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Evalution of the exposure to pollutants in the drinking water of So-ava municipality using biomarkers: epidemiological study

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Abstract

The previous works revealed the existence of the risks of exposure to pollutants through the consumption of the water in the localities of Ahomey and Houédo in Sô-Ava. It was thus advisable to look for biomarkers, indicators of exposure to these toxics. In this context, a survey was conducted at first to identify the characteristic disorders connected to exposure to xenobiotics. This survey was completed by biochemical and hematological analyses of blood and urine of the investigated. The results revealed that: the characteristic clinical signs to lead and/or cadmium poisoning existed within the population; in particular, stomach pain, , symptoms connected to the reproduction, sign of bone pain, mental retardation, , asthenia, arterial high blood pressure, sight impairment, excessive perspiration, neurological and renal signs. Also in exposure areas, correlation between blood lead and lead in drinking water was high but that correlation was weak for cadmium. Besides, 80 % of the investigated

presented a concealed anemia. Other hematological and biochemical values were within the standards, and there was no major disturbance at this level. In respect of urine analyses, there was blood in the urine of 45.16 %, leukocytes of 38.71 %, proteins of 41.94 % and nitrites of 80 % of the exposed population. No glucose was found in the urine but the excretion of calcium is sometimes very alarming with certain individuals especially in the localities where the exposure is high.

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Introduction

The presence of lead in the water of consumption is usually attributable to the phenomenon of corrosion which arises in the structural components of the distribution pipelines and in the domestic piping which contain some lead (Gardels and Sorg, 1989). In Sô-Ava, the contamination of drinking water is due to several factors in particular spills of petroleum products in the lake, the domestic and biomedical garbage dumps on the edge of the lake and the contributions of Ouémé river through the runoff residues of pesticides (Kinsiclounon *et al.,* 2013).

The poisoning with lead is manifested in several ways. From a blood concentration of 10 µg / dL of lead, there is inhibition of enzymes responsible for the synthesis of heme. This inhibition is total with 90 µg/dL (Landrigan and Todd, 1994).

The main targets of lead toxicity on the peripheral nervous system are motoneurones, which undergo a segmental demyelization and an axonal degeneration slowing down the electric conduction.Through the drinking water they consume the populations of the lakeside municipality of Sô-Ava are exposed to lead, cadmium, to total iron and nitrates (Kinsiclounon *et al.,* 2013a; Kinsiclounon *et al.,* 2013b).

The search of biomarkers is a part of the assessment method to exposure to toxics. The quantitative estimation of this exposure had shown that children are more exposed than adults. It was then necessary to confirm these theoretical estimations by the search of biomarkers, particularly effects biomarkers connected to these contaminants. Biomarkers are classified in three categories: exposure, effect and susceptibility biomarkers (Lagadic *et al.,* 1997).

Exposure biomarkers are indicators of contamination of biological systems by one or several xenobiotics. They are generally involved in the mechanisms of cellular defense (i.e.: antioxidant enzymes) and of detoxification of xenobiotics. The effect biomarkers correspond to biological changes which according to the intensity of the responses can statistically or mechanistically be associated to some pathology or an altered physiological state. As far as the susceptibility / sensibility biomarkers are concerned they indicate the inherent or acquired capacity of a body to respond to stress induced by the exposure to a xenobiotic.

Materials and methods

Identification of toxicity biomarkers in blood and urine of the populations

This identification is going to cross the surveys data on the pathologies chararacterising nitrogenous compound and heavy metals recorded and the level of contamination of the body of the investigated subjects. The level of contamination of the body by heavy metals and nitrogenous by-products is known by diagnosis - through interviews, blood and urine analyses for certain bio-indicators of toxicity from the investigated: That is:

Blood

Blood cells counts and empty stomach blood analyses for glucose, urea, creatinine, uric acid, total cholesterol, transaminases SGOT + SGPT, iron, ferritin, saturation coefficient , of lead and cadmium.

Urine

Content in glucose, proteins, calcium, nitrites, red blood corpuscles or hemoglobin, leukocytes, bilirubin and of pH measure.

Sampling of biological fluids

The qualitative and quantitative analyses of blood and urines are very delicate and precision and accuracy of the analyses and the interpretation of the results depend on sampling methods and apparatus.

