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Research Article

ARSENIC CONTENT IN TISSUES, FUNGUS COMBS, HERBS, TERMITE MOUNDS AND SOILS OF TERMITES OF THE GENUS *MACROTERMES* AND ASSESSMENT OF THE HEALTH RISK ASSOCIATED WITH THEIR FOOD USE IN THE INDUSTRIAL ZONE OF THE SOCIETE NOUVELLE DES PHOSPHATES DU TOGO (SNPT)

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ABSTRACT

Arsenic is present in different environmental matrices of the phosphate mining and processing area of the Société Nouvelle des Phosphates du Togo; including termite tissues, termite waste, termite mounds, termite Fungus combs, soils and nearby herbs. Arsenic levels vary across matrices, with termite waste having the highest concentration (approximately 27.01 mg/kg) compared to the outside herbs, which have the lowest. Phosphate mining sites, including Hahotoé-Kpogamé and Akoumapé, have higher levels of As contamination in soils and termite tissues compared to the phosphate processing site. A positive correlation is observed between As levels in termite tissues and termite waste; suggesting an ability of termites to eliminate arsenic via their waste. Regarding health risks, the hazard quotient (HQ) for arsenic in termites exceeds 1, and for non-threshold effects, the excess individual risk (EIR) assessment gives EIR > 10^{-4} in both adults and children; which would mean that termite consumption poses a significant health risk, including cancer risks for human populations, particularly children. However, it should be noted that the estimation in this study includes total As (organic and inorganic). Since the toxicological reference values were calculated on the basis of inorganic arsenic, which presents more hazard and whose fraction represents 10% of total As, the daily exposure dose values for adults and children represent $1.40.10^{-4}$ mg/kg/d and $1.02.10^{-4}$ mg/kg/d of inorganic arsenic, respectively. These doses will then become lower than the toxicological reference value (TRV) with the consequence of hazard quotient (HQ) lower than 1 and resulting in low risks of carcinogenic effects. It is therefore necessary to specifically determine the inorganic fraction to refine the risk assessment and to extend this study to other trace elements in the prospected area.

Keywords: Termites of the Genus *Macrotermes*, Industrial Zone of SNPT, Arsenic, Health Risk.

INTRODUCTION

Termites are insects belonging to the order Blattoptera. They are widely distributed throughout the world, particularly in tropical, subtropical and semi-arid regions (Eggleton, 2000; Trabi *et al*., 2020). They are considered consumers of dead wood and their main damage is to processed wood. In recent decades, special attention has been paid to the study of termites because they participate in many ecosystem services including the decomposition of organic matter, the evolution of the physicochemical structure of soils (Mora *et al*., 2005; Trabi *et al*., 2010) and also because of the important place they occupy in tropical ecosystems, given their economic interest (Kotoklo, 2010). These termites also have important nutritional values for

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the rapid development of young poultry. The winged forms of some species, dried directly in the sun and lightly fried in their own fat (Ekpo, 2007) are highly appreciated by the populations. The work carried out by Niaba *et al*. (2018) on the nutritional potential of the winged reproductive of the termite *Macrotermes subhyalinus* captured in Abobo-doumé, Ivory Coast revealed that *Macrotermes flour subhyalinus* is an important source of protein, fat with a high energy value of 581.5 (kcal/100g DM).

In Togo, some studies carried out on termites have focused on the specific diversity of termites (Gbenyendji *et al., 2011),* termite stands and their impacts on plant resources (Anani *et al*., 2008; Anani *et al*., 2010) without being interested in the chemical quality of termites. Environmental pollution problems caused by anthropogenic activities are major issues at the national level and taken into account in sustainable development policies. For several decades, the scientific community has paid great attention to the dispersion of pollutants in the environment. Trace elements are one of the main environmental pollutants due to their high toxicity, accumulation in living organisms, persistence and dispersion in the environment (Picot, 2012; Aina *et al*., 2012; Salem *et al*., 2014; Badassan *et al*., 2020; Ouro-sama *et al*., 2021).

