

Full Length Research Paper

Oxidative stress in human due to metallic traces elements bioconcentration in three coastal villages near phosphate treatment factory in Togo

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Abstract

Human contamination by toxic heavy metals, due to pollution by phosphate waste rejection from SNPT factory in Kpémé, concern also the neighbouring villages. Through this study, we wanted to determine the level of the bioaccumulation of cadmium and lead and its impact on human oxidative stress in three villages near Kpémé. The quantity of Cd and lead in human blood were determined in Gbodjomé (control area), Agbodrafo, Goumoukopé and Aného (neighbouring villages of the factory) by atomic absorption spectrophotometry, as well as some biomarkers of oxidative stress (MDA and FRAP). The results show human contamination by heavy metals, causing a lipid peroxydation and a decrease of Ferric Reducing Activity of Plasma. The contents of lead in blood were more important in relation to those of the cadmium. The concentrations variation was as follows: $87.27 \pm 12.57 \mu\text{g/L}$ to $327.88 \pm 32.13 \mu\text{g/L}$ for lead and $01.01 \pm 0.11 \mu\text{g/L}$ to $11.68 \pm 2.38 \mu\text{g/L}$ for cadmium. Generally speaking, the heavy metals contents in the blood and the lipids peroxydation were significantly more important in Agbodrafo, Goumoukopé and Aného areas in relation to Gbodjomé area ($P < 0.0001$). The antioxidant power of the blood plasma was on the contrary significantly lower in the pollution area in relation to the control area ($P < 0.0001$). It results of this study that environment pollution caused by SNPT factory affects as well surrounding villages' populations.

Keywords: SNPT, cadmium, lead, bioaccumulation, blood, MDA, FRAP.

INTRODUCTION

Phosphate waste rejections in Kpémé by "Société Nouvelle des Phosphates du Togo (SNPT)" factory, affect

first the surrounding areas (Mélila et al., 2012a). Indeed, the effluents thrown out by this factory in the sea at Kpémé are drained on both sides upstream (in Agbodrafo) and downstream as far as Aného through Goumoukopé. Those effluents contain metallic trace elements such as cadmium and lead which are

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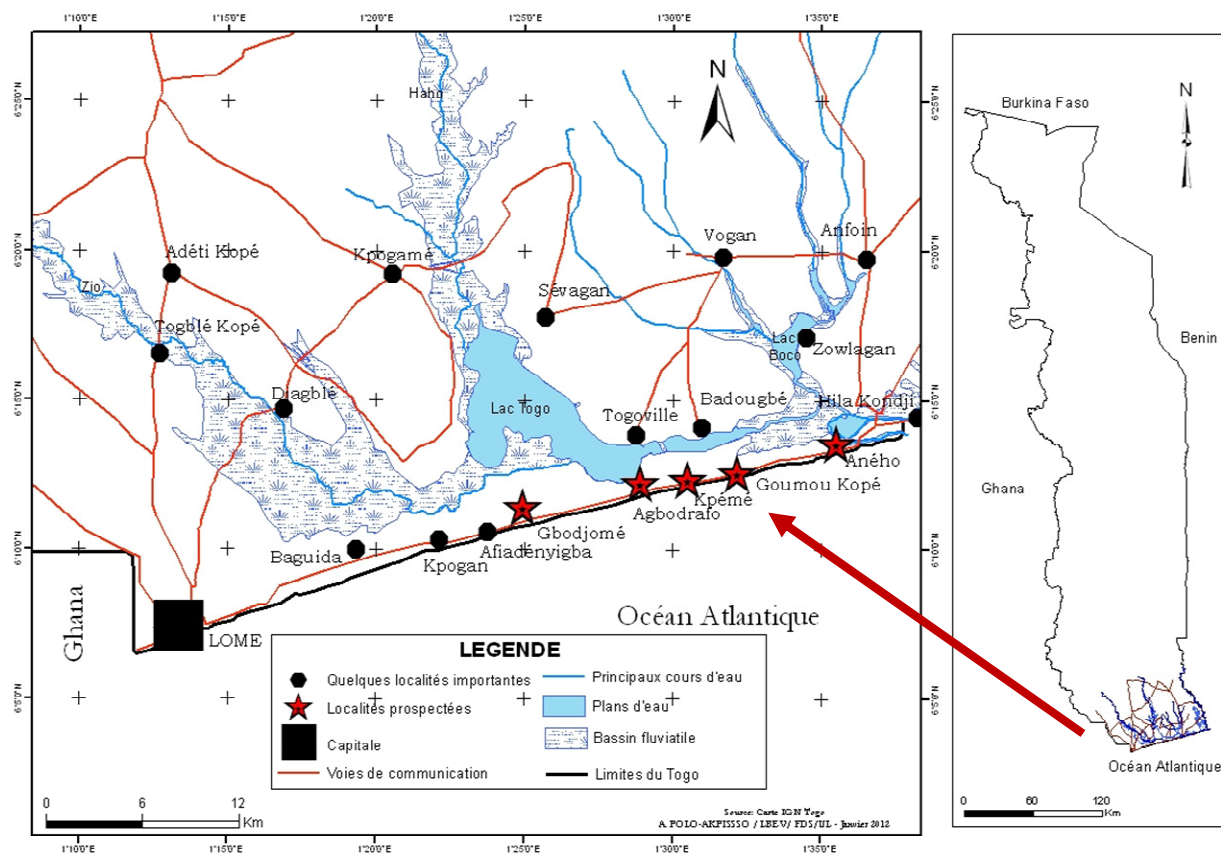


Figure 1. Indication of the study areas concerned by the phosphate waste rejection

bioaccumulated by marine fauna species in Kpémé as well as in surrounding areas (Gnandi et al., 2006). Moreover, through the dust rejected in the atmosphere by this factory, agricultural and market garden produces grown near the factory are also infected by these metals (Gnandi et al., 2009). Human may be infected by trophic ways through food chain but also, by skin and respiratory ways (Mélila et al., 2012b). However, although studies have proved Togolese coast people exposure to toxic heavy metals, no scientific study has undertaken to evaluate the rate of these metals in the blood and the impact of the bioaccumulation of these metals on human. And yet, trace metallic elements constitute the main oxidative stress factor in mammals by the production of free radicals and the cardiovascular diseases (Servais et al., 2003; OMS, 2004; Yildirim et al., 2011; Mélila et al., 2012a). The heavy metals oxidize lipids and proteins and form deterioration products with high radical activity which produces reactive oxygen species (ROS) (Favier, 1997, 2003). So, oxidative stress is an imbalance for the production of ROS which lead to an increased oxidation of cellular components (Favier and Goudable, 1997; Servais et al., 2003). The aim of this study is to determine the level of human intoxication by Cd and lead, which are

the main toxic heavy metals contained in the food eaten, and the oxidative stress bred by these metals in three villages near Kpémé, area of Togolese phosphate treatment. Thus not only do, we report for the first time Cd and lead contents in human blood in Agbodrafo, Goumoukopé and Aného; but also, the variation of the impact of these metals on oxidative stress in human. For this, we refer to the Ferric Reducing Ability of Plasma "FRAP" and to malondialdehyde (MDA) production, which are identified by studies (Farombi et al., 2007; Legault, 2007; Agbonon and Gbéassor, 2009) as oxidative stress indicators and biomarkers of environment pollution.

MATERIALS AND METHODS

The study surroundings (framework)

The study area is situated in the South side of Lomé - Aného national road, from Gbodjomé village (West) to Aného (East) through Kpémé covering a distance of about 20 km. By its geographic situation, it is part of Lakes prefecture in the maritime region of Togo (figure 1). The essential characteristic of this geographic space is the

presence of the SNPT factory.

Materials

It's about blood samples taken on subjects in the polluted areas of Agbodrafo, Goumoukopé and Aného and in control area of Gbodjomé, situated upstream from SNPT factory about 11 km and which isn't directly affected by this pollution.

Laboratory apparatus and materials

Laboratory reagents such as β -2-mercaptoethanol, Triton X100, hydrogen peroxide, Tris-HCl buffer, chlorhidric acid (HCl), Ethylene-diamine-tetra-acetic acid (EDTA), provided by LABOSI-OULSHY-LE-CHATEAU-France; n-butanol and absolute ethanol, Bradford kit, Bovine Serum Albumin (BSA) which are provided by Biolabo S.A. FISMES (France); Natrium dodecyl sulfate (NDS), Malondialdehyde (MDA), Thiobarbituric acid, 1,1-diphenyl-2-picril hydrazine (DPPH), 2,4,6-tripyridyl-S-triazine (TPTZ) which are provided by SIGMA Chemical (St Louis, USA); Potassium chloride (KCl) comes from Fisher Bioblock Scientific (France), Cadmium and Lead standard solutions come from Merck KGaA (Durmstalt, Germany).

Sampling

The study was designed to compare the parameters of people of similar ages ($P = 0.3732$) included between 18 and 50 years old with an average of 27.66 ± 6.84 years in Gbodjomé (control); 26.87 ± 7.02 years in Agbodrafo; 26.97 ± 6.81 years in Goumoukopé and 27.24 ± 6.32 years in Aného (polluted areas) having similar alimentary habits and who evolve in the same circumstances. So, an investigation has allowed to identify and to select four groups of 100 persons, men and women non-smokers whose animal proteins usual source is sea produces taken in each locality. The persons are chosen at random and the exclusion criteria concerned contamination sources other than food consumption contaminations from the pollution induced by phosphate treatment factory of Kpémé.

About 10 ml of blood were collected from each subject on an empty stomach since 12 hours (night fast) after a shower (without work clothes for the factory agents), and distributed into two tubes of 5 ml guaranteed without lead nor cadmium (included cork), one with anti-coagulant (EDTA) and another without anti-coagulant and conveyed in an icebox at an ambient temperature to analysis laboratory the same day. The collections are performed with precaution relatively to instruments used (needles, tubes, antiseptic corks, gloves) as such a study requires

(Goullé et al., 2005). The blood collected on anticoagulant has served to dose Cd and lead, and the one collected in the tubes without anticoagulant for the dosage of oxidative stress indicators.

Methods

Samples mineralization and traces elements (Cd, Pb) determination

5 ml of each sample added to 2 ml of concentrated sulphuric acid and 1.5 ml of concentrate nitric acid are introduced in borosilicated glass bottle, closed with caps in order to avoid eventual leakage. The bottles are then heat in a double-boiler at 80°C for 2 hours. The product obtain is diluted with distilled water up to 50 ml. The solution obtained is used for an atomic absorption spectrophotometer Varian® reading interfaced with a micro-computer.

Protein and oxidative stress bio-indicators dosage

The samples reserved for this purpose, are centrifuged at 3000 rpm during 15 minutes at the ambient temperature the same day. So the plasma is utilized to determine the MDA concentration and the FRAP activity. Total proteins content of samples has been estimated by Bradford (1976) method with Bovine Serum Albumin (BSA) as standard. Lipids peroxidation (MDA) has been determined by acid substances measurement which reacts in thiobarbituric acid (TBARS) as described by Satoh (1978). FRAP activity is determined by measuring Fe^{2+} ions concentration in the blood as described by Agbonon and Gbéassor (2009).

Statistic analysis

All the results are expressed as mean \pm SEM and analyzed with Graph Pad Prism software version 4, 00. The analysis of the variance has been performed with unpaired Student t test. $P < 0.05$ is considered to be significant.

RESULTS

Quantity of Cd and lead in human blood

Cadmium and lead content in the blood of human from Gbodjome, Agbodrafo, Goumoukope and Aneho are denoted in table 1. Quantity of Cd and lead in human blood in Agbodrafo, Goumoukopé and Aného (pollution areas) are more increased than those of Gbodjomé and

Table 1. Cadmium and lead levels in blood depending on localities

Localities	Cd ($\mu\text{g/l}$)	Lead ($\mu\text{g/l}$)
Gbodjomé (control)	01,01 \pm 0,11	87,27 \pm 12,57
Agbodrafo	07,73 \pm 1,23 ***	165,66 \pm 19,92 ***
% of difference	665	89
Goumoukopé	11,68 \pm 2,38 ***	327,88 \pm 32,13 ***
% of difference	1056	275
Aného	03,59 \pm 1,08 ***	167,31 \pm 21,57 ***
% of difference	255	91

Results are expressed as mean \pm ESM of 100 samples.
Different significantly: *** P < 0.0001.

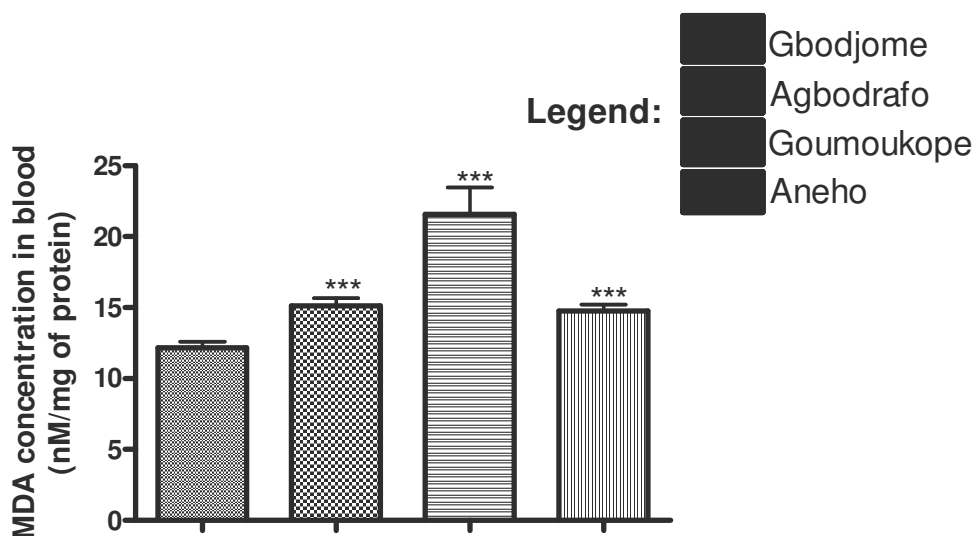


Figure 2. Level of malondialdéhyde (MDA) in human blood according to localities. Values are expressed as mean \pm ESM of 100 samples. Significantly different vs control: *** P < 0.0001.

more increased than limit values (Goullé et al., 2005). Generally, lead content are higher than cadmium's (table 1).

Malondialdehyde (MDA) level

MDA level has significantly increased in the blood of subjects from Agbodrafo, Goumoukopé and Aného ($p < 0.0001$) in relation to Gbodjomé, with a great increase in Goumoukopé. The lipid peroxidation percentage increase is 24, 05% in Agbodrafo; 77.09% in Goumoukopé and 21.18% in Aného (figure 2).

Ferric reducing antioxidant power of plasma

FRAP values have significantly decreased in the blood of subjects from Agbodrafo, Goumoukopé and Aného ($p < 0,0001$) in relation to Gbodjomé, with a more noticeable

decrease in Goumoukopé. The FRAP value decreased percentage in the blood is 50.18% in Agbodrafo; 74.57% in Goumoukopé and 35.39% in Aného (figure 3).

DISCUSSION

The industrial activities of SNPT in the treatment of Togolese phosphate induce air and marine pollution on Togolese coast at Kpémé and in neighbouring localities (Gnandi and Tobschall, 1999; Tchangbédji et al., 2003; Gnandi et al., 2005). Recent studies showed that fishing and agricultural produces eaten by human in the polluted areas are infected by toxic heavy metals from industrial waste (Gnandi et al., 2006; Mélila et al., 2012b). Other studies have shown the impact of this pollution on marine biodiversity (Mélila et al., 2012a). However, the evaluation of different concentrations of metallic trace elements such as Cd and lead in the blood of human who lives in such a polluted atmosphere and eats infected

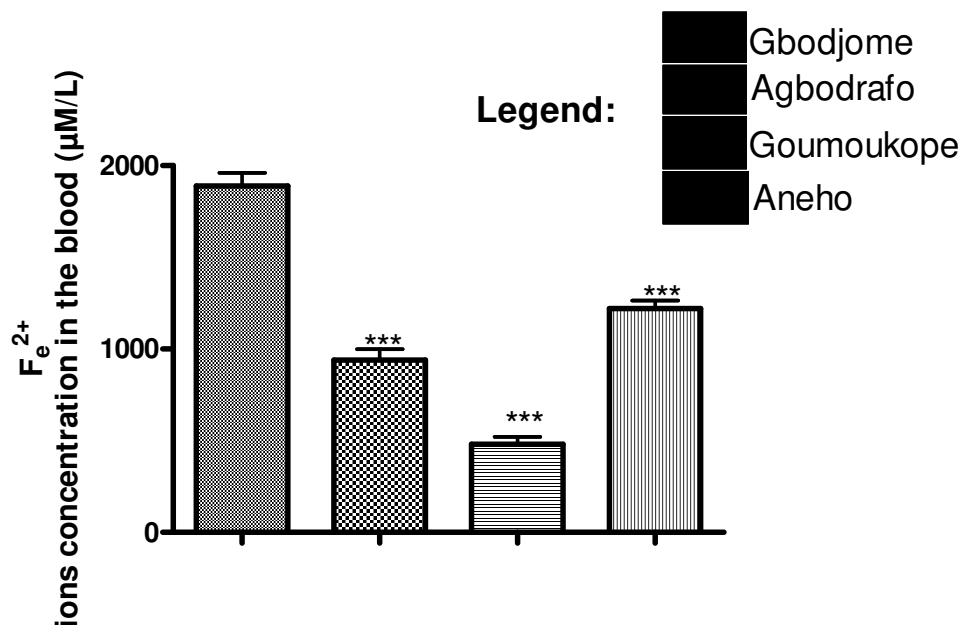


Figure 3. Fe²⁺ ions concentration (FRAP value) in the blood according to localities. Values are expressed as mean \pm ESM of 100 samples. Significantly different vs control: *** P < 0.0001.

foods has not been done in areas near Kpémé yet. Also, there is no datum of human, on oxidative stress biomarkers, bioindicators of environmental pollution (Farombi et al., 2007) and the consequence of toxic heavy metals bioaccumulation (Yildirim et al., 2011) in the polluted areas, especially in Agbodrafo, Goumoukópé and Aného, localities near Kpémé. Our results denote that Cd and lead quantities in human blood are increased in subjects living in Agbodrafo, Goumoukópé and Aného, in relation to Gbodjomé. This led to a lipids peroxidation as well as a decrease of antioxidant power of blood plasma.

The Cd and the lead concentrations in the blood are more increased in Agbodrafo and Goumoukópé because of their position in relation to the SNPT factory. Indeed, Agbodrafo and Goumoukópé are the bordering villages of Kpémé. In such a position, human can be infected by the consumption of sea produces which accumulate these heavy metals through the mud and wastes which are rejected (Gnandi et al., 2006; Mélila et al., 2012a) and by the consumption of farm produces grown near the factory which bioaccumulated trace metallic elements through dust rejected in the air (Gnandi et al., 2009). Indeed, previous studies (Gnandi et al., 2006, 2009; Mélila et al., 2012a) showed that metals concentrations in sea produces, vegetables and farm produces in these areas are higher than the norm admitted for consumption (OMS, 2004), denoting a contamination of fishes and farm foods by heavy metals. These food consumers can then be vulnerable to the toxicity of heavy metals. The high bioaccumulation of Cd and lead by human in

Agbodrafo and Goumoukópé could also be due to breeding and the contact with an atmosphere polluted by dusts and gas rejected by the Togolese phosphate ore treatment which contains these metals (Mélila et al., 2012b). In Aného, Cd and lead concentrations are lower than those of Agbodrafo but mostly than those of Goumoukópé; nevertheless, Cd and lead levels in blood remain higher than control area (Gbodjomé). These data show that Aného's people are also infected by the heavy metals through contaminated food eating. These results also show that this area is less affected in relation to the bordering localities of phosphate ore treatment area and confirm the previous results. Indeed, Gnandi et al. (2006) had indicated that marine pollution from wastes rejection and phosphate mud decreases progressively as we go away from the factory. The residents of Aného might be eating fishing produces less infected than people from Agbodrafo and Goumoukópé. The high level of pollutants concentrations in Goumoukópé might be due to the fact that mud and wastes are rejected there in addition to rejection in Kpémé. Also, in relation to his downstream situation, the wastes and the mud discharged in Kpémé are drained towards this locality and so, human might be eating fishes more infected. Generally speaking, blood concentrations in Cd and Pb in Agbodrafo, Goumoukópé and Aného are superior to limit level authorized by World Health Organization (Goullé et al., 2005); this fact denotes availability of the body to the bioaccumulation of trace metallic elements such as Cd and lead. In our study, the lead content is more increased compared with the cadmium content, this is explained by the fact that the

foods eaten concentrate more lead than Cd as the previous studies have shown (Gnandi et al., 2006; Gnandi et al., 2009), but also by the fact that wastes, mud and dusts might contain lead at high tenors than Cd ones. This bioaccumulation of Cd and lead from infected foods consumption at the concentrations superior to limit level authorized by World Health Organization can intensely alter human health (OMS, 2004; Gnandi et al., 2008). Indeed, Cd and lead are toxic elements after quicksilver and the target organs are kidney, liver, the nervous system and blood. These metals can also be sources of cardiovascular diseases and cancer (OMS, 2004). Moreover, the presence of toxic heavy metals in the body are sources of free radicals inducing the ROS production (Servais et al., 2003; Yildirim et al., 2010; Pandey et al., 2003) and then the oxidative stress (Nordberg and Arner, 2001; Farombi et al., 2007; Yildirim et al., 2011; Mélila et al., 2012b). Our results have shown a significant raise of lipid peroxidation in the blood in the polluted areas (Agbodrafo, Goumoukopé and Aného) compared to the control area (Gbodjomé). This increase can be attributed to the accumulation of Cd and lead in the blood as our data indicate. Indeed, the accumulation of these metals catalyses ROS which are able to damage tissues such as DNA, proteins and lipids (Livingstone, 2001; Pandey et al., 2003; Farombi et al., 2007; Yildirim et al., 2011). The increase of blood level of MDA and the decrease of ferric reducing antioxidant power (FRAP) of plasma noticed in Agbodrafo, Goumoukopé and Aného areas compared with Gbodjomé area, show a struggle of the body against oxidative stress extension induced by the heavy metals. This pathologic condition is more acute in Goumoukopé area where we notice more the increase in MDA (74%) and more the decrease of antioxidant potential (77%) in people who form the subject of our study. These results are in accordance with Agbonon and Gbéassor (2009) ones, who have noticed a perceptible decrease of total potential antioxidant of plasma with an increase of MDA level in the blood on Wistar rats poisoned by CCl_4 .

CONCLUSION

The previous studies showed that environment pollution of Togolese coast by wastes of SNPT factory concerns not only Kpémé area but also the neighbouring localities. Thus, the marine fauna bioaccumulated heavy toxic metals from the effluents rejected there. The farm produces grown near this factory too bioaccumulated them from the gas and dusts that are rejected in the atmosphere. This phenomenon is noticed in Kpémé area as well as in Agbodrafo, Goumoukopé and Aného. This situation makes man who is at the end of the trophic chain vulnerable in relation to the heavy toxic metals contained in the food consumed. This study shows that human body presents availability to the bioaccumulation

of trace metallic elements according to the environment pollution extent. The high concentrations of Cd and lead in the blood of subjects in Agbodrafo, Goumoukopé and Aného areas compared with Gbodjomé area are due, on the one hand to the consumption of infected foods from rejected wastes in the sea and in the atmosphere, and on the other hand to direct contamination by respiratory tract and by cutaneous absorption because of SNPT industrial activities. This bioaccumulation of Cd and lead by exposed subjects is more acute in Goumoukopé, high in Agbodrafo and less high in Aného, showing that this contamination is due to the SNPT industrial activities. The impacts of the industrial pollution on human decreased progressively when we go away from the factory. This metals contamination to human could be the cause of the lipids peroxidation extension and of the significant decrease of total potential antioxidant of blood plasma observed in Agbodrafo, Goumoukopé and Aného compared with Gbodjomé indicating an induction of oxidant stress to exposed persons. This study furthermore shows that people of neighbouring localities of Kpémé also, are affected by the pollution caused by Togolese phosphate ore treatment factory.

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