Choice of participants

The investigation was conducted during April and May, 2012, on a sample of 31 cases taken at random in the villages of Ahomey Gblon, Ahomey Lokpo, Houédo Gbadji and Sô-Tchanhoué where the resident populations are the most exposed to the xenobiotic, through the contaminated drinking water.

Blood and urine were the analyzed biological fluids and were collected from people selected according to an accidental sampling after a survey (by questionnaire attached in appendix). Beforehand, ethical approval from the research ethics committee was obtained before the commencement of the investigation. Written informed consent for participation of the people was received from all participants.

Alternately, each of them received a plastic clean flask for urine collection.

Samplings, identification, processing and preservation of the biological samples

Venous blood was collected on an empty stomach with the help of the Vacutainer method (vacuum sampling). Two tubes of 5 mL with anticoagulant and a dry tube of 5 mL were used by everyone. The anticoagulants used were: the ethylenediaminetetraacetic acid (EDTA) for blood cells count (NF), analysis of lead and cadmium then sodium fluoride for the analysis of glycemia and creatinine. The dry tube was used for the analysis of other biochemical analyses. These blood samples correctly identified (by an anonymous code) were centrifuged at 2000 tours for 15mn for serum separation and preservation at 4 °C was done.

Urines were collected in flasks of 5 ml between 11 am and 5 pm according to the method recommended by WHO for the search of bilharziasis eggs because it is in this period of time that *Schistosoma hermatobium* lays eggs in the bladder (WHO, 1998).

All the tubes of blood and flasks of urine and the survey papers regarding each person had the same anonymous code till the end of the study. The transport of samples was carried out in icebox at 4°C before being preserved at - 20°c at laboratory.

Urea and creatinine are waste of the metabolism which inform about the purifying function of the kidney, the main target of heavy metals. Any nephrotoxic substance (lesion of tubules) - even in the short term - may modify the blood concentrations of this two waste matter in the sense of an increase (they are reabsorbed in the damaged tubules and return to the blood, resulting in their increase).

Calcium and iron are in competition with heavy metals (Pb, Cd, Hg): any excessive presence of these oligo elements in urines (or stool) can be a proof of the action of heavy metals which would have occupied the receptors of these elements on the sites of the inactive enzymes.

The transaminases synthesized in liver increase to indicate the contamination of the body with heavy metals, especially with lead and cadmium.

These analyses were completed by the analyses of glycemia and NF in order to a possible problem with metabolism of glucose and anomalies of blood cells (red and white blood cells) owed to the exposure to identified xenobiotics. These parameters were analyzed in the laboratory of biomedical analyses of the university of Abomey-Calavi.

Analyses of toxicity biomarkers in blood

Analyses methods

Blood tests were conducted according to Elitech protocols (Clinical System) certified by European society of Standardization. Molecular Absorption Spectrophotometer of Screen Master make was used for analysis of glycemia, uremia, creatinemia, transaminases, uric acid, calcemia and cholesterols on one hand and automated hematology of RAYTO model RT-7200 brand was for blood counts (NF) in the laboratory (LAB / CAMPUS) of the Research for Health and Development Institute on the other hand.

As regards for hemochromatosis, the analysis of ferritin was conducted by means of MINI VIDAS using Bio Mérieux Kit in the Haematology Laboratory of the University of Abomey-Calavi Teaching Hospital.

-the analysis of serum iron or transferrin was carried out with automated biochemistry of Cyan Plus make with Bio Mérieux kit of the Hubert Koutoukou Maga National university Hospital of Cotonou. The analysis of serum iron or transferrin was completed by the calculation of the coefficient of saturation (CS) to explore the circulating compartment. The total potentiality of transferrin to bind iron is called total capacity of binding (TCB).They are calculated by the following formulae:

CTB = CLF + sideremy

(TCB) = TRF (g/l) x (2/80000) x 106 = µmol iron/l

(TCB) = TRF (g/l) x 25 = µmol iron/l

Normal values of TCB ranging between 30 and 60

CS= ([serum iron]/CTB) x100; CS = coefficient of saturation

CS = 30+or- 5% [23-45]

CS < 20% → deficiency

CS > 55% → Overload

- Concerning the analysis of transaminases SGOT and SGPT on one hand, and glycemia, cholesterols, uric acid on the other hand, for the poisoning with nitrites, because of the alteration of the hepatic functions, the methods used were respectively the enzymatic kinetics at 340 nm and the colorimetric method in the final stage (505 nm).

-For calcemia, it was about a colorimetric and complexometric method at 600 nm.

-Analysis of uremia was carried out with an enzymatic kinetic, method at UV (340 nm).

- Creatinemia was measured by the colorimetric and kinetic method of Jaffé (505 nm).

-Blood count was conducted with the help of the automated hematology.

-All the manipulations were checked by serum multiparametric Elitrol 1 of Elitech brand.

Lead blood level was measured at toxicology laboratory of the Beninese Ministry of Health.

The level of calcium in urine was measured with the same reagent as in the blood. The reference values used for the interpretation of each of the parameters are from Blague-Belar *et al.,* (1991) and Loko (2001).

The analyzed variables in urine

The analysis of some parameters in urine was conducted by using strips (TC Teco diagnostics). Thus glucose, proteins, calcium, nitrites, red blood corpuscles or hemoglobin, leukocytes, bilirubins were analyzed and the pH measured

Results

Results of survey

The cohort of 31 subjects in study was made of 61 % of men and 39 % women. The mean age was of 30 years old for the adults and 12 years old for children. As profession, the women were essentially housewives, and men were farmers. Concerning the origin of the investigated, except for the case of a woman who had been living there for 2 years, the newest inhabitants in these localities already counted 12 years of residence. They were all Beninese.

Blood lead /Blood cadmium and quality of the drinking water

Figures 1 show the evolution of the blood lead and the blood cadmium related to the contents of lead and cadmium in the drinking water of the localities of Sô-Ava.

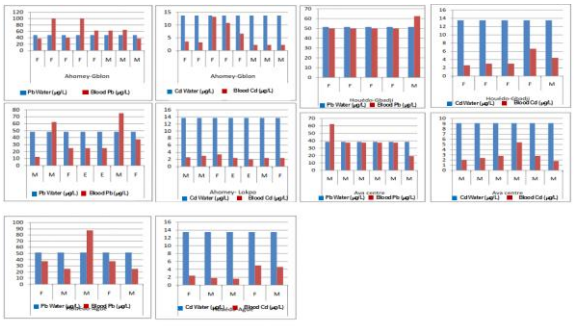

Fig. 1. The evolution of blood lead and blood cadmium related to the contents of lead and cadmium in the drinking water.

The average blood lead and blood cadmium were respectively 49.7 and 3.884 µg / l against 49.95 and 13.6 µg / l in the water in the localities where the exposure was high; while the blood lead and the blood cadmium were respectively 38.61 and 2.86 µg / l against 38.5 and 9.1 µg / l in the water of Sô-Ava. For the whole region the global mean values were: 44.15 ± 18.66 µg / l against 44.22 and 3.37 ± 2.05 µg / l compared to 11.35 mg / l. Yet, the blood lead threshold effect concentration where no clinical signs are observed is connected to the daily allowed intake (DAI) which is reached with a blood lead of 20 µg / L, that is is DAI of 3.6 µg / kg (WHO, 1986).

Connection between contents in lead / cadmium of water, blood lead/ blood cadmium and clinic symptomatology

The relation between contents in lead / cadmium of water, blood lead / blood cadmium and the symptomatology is expressed in Table 1.

Table 1. Connection between contents of lead / cadmium of water, blood lead/blood cadmium and clinical symptoms.

Villages	**Sex**	**Main symptoms recorded (during surveys)**	**Blood lead(µg/L)**	Pb in water **(µg/L)**	**Blood cadmium (µg/L)**	Cd in water **(µg/L)**
Norme Standard			**< 10**	**40**	< 2 µg.L-1 (18 nmol.L-1)	**5**
A.Gblon	F	F High blood pressure, colics, miscarriage, sleep disorder	37.5		3.5	
	F	F Miscarriage, parasthesia of hands	100	48.4	3.2	13.7
	F	Death of two newborns and miscarriage	40		13.2	
	F	Hyperactivity	100		10.8	
	F	Three dead babies, miscarriage, diarrhea, colics, sleep trouble, heavy breath, asthenia, bone pain, losing weight.	62.5		6.6	
	M	Apathy,asthenia, abdominal colics, breating disorders	62.5		2.2	
	M	Apathy, asthenia, colics, diarrhea	65.0		2.2	
	M	Headache, sleeping disorders, constipation, two mischarriages and one stilborn child	37.5		2.2	
Lokpo	M	Osseous pains, asthenia, apathy, low blood pressure, miscarriage (his wife), one stillborn infant,	12.5		2.6	

		colics				
	M	Headache, asthenia, kidney pains, dental pain, stillborn child, diarrhea,colics	62.5		3.0	
	F	Asthenia, excessive perspiration, stillborn child, osseous pains	25.0		3.4	
	E	13-year-old child dismissed from school after repeated failure in the primary school	25.0		2.4	
	E	13-year-old child removed from school after repeated failure in the primary school	25.0		2.0	
	M	Apathy,asthenia, infertility (male infertility)	75.0		2.4	
	F	Difficulty to urinate, headache, loss of twins	37.5		2.4	
Ouédo Agué	F	Headache,asthenia, loss of two children	37.5		2.4	
	M	23 years old man, nothing worth reporting, but was dismissed from primary school	25.0	51,5	1.8	13,5
	M	asthenia Sight impairment	87.5		1.6	
	F	Asthenia, Miscarriage, hgh blood pressure,	37.5		5.0	
	M	Colics, miscarriage,asthenia	25.0		4.6	
	F	Asthenia, infertility	50.0		2.6	
	F	Asthenia, miscarriage, sleeping disorders	50.0		3.0	
Ouédo-Gbadji	F	Asthenia, miscarriage, loss of child	50.0		3.0	
	F	Parasthesia and itching, miscarriage, dead child	50.0		6.6	
	M	Asthenia, dizziness, two miscarriages, two dead children	62.5		4.4	
			49.7± 23,53	**49.95**	**3.884± 2.80**	13.6

m ± SD									
Ava center	M	Locality	selected	as	62.5			2.0	
(control	M	control			37.5			2.4	
locality)	M				37.5		38.5	2.8	
	M				37.5			5.4	9.1
	M				37.5			2.8	
	M				19.2			1.8	
					38.61± 13.80			**2.86± 1.30**	
m ± SD					**44,15 ± 18.66**		**44.22**	**3.37 ± 2.05**	**11.35**

Analysis of the clinical symptomatology relative to heavy metals poisoning

- *digestive symptoms*

37 % of the investigated individuals in Ahomey-Gblon, 57 % in Ahomey-Lokpo, 64 % in Houédo-Aguékon and 73 % in Houédo-Gbadji presented abdominal pains. The signs were essentially colics.

- *Neurological symptoms*

62 % of the investigated in Ahomey-Gblon, 42 % in Ahomey-Lokpo, 85 % in Houédo-Aguékon and 76 % in Houédo-Gbadji presented more or less neurological disorders such as :sleep disorders, shivers or headaches.

- *Reproduction symptoms*

The sign looked into was: «Did you have at least a miscarriage or stillborn children?» The result was the same everywhere. Sometimes there were two miscarriages and stillborn. Only a relatively recent woman in the area of Ahomey-Gblon and a young woman of Houédo-Gbadji have never had a miscarriage. That of Houédo-Gbadji was operated during her first gesture and can never conceive.

- *Osseous Signs*

25 % of the investigated in Ahomey-Gblon, 42 % in Ahomey-Lokpo, 0 % in Houédo-Aguékon and 0 % in Houédo-Gbadji presented pains in the bone. The signs were essentially irritations and osseous pains.

- *Signs connected to mental retardation*

We found an appearance of "mental retardation" with some children. For the children, the data do not allow concluding on a mental retardation, but these children, on average in their twelve, had dismissed from school because of poor performance.

- *Renal signs*

28 % of the investigated in Ahomey-Lokpo complained of disorders connected to micturation, 0 % in Houédo-Aguékon and 0 % in Houédo-Gbadji presented disorders connected to the renal system according to their statements. It essentially implied difficulties to urinate.

- Other

We found asthenia with 14 % of the cases generally, some cases of high blood pressure, sight impairment and excessive perspiration.

Stomach pains and abdominal distress, sleep disorders and nervous issues, osseous pain, difficulty to urinate, miscarriage or stillborn child, retardation at school, excessive perspiration were the recorded symptoms among the population

This information could be a matter of symptoms of diverse diseases, that's why it is important to proceed in tests of blood and urine to search for biological signs indicating poisoning with heavy metals.

Connection between blood lead/blood cadmium and biochemical parameters

Table 2 presents the results of blood lead, blood cadmium and biochemical variables.

Table 2. Connection between blood lead/blood cadmium and biochemical parameters.

Parameters	blood lead (µg/L)	Blood cadmium (µg/L)	Glycemia (g/L)	Uremia (g/L)	Creatininemia (mg/L)	Uricemia (mg/L)	Total Cholesterol (g/L)	S GOT (UI/L)	S GPT (UI/L)
Reference valuese	< 40	< 5	0.70-1.10	0.10-0.40	8-14	30-57	1.50-2.50	< 46	<49
m ± SD	44,15 ± 18,66	3,37 ± 2,05	0,73 ± 0,09	0,20 ± 0,05	10,90 ± 1,81	45.13 ± 12.99	1,63 ± 0,37	24,97 ± 18,15	20,71 ± 9,47

The values of the uricaemia recorded with the investigated were sometimes above to the permissive value which is 30-57 mg / L. The extreme values were 58, 60, 77 and of 80 mg / L and the mean value was 45.13 ± 12.99 mg / L. For transaminases which are indicators of poisoning or eliminating hyperactivity of the liver, the extreme values were recorded at the level of an individual whose blood lead was of 62.5 µg / L. At this level were recorded 110 UI / L for S GOT and 51 UI / L for S GPT. Set apart these few observations, no significant variations were recorded with the biochemical parameters within the investigated.

Relation between blood lead / blood cadmium and urinary analyses

The relation between blood lead / blood cadmium and urine tests are represented in Table 3.

Table 3. Connections between blood lead / blood cadmium and urine tests.

Villages	Blood Pb (µg/L)	Blood Cd (µg/L)	Glucose (mg/dL)	Proteins (mg/dL)	Calcium (mg/L)	RBC (RBC c/µL)	WBC WBCc/µL)	Bilirubins (mg/dL)	pH
Standard m ± SD	< 40 44.15 ± 18.66	< 5 3.37 ± 2.05	0 0	0 13.55 ± 36.29	194.5 ± 138.7	0 47.4 ± 95.9	0 37.10 ± 21.28	0 0.06±0.17	5.0 6.88 ± 1.06

The results of analyses of the urine did not show significant anomalies, but the excretion of calcium was sometimes very alarming with certain individuals, especially in the localities where the exposure was high. Also, there was presence of blood in the urine in forms of red blood cells and leukocytes which could be linked to nitrites poisoning or infection with parasites. Indeed, the bilharziasis is a parasitosis which has as one manifestation the presence of the blood in the urine. Moreover, it was reported that cases of bilharziasis were frequent in the investigated localities.

Relation between blood lead and sideropenic anemia

The relation between blood lead and sideropenic anemia is summarized in Table 4.

Table 4. connection between blood lead and sideropenic anemia.

Parameters	Blood Pb (µg/L)	Hb (g/dL)	Serum iron	Binding	Saturation

			(mg/L)	Capacity (mg/L)	Coefficient (%)
Reference values	< 40	14	0.60-1.60	2.5-4.0	25-45
m ± SD	44.15 ± 18.66	13.02 ± 1.47	0.81 ± 0.09	6.23 ± 0.05	0.13 ± 1.81

The results of these analyses showed that there was a benign anemia in areas with high exposure, as well as in Sô-Ava. The presence of blood in urines is a track to be explored in order to explain this anemia, but also the level of serum iron.

Relation between blood lead / blood cadmium and Blood Count

The relation between blood lead / blood cadmium and Blood Count is summarized in Table 5.

Table 5. connection between blood lead / blood cadmium and Blood Count.

Parameters	Blood Pb (µg/L)	Blood Cd (µg/L)	Hb (g/dL)	RBC 10^6	MCV	WBC 10^3	PN (10^3/µL)	PE (10^3/µL)	PB (10^3/µL)	Lym (10^3/µL)	M (10^3/µL)	Plts(10^3/µL)
Reference values	< 40	< 5	14	10^6		0^3						
m ± SD	44.15 ± 18.66	3.37 ± 2.05	13.02± 1.47	4.77± 0.53	84.62± 6.50	5.30± 1.41	2.21 ± 0.68	0.35 ± 0.32	0.003 ± 0.013	2.4 ± 0.85	0.34 ± 0.13	241.8± 66.9

According to the results, 80 % of the investigated presented a benign anemia without other etiology found rather than the poisoning with lead and cadmium, the malaria not having been investigated nor other parasites. The average hemoglobin was 12.8 g / dL in areas with high exposure and 13.02 g / dL average in all localities. Given the reduced number of studied cases, this difference is not interpretable. There was no significant difference with blood count.

Exposure to nitrites and urinary infections

Table 6. percentage of the appearance of blood, the leukocytes, the nitrites and the proteins in urine of investigated individuals exposed to xenobiotics (n=31 people).

Parameters	Blood	Proteins	Nitrites	Leukocytes
% positives	43 %	45%	85%	40 %

Urine analysis revealed the presence of nitrites (85%), appearance of blood with 43 %, leukocytes with 40 %, glucose with 0 % and proteins with 45 % of the investigated. These symptoms added to the results of blood parameters are proofs of an exposure to nitrates and an entero-bacteriological infection.

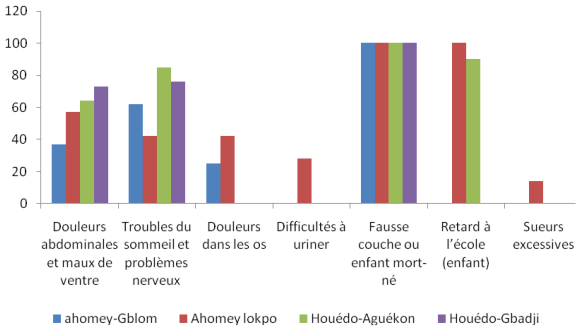

Fig. 2. Distribution of the symptoms within the investigated population.

Lead and cadmium were not the only xenobiotics to which the population of Sô-Ava was exposed. They

were exposed to nitrates and its by-products as well. The evaluation of the toxic effects of these substances on the health of the residents of this locality did not show a significant disturbance at the level of the biochemical and hematologic analyses, in spite of the renal inflammation observed with 80 % of the investigated at the level of the urine analysis. Before drawing any conclusion, it is necessary to carry out an experimental study on animals.

Discussion

Given the fact that exposure to cadmium do not present a real risk within the investigated people, the discussion of all these results focuses on lead content in water and in the blood for the determination of the signs connected to the lead poisoning (Titon, 2010). The blood lead (on total blood) is the best indicator of recent exposure to lead when the exposure is stable (Casas, 2005). This is the result of a one-off measure, not measuring the total load of lead within the body (Symanski and Hertz, 1995) away from any contact with lead, it underestimates the lead pool; during days which follow a massive contamination, it surpasses it. It rises from the beginning of the exposure; it varies according to the peaks of exposure to reach a state of equilibrium three months after the beginning of the exposure (Beauchamp, 2003). The blood lead of the individuals who were not exposed to a specific lead source was lower than 40 µg / L. A variation of more than 10 µg / L between 2 successive blood lead corresponds to a significant evolution (Symanski and Hertz 1995).

As a general rule, the blood lead in men is 80 µg / L and 63 µg / L in women. The infantile lead poisoning - the disease which should be declared - is defined by a blood lead higher or equal to 100 µg / L in an individual below 18 years old (Boutron and Patterson, 1983). Lead poisoning is defined by the observation of a blood lead higher or equal to 40 micrograms per liter (Symanski and Hertz, 1995). The consideration of this disease is made difficult because it is a sort of disease with few specific symptoms. It can thus go unnoticed easily. Only the analysis of lead in blood can assert it (Titon, 2010). The results showed that there was some lead in the blood of the investigated people and this is connected to certain bio-indicator factors of toxicity. This lead analysis was crossed with the data of inquiries on metals characteristic pathologies recorded and the level of contamination of investigated subjects. The level of contamination with lead and cadmium is known by the identification through conversations, analyses of toxicity bio-indicator parameters. The mean blood lead was 49.7 ± 23.53 µg / L in localities with high pollution and of 38.61 ± 13.80 µg / L in the control locality, which is Sô-Ava center. In exposure areas, the moderate average for cadmium was 3.884 ± 2.80 µg / L against 2.86 ± 1.30 µg / L in Sô-Ava. Thus the blood cadmium did not exceed the permissive value while the blood lead widely exceeded the permissive value in the highly exposed areas, which is not the case for Sô-Ava center. Yet, the blood lead threshold effect concentration where no clinical signs are observed is connected to the daily allowed intake (DAI) which is reached with a blood lead of 20 µg / L, that is DAI of 3.6 µg / kg (WHO, 1986). Indeed, the consumption by man of food contaminated with lead which can cause a blood concentration of 10 µg / dL From a blood concentration of 10 µg / dL of lead, there is inhibition of enzymes responsible for the synthesis of heme. This inhibition is total with90 µg/dL (Landrigan et odd, 1997). It is considered that the blood lead increases from contributions of 5 µg / kg / j through food intake. It is known that a blood lead lower than 60 µg / dl could reduce intelligence and cause changes in the behavior pattern (Vaidya and Rantala .,1996). The same level of lead could produce a specter of toxicity in which the clinic symptoms such as encephalopathy, renal insufficiency and anemia had their sub-clinic counterparties in a decrease in intelligence, an altered functioning of renal tubules, high rates of protoporphyrin and osseous disorders (Silbergeld *et al.,* 1988). Our results showed that there were signs of toxicity according to the information supplied by the investigated people with regard to the clinical symptomatology. All the signs known for lead poisoning were counted: anemia, abdominal pains,

constipation, drinking disorders, anorexia, vomiting, behavior disorders, apathy or irritability, hyperactivity, attention deficit and sleep disorders (Roos *et al.,* 2006), bad psychomotor development, decrease of the cognitive performances and especially miscarriages (Symanski *et al.,* 1995; Silbergeld *et al.,* 1988). In the study area and particularly in areas with intense exposure, the recurring signs after inquiries were anorexia, digestive disorders and stillborn or miscarriages which are the archetypal signs of poisoning with lead. Besides, the biochemical, hematologic and urine analyses of investigated essentially urea, creatinine, transaminases, total cholesterol, urine calcium, blood count, serum iron, hemoglobin, glycemia and transaminases showed variability with regard to the reference values. Urea and creatinin are wastes of the metabolism which give information about the purifying function of the kidney, the main target of heavy metals (Casas, 2005). Any nephrotoxic substance (lesion of tubules) even in the short term may modify the blood concentrations of these two wastes in the sense of an increase (they are reabsorbed in the damaged tubules and return to the blood, resulting in their increase), (Roos *et al.,* 2006). The results showed that most of the values of the variables were within the normal ranges apart some isolated cases. In respect of transaminases, the permissive value (49 g/l) was exceeded only at the level of a man from Houédo-Gbadji. The latter has moreover a blood lead above the permissive limit.

The results of analyses of urine did not show significant anomalies but the excretion of calcium was sometimes very alarming with certain individuals especially in the localities with high exposure. Cadmium and lead are in competition with calcium in the body. Calcium just like iron are in competition with heavy metals (Pb, Cd, Hg): any excessive presence of these trace elements in urine (or stools) is a proof of the action of heavy metals which would thus occupy the site of these elements on the sites of the enzymes making the enzymes inactive (Roos et al ., 2006). As a result, the urinary excretion of the latter is a sign of poisoning with lead and cadmium. Also excretion of iron results in iron deficiency anemia (Gonnot, 2009). The anemia through poisoning with lead results from the decrease of the life expectancy of erythrocytes and from the reduction in the synthesis of heme by enzymatic inhibition. Furthermore, the fixation of lead to thiols and phosphates of the membranes triggers an increase of the membrane fragility and an alteration of its permeability. This effect, followed by an inhibition of the active transport by inhibition of ATP Na +/K + dependent, acts on the viability of erythrocytes (Gonnot, 2009). Lead inhibits 3 enzymes: *delta-aminolevulinic acid dehydratase* (*ALAD*), coproporphyrinogen oxidase and *ferrochelatase*. The result is respectively an accumulation of aminolevulinic acid (AA), an increase of coproporphyrinogen and a decrease of the quantity of heme formed coupled with an increase of the rate of protoporphyrin (Murozumi *et al.,* 1969). Protoporphyrin in excess takes the place of the heme in hemoglobin and binds zinc on the site usually occupied by iron. By negative feedback via the heme, lead also affects the activity of the ALA synthetase and the synthesis of the globinic portion. As a consequence, the urinary excretion of ALA and coproporphyrin is increased; protoporphyrin and coproporphyrinogen accumulate in erythrocytes. A decrease of the rate of hemoglobin is observed as soon as the blood lead reaches 400 µg / l, but the anemia is generally the fact of the massive contaminations (blood lead > 800 µg / l, 3.9 µmol / L) or of a deficiency related to iron therapy (particularly with children). Lead anemia is generally moderated and typically normocytic, sideroblastic and discreetly hypersiderimic anemia. With children it is often hypochromic and microcytic, because iron deficiency is frequently associated with it (Gonnot, 2009).

In the case of our study, the low mean blood lead of the investigated could not explain the occurrence of anemia. Studies with more important samples, with clear inclusion criteria are necessary to establish the link between anemia and blood lead. The other

possible cause for the presence of blood in urine is parasitic infection. Indeed, bilharziose is a parasite that causes an appearance of blood in urine. Moreover, the microbiological analysis of urine showed that 82 % of the investigated suffer from bilharziose. In fact, *Foy* and Nelson (1963) reported that the anemia in the early stage of schistosomiasis is probably due to blood loss. Walker *et al.,* (1954) reported that *S. mansoni* infection did not significantly lead to blood loss or to iron-deficiency anemia in South Africa. Taha *et al.,* (1957), from Egypt, reported anemia in patients with bilharzial colonic and rectal polyps (TA). Farid *et al.,* (1966) for the first time reported blood loss in two patients with bilharzial colonic and rectal polyps, and later confirmed these studies in another 10 patients (1967)**.** They emphasized that, though the mean daily blood loss of 12.5 ml and iron loss of 3.3 mg may not be high and may not lead to overt anemia, it certainly can lead to depletion of the body iron stores. These authors stressed the importance of the fact that other helminthic infections besides hookworm may in certain geographical areas lead to chronic intestinal blood loss. In respect of *S. haematobium,* Gerritsen et al. (1953), using chemical analysis, measured the urinary blood loss in eight African patients suffering from advanced chronic *S. haematobium* infection. They calculated the urinary blood loss as ranging from 1.3 to 6.1 ml/day. Mahmood (1966) used in vivo 59Fe-labeled red blood cells to measure the urinary blood loss in eight patients infected with *S. haematobium* and reported a loss ranging from 0.44 to 6.0 m!/day. Farid et al. (1968), using the same method, measured the urinary blood loss in nine patients with *S. haematobium* infection and severe hematuria and reported it as ranging from 2.6 to 126 mI/day with a mean daily iron loss ranging from 0.6 to 37.3 mg. They noted that though the urinary blood loss in *S. haematobium* infection can be severe it usually lasts only for short periods and is not a constant steady loss as in hookworm infection. Other workers from Egypt (Ata, 1961) have repeatedly noted that the severest iron-deficiency anemia occurred in farmers with obvious hematuria caused by *S. haematobiurn* and combined *S. mansoni* and *A.duodenale* infections. In concluding it is important to understand that human parasites may cause anemia by different mechanisms than simply direct blood loss and this could explain the anemia cases reported in the present study.

Additionally cadmium could disrupt the absorption of iron by competing with its carrier. Moreover, in the case of human beings we noticed that women suffering from an iron deficiency absorb up to 20 % of ingested cadmium because cadmium is transported by hemoglobin even if once in the liver, its action is often inhibited because it is bound to metallothioneines. Yet it is thanks to iron that the formation of red blood cells takes place. Consequently, this iron deficiency results in an anemia called iron deficiency anemia which arises when the production of hemoglobin in the blood stops (Gonnot, 2009). For the total cholesterol, all the values recorded were within permissive ranges (2.5 g/l).

Conclusion

At the end of the study of the toxic effects of the xenobiotic on the health of the populations of the lakeside municipality of Sô-Ava, we can notice that the works did not show a significant disturbance at the level of biochemical and hematological analyses. However, urine analysis showed a renal inflammation with 80 % of the investigated. Lead and cadmium were not the only xenobiotic to which the population of Sô-Ava could be exposed. More thorough studies will allow us to confirm these results. Anyway, at the present stage, the results enough worrisome so that a monitoring of the pollution of the water of swamps and the drilling consumed by the populations of Ahomey and Houédo start with a series of raising awareness to protect public health.

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