Hahotoé-kpogamé phosphate mining and processing area in Togo has been the subject of several studies with evidence of the presence of inorganic pollutants in water, sediments and their bioaccumulation in the tissues of plants, fish, mussels and even the impacts of fluorosis on the teeth of populations in the phosphate mining area (Mélila *et al*., 2012 ; Mélila *et al*., 2013 ; Gnon *et al*., 2017; Solitoke *et al*., 2018; Ouro-Sama *et al*., 2019, 2021, 2022). Other work has led to the assessment of health risks associated with the consumption of certain fishery products (Ouro-sama *et al*., 2014& 2021) in particular fish and mussels without being interested in insects which represent an important link in the balance of ecosystems and the contribution of significant nutritional value. However, in this phosphate mining area, the habitats and populations of termites of the genus *Macrotermes* have been identified and these termites are usually collected to feed poultry which feast on these insects. They are also edible to humans, especially the winged forms of some species which, during the rainy season, are captured at night, grilled on embers and crunched like peanuts. This work has a dual objective : firstly, to assess the level of As pollution in several matrices (termite tissues, termite waste, Fungus combs, termite mounds, external soils and external Herbs of the termite mound) and secondly, to assess the health risk linked to the consumption of these termites by populations. As was chosen because of its toxicological characteristics and the health risk it poses to the consumer at the end of the food chain. In addition, as is not only a non-essential element, but it is also a potent poison whose toxicity varies depending on the oxidation state (Qasim, 2015).

MATERIALS AND METHODS

Area of Study

The study area is part of the coastal zone. It is located between the maritime limit of the Exclusive Economic Zone (EEZ) and the continental limit located 50 km beyond the zone of influence of the average tide. This continental limit coincides with the Maritime Region which is located between the latitudes North 6° 01' and 6° 05' and the longitudes East 0° 70' and 1° 40' with an area of 6395 km² or 11.2% of the Togolese territory (MERF, 2007). It is this area that covers the phosphate extraction and processing activities of Hahotoé-Kpogamé. The project area has a subequatorial or Guinean climate, characterized by alternating rainy seasons and dry seasons: a long rainy season from mid-March to mid-July; a short dry season from mid-July to mid-September; a short rainy season from mid-September to mid-November; and a long dry season from mid-November to mid-March. The average annual temperature throughout the area is 27°C. The hottest period of the year is concentrated between the months of February, March and April with temperatures oscillating around 28°C (UNIDO/TGO, 2007). The study sites and geographic coordinates are presented in the Table 1 and Figure 1.

Table 1. Sample Collection Sites and Geographic Coordinats.

Place	Contact details		
	N	E	Alt (m)
Goumoukopé1	06°13'13,7"	001°32'03,0'	14
Goumoukopé2	06°13'16,7"	001°31'59,4"	13
Kpémé1	06°13'04,2"	001°31'28,3"	15
Kpémé2	06°13'02,5"	001°31'26,7"	15
Kpémé3	06°12'36,2'	001°31'01,6'	10
Kpémé4	06°13'12,4"	001°29'34,2"	15
Kpémé5	06°13'13,1"	001°29'36,4	16
Hahotoé1	06°21'11,5	001°23'57,6"	16
Hahotoé2	06°22'14,6"	001°24'35,0"	58
Akoumapé1	06"22'39,1"	001°25'12,6"	59
Akoumapé2	06°24'14,5"	001°26'00,5"	60
Akoumapé3	06°27'06,5"	001°26'57,2	82
Vogan	06°20'57,2"	001°31'04,4	54

Sampling

The study was carried out on 266 samples of 06 different matrices composed of termite tissues (65), termite waste (41), Fungus combs (38), termite mound (40), external soils (41) and external herbs of the termite mound (41). During sampling, the team was equipped with hoes, mauls, laboratory forceps, sterilized plastic bags, sterilized plastic boxes and trays. All these equipments were previously disinfected and dried in the laboratory before use. In addition to these tools, the team had a box of alcohol to regularly clean the forceps that were used for termite collection. The samples considered were taken several times (at least three times) at the level of the identified termite mounds.

For each termite mound, physical parameters such as diameter and height of the termite mound are measured and recorded in a notepad. In addition to these two parameters, images and geographic coordinates are taken respectively using a digital camera and a Garmin type GPS. After recording the physical parameters of a termite mound, it is fragmented using a hoe and blocks of termites are distributed on trays. Each block is shaken vigorously, causing the termites to fall into the trays. The latter are then collected using tweezers and stored in sterilized plastic boxes reserved for this purpose. Each box is labeled directly in the field. Other elements such as soil, termite mound, Fungus combs and herbs are also collected at the same time in sterilized plastic bags. For waste, this is obtained by lightly scraping the internal part of the termite mound. At the Laboratory of Organic Chemistry and Natural Substances of the University of Lomé, samples are stored in a freezer at a temperature of -20 °C.

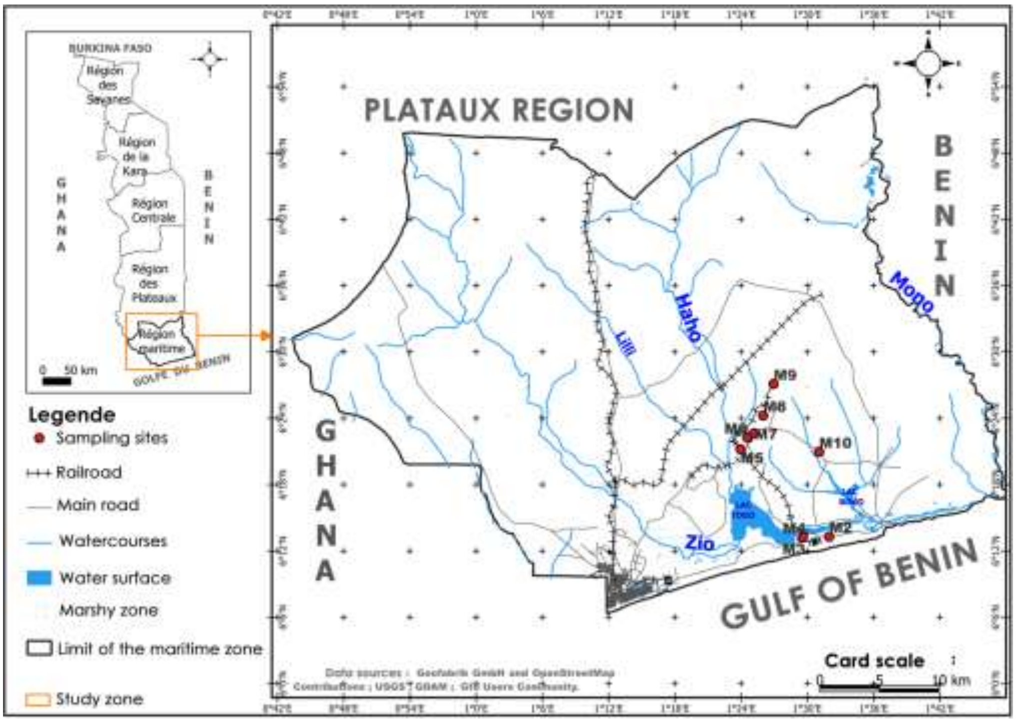

Figure 1. Map Showing the Locations Surveyed.

Mineralization and Solubilization and Dosage

The analysis of the different samples was carried out at the analysis laboratory of the Togolese Institute of Agronomic Research (ITRA). The termite and herbs samples were mineralized by nitric acid attack. This mineralization was done in a closed and hot environment (150 °C). Five (5) g as a test sample was used for each type of sample with a recovery volume of 50ml. The samples, after attack with nitric acid and heating, are filtered using filter paper. The filtrate obtained contains the chemical elements to be measured. The elements sought in the filtrate were measured using a flame atomic absorption spectrophotometer (Spectr AA brand).

Health Risk Assessment Methodology

The health risk assessment followed four main steps in accordance with the methods described by the USEPA. It resulted in the calculation of the daily exposure dose (DED). The exposure scenarios where the individual is most exposed (maximalist hypothesis) will be used. The formula used to assess the average quantities of termites ingested per day was calculated according to the following equation (Hounkpatin *et al*., 2012):

Equation 1: $QAvg. = \sum[\text{Qmin. x \%(adult or child)}]/100$

QAvg.: average quantity of termites consumed per day;

Qmin.: minimum quantity (g) of termites consumed per day;

%(adult or child): percentage of adults or children corresponding to the different quantities;

100: number of individuals surveyed.

The oral toxicological reference value (TRV) used is that produced by the United States Environmental Protection Agency (USEPA). These are indices characterizing the link between human exposure to a toxic substance and the severity of an observed harmful effect.

The threshold effect value for arsenic is 3.10^{-4} mg/kg/day and for non-threshold effects is 1.5 mg/kg/day. The estimated exposure frequency is 180 days, i.e. at least once a day. This period covers the rainy periods during which winged termites are accessible and captured.

The daily exposure dose (DED) of arsenic linked to the consumption of termites is determined as follows:

$$DJE = \frac{C \times Q \times F}{P}$$

Where DJE = Daily Dose of Exposure to As (mg/kg/day); C = Concentration of As measured in termite tissues (mg/kg); Q = Quantity of termites ingested per day (kg/day); F = Frequency of exposure, it is unitless; P = Body weight of the target (kg).

The average body weight of children aged 3 to 16 is 28.7 kg according to the work carried out in the coastal area by Djadou *et al*. (2017) . That of adults (22 to 60 years) is 67.64 kg (Aduayi-Akue, 2015) .

For threshold effects, the risk characterization is expressed by the hazard quotient (HQ). It is calculated for the oral route of exposure as follows:

$$HQ = \frac{DJE}{DJA}$$

Where DJE = Daily Dose of Exposure (mg/kg/day); DJA = Acceptable Daily Intake (mg/kg/day).

If HQ < 1, the occurrence of a toxic effect is very unlikely;

If HQ > 1, the occurrence of a toxic effect cannot be excluded.

For non-threshold effects, the excess individual risk (ERI) represents the probability for an individual to develop a cancer associated with the substance exposed during his or her entire life. It is evaluated by the following relationship (Monferran *et al* ., 2016) :

$$ERI = \frac{C*Q*F*T*ERUo}{M*Tm} = (DJE * ERU)\frac{T}{Tm}$$

ERUo: Excess Unit Risk by oral route (µg/g/d)

T: Duration of exposure (year)

Tm: Average period of entire life (year)

C: As concentration in termite tissues (µg/g)

Q: daily quantity of termite ingested (kg/day)

F: exposure frequency (days/year)

M: Body weight (kg)

DJE: Daily Dose of Exposure (µg/g/d)

The exposure duration (T) for non-threshold effects is defined as 30 years and the lifetime (Tm) is considered to be 70 years according to USEPA (1991) .

According to research conducted by USEPA (2010) in health risk assessment, when the values of:

ERI < 10^{-6}, the excess individual risk is negligible;

10^{-6} < ERI < 10^{-4}, the excess individual risk is acceptable;

ERI > 10^{-4}, the excess individual risk is unacceptable.

Statistical Analysis

The data were initially processed using Excel software. Analysis of variances (ANOVA) followed by the multiple comparison test (Tukey HSD) were used to assess interspecific variations in arsenic contamination in the different matrices analyzed in this study. Pearson correlation analysis was used to assess the relationships between arsenic concentrations from one matrix to another. These analyses were carried out using R software. These tests have been used in several studies for the comparison of multiple data and to highlight the existing links between variables (Salem *et al*., 2014; Jayaprakash *et al*., 2015; Ouro-sama *et al*., 2021).

RESULTS AND DISCUSSION

The Table 2 presents the physical characteristics in terms of circumference and height of the termite mounds that were the subject of this study. Analysis of the Table 2 that the circumference of the termite mounds studied varies from 0.87 to 11 m with an average of 6.25 ± 3.20 m and the height of the termite mounds varies from 0.5 to 2.25 m with an average of 1.46 ± 0.55 m. Analysis of the standard deviation values shows that the physical parameters (circumference and height) vary greatly from one termite mound to another. These differences can be explained either by the varied age of the termite mounds or because of the disparate stages of external degradation as Traoré (2008) pointed out in his study on the impact of epigeal termite mounds on the regeneration and dynamics of savannah ecosystems in Burkina Faso.

The results from the characterization of arsenic in the different matrices of termites of the genus *Macrotermes* were recorded in statistical form in Table 3. For each element, the minimum, maximum, mean and standard deviation of the results were presented. The analysis of the table shows firstly that the as contents vary from one matrix to another and secondly, the arsenic contents in the different matrices vary from low (external herbs) to moderately high (termite waste). In termite tissues, the average contents are 3.25 ± 3.24 mg/kg. In waste, the average content is 27.01 ± 8.64 mg/kg. The contents measured in the Fungus combs have an average value of 2.77 ± 3.18 mg/kg and those measured in the termite mound, soils and external herbs have values of 11.79 ± 4.97 mg/kg respectively. 16.13 ± 9.83 mg/kg and 2.51 ± 3.30 mg/kg.

Table 2. Circumference and Height of Termite Mounds of the Genus *Macrotermes in the* Hahotoé-Kpogamé Phosphate Extraction and Processing Area in Togo.

Code	Place	Size	
		Circumference (m)	Height (m)
M1	Goumoukope1	10	1.5
M2	Goumoukopé2	8	1.5
M3	Kpeme4	0.87	0.5
M4	Kpeme 5	11	1.8
M5	Hahotoé 1	4.7	1.98
M6	Hahotoe 2	6	0.6
M7	Akoumape 1	4	2.25
M8	Akoumape 2	5.4	1.5
M9	Akoumape 3	3.5	1.5
M10	Vogan	9	1.45
Average		5.68	1.23
Standard deviation		3.43	0.64

Arsenic Contents in Termite Tissues, Waste, Fungus Combs, Termite Mounds, External Soils and External Herbs of Termites of the Genus *Macrotermes*

High levels of trace elements in polluted environments can cause toxic effects on the reproduction, growth, and feeding of organisms living in these biotopes (Grelle, 1998). In the absence of data on As levels in winged termites, the levels in the present study were compared with the As levels measured in insects in general. This comparison shows that the As levels in the tissues of termites in the Hahotoé-Kpogamé phosphate mining and processing area in Togo are less concentrated than the As levels in the tissues of *T. clotho insