zea mays* pollution by heavy metal in the Phosphate treatment area of Kpeme (southern Togo), Int J Biol. Chem. Sci. 8 (2015) 2347–2355 (in French).
[16] G. Tanouayi, K. Gnandi, H. Ahoudi, K. Ouro-Sama, Metallic contamination of surface water and groundwater in the exploitation zone of Hahotoe-Kpogame phosphates (South-Togo): case of cadmium, lead and nickel, Larhyss Journal 21 (2015) 25–40 (in French).
[17] WHO, Fluoride and health. Monograph Series N° 59, chapter VI, WHO, Geneva, 1972, 29p Available on the website: http://shodhganga.inflibnet.ac.in/bitstream/10603/124128/16/16_references.pdf, (Accessed 16 January) (in French).
[18] M. Torra, M. Rodamilans, J. Corbella, Serum and urine fluoride concentration: relationships to age, sex and renal function in a non-fluoridated population, Sci Total Environ. 220 (1998) 81–85.
[19] M. Sowers, G.M. Whitford, M. Kathleen-Clark, M.L. Jannausch, Elevated serum fluoride concentrations in women are not related to fractures and bone mineral density, J. Nutr. 135 (2005) 2247–2252.
[20] Z.Z. Guan, Y.N. Wang, K.Q. Xiao, D.Y. Dai, Y.H. Chen, J.L. Liu, P. Sindelar, G. Dallner, Influence of chronic fluorosis on membrane lipids in rat brain, Neuro Toxicol Teratol. 20 (1998) 537–542.
[21] J.A. Varner, K.F. Jensen, W. Horvath, R.L. Isaacson, Chronic administration of Aluminum fluoride or sodium-fluoride to rats in drinking water: alterations in neuronal and cerebrovascular integrity, Brain Res. 784 (1998) 284–298.
[22] I. Menoyo, A. Rigalli, R.C. Puche, Effect of fluoride on the secretion of insulin in the rat, Arzneimittelforschung 55 (2005) 455–460.
[23] National Research Council (NRC), Fluoride in drinking water: a scientific review of EPA’s standards. Committee on Fluoride in Drinking Water, The National Academies Press, Washington DC, 2006.
[24] WHO, Environmental Health Criteria 227: FLUORIDES, WHO, Geneva, 2002, 290p Available on the website:*whqlibdoc*.who.int/ehc/WHO_EHC_227.pdf, (Accessed 16 February 2018).
[25] M. Melila, R. Rajendran, K.L. Awaga, G. Arumugam, M. Kpemissi, S. Agbere, G. Lazar, K. Amouzou, 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, J. Trace Elem. Med. Biol. 56 (2019) 13–20, https://doi.org/10.1016/j.jtemb.2019.07.002.
[26] E.B. Bassin, D. Wypij, R.B. Davis, M.A. Mittleman, Age-specific fluoride exposure in drinking water and osteosarcoma (United States), Cancer Causes Control. 17 (2006) 421–428.
[27] A. Prystupa, J. Sak, P. Kicinski, A. Stenzel-Bembenek, A. Błazewicz, Serum Concentration of Fluoride in Patients with Alcoholic Liver Cirrhosis from the Lublin Region in Eastern Poland, Int. J. Environ. Res. Public Health 18 (2021) 1115, https://doi.org/10.3390/ijerph18031115.
[28] I. Błaszczyk, E. Birkner, I. Gutowska, E. Romuk, D. Chlubek, Influence of methionine and vitamin E on fluoride concentration in bones and teeth of rats exposed to sodium fluoride in drinking water, Biol. Trace Elem. Res. 146 (2012) 335–339.
[29] H. Zuo, L. Chen, M. Kong, L. Qiu, P. Lü, P. Wu, Y. Yang, K. Chen, Toxic effects of fluoride on organisms, Life Sci. 198 (2018) 18–24.
[30] Y. Lu, Q. Luo, H. Cui, H. Deng, P. Kuang, H. Liu, J. Fang, Z. Zuo, J. Deng, Y. Li, X. Wang, L. Zhao, Sodium fluoride causes oxidative stress and apoptosis in the mouse liver, Aging 9 (2017) 1623–1639.
[31] C. Liang, Y. Gao, Y. Zhao, R.K. Manthari, J. Ma, R. Niu, J. Wang, J. Zhang, J. Wang, Effects of fluoride and/or sulfur dioxide on morphology and DNA integrity in rats’ hepatic tissue, Biol. Trace Elem. Res. 183 (2018) 335–341.
[32] Q. Niu, P. He, S. Xu, R. Ma, Y. Ding, L. Mu, S. Li, Fluoride-induced iron overload contributes to hepatic oxidative damage in mouse and the protective role of grape seed proanthocyanidin extract, J. Toxicol. Sci. 43 (2018) 311–319.
[33] G. Tanouayi, K. Gnandi, K. Ouro-Sama, A.A. Aduayi-Akue, H. Ahoudi, Y. Nyametso, H.D. Solitoke, Distribution of Fluoride in the Phosphorite Mining Area of Hahotoe–Kpogame (Togo), J Health Pollut. 10 (2016) 84–94.
[34] OMS/IOMC, Guide succinct des méthodes de dosage du plombdans le sang, 21p., 2013.
[35] S. Jianxun, W. Qiuyu, W. Jinyu, J. Shuqiang, Z. Hongmin, The influence of fluoride ions on the equilibrium between titanium ions and titanium metal in fused alkali chloride melts, Faraday Discuss. 190 (2016) 421–432.
[36] A.C. José, C.P. Rodolfo, Investigation on the biological effect of fluorine present on the surface of titanium implants. Analysis of the reaction fluoride-titanium oxide, Actual. Osteol. 13 (1) (2017) 37–45.
[37] VIDAL – Analysis, Analyzes of Medical Biology, Usual values/Interpretation of biological results and usual values, Available on the website: https://www.vidal.fr, info-practices, (Accessed 24 February 2018) (in French), 2018.
[38] Q. Yao, Q. Lin, S.-A. Yan, M. Huang, L. Chen, Dietary risk assessment of fluoride, lead, chromium, and cadmium through consumption of Tieguanyin tea and white tea, Food Sci. Technol, Campinas. 41 (3) (2021) 782–789.
[39] Y. Li, S. Wang, Z. Nan, F. Zang, H. Sun, Q. Zhang, W. Huang, L. Bao, Accumulation, fractionation and health risk assessment of fluoride and heavy metals in soil-crop systems in northwest China, Science of The Total Environment 663 (2019) 307–314.
[40] N.C. Yildirim, M. Yurekli, N. Yildirim, Investigation of some antioxidant enzymes activities depending on adrenomedulin treatment and cold stress in rat liver tissue, Turkist J Biochem. 35 (2010) 138–142.
[41] H.-J. Tsai, P.-Y. Wu, J.-C. Huang, S.-C. Chen, Environmental Pollution and Chronic Kidney Disease, Int J Med Sci. 18 (5) (2021) 1121–1129.

[42] G.Y. Yeh, D.M. Eisenberg, T.J. Kaptchuk, R.S. Phillips, Systematic review of herbs and dietary supplements for glycemic control in diabetes, Diabetes Care 26 (2003) 1277–1294.
[43] P. Lawson-Evi, K. Eklu-Gadegbeku, A. Agbonon, K. Aklikokou, E. Creppy, M. Gbéassor, Antidiabetic activity of *Phyllanthusamarus* Schum. And Thonn. (Euphorbiaceae) on alloxan induced diabetes in male Wistar rats, J App Sci 11 (2011) 2968–2973.
[44] M. Ferguson, V. Vaidya, J. Bonventre, Biomarkers of nephrotoxic acute kidney injury, Toxicology 20 (2008) 182–193.
[45] G. Hythurbide, A. Hertig, Increased in creatinine: knowing how to interpret a creatinemia to assess kidney function is essential, Review of the Practitioner General Medicine Tome 26 (876) (2012) 152–153 (In French).
[46] C.W. Schmidt, W. Leuschke, Fluoride content of deciduous teeth after regular intake of black tea, Dtschstomatol 40 (10) (1990) 441.
[47] F. Marumo, S. Iwanami, High fluoride concentrations in the serum and bone of patients with chronic renal failure, Fluoride 34 (3) (2001) 213.
[48] W. Lee, Drug-induced hepatotoxicity, Alimentary pharmacology & therapeutics 7 (1993) 477–485.
[49] M. Sturgill, G. Lambert, Xenobiotic-induced hepatotoxicity: mechanisms of liver and methods of monitoring hepatic function, Clin Chem. 43 (1997) 1512–1526.
[50] F. Kargin, H.Y. Cogun, Metal interactions during accumulation and elimination of zinc and cadmium in tissues of the fresh water fish, Tilapia *nilonitica*, Bull Environ Contam Toxicol. 63 (1999) 511–519.
[51] E.J. Werder, J.I. Beier, D.P. Sandler, K.C. Falkner, T. Gripshover, B. Wahlang, L. S. Engel, M.C. Cave, Blood BTEXS and heavy metal levels are associated with liver injury and systemic inflammation in Gulf States residents, Food and Chem. Toxicol. 139 (2020) 111242, https://doi.org/10.1016/j.fct.2020.111242.
[52] Y. Zhao, Y. Li, J. Wang, R.K. Manthari, J. Wang, Fluoride induces apoptosis and autophagy through the IL-17 signaling pathway in mice hepatocytes, Arch. Toxicol. 92 (2018) 3277–3289.
[53] P. Girardi, E. Merler, A mortality study on male subjects exposed to polyfluoroalkyl acids with high internal dose of perfluorooctanoic acid, Environ. Res. 179 (2019) 108743.
[54] M. Melila, W. Poutouli, K. Amouzou, G. Tchangbédji, M. Tchaou, A. Doh, Assessment of the impact of the discharge of phosphate waste in the sea on the marine biodiversity in three coastal localities in Togo, using biomarkers of oxidative stress in *Sphyraena barracuda* (HECKEL, 1843), Int. J. Biol. Chem. Scien. 6 (2012) 820–831, https://doi.org/10.4314/ijbcs.v6i2.24 (In French).
[55] A.A. Aduayi-Akue, Assessment of soils and local variety of maize *Zea mays* pollution by heavy metal in the Phosphate treatment area of Kpeme (southern Togo), Int. J. Biol. Chem. Scien. 8 (2014) 2347–2355, https://doi.org/10.4314/ijbcs.v8i5.37 (In French).
[56] Miquel G., Revol H., Effects of heavy metals on the environment and health (OPECST report). Available on the website: https://www.senat.fr, accessed on January 16, 2018 (In French).
[57] R.A. Lourenço, T. Combi, M.A. da Rosa, S.T. Sasaki, E. Zanardi-Lamardo, G. T. Yogui, Mysterious oil spill along Brazil's northeast and southeast seaboard (2019–2020): trying to find answers and filling data gaps, Mar. Pollut. Bull. 156 (2020), 111219, https://doi.org/10.1016/j.marpolbul.2020.111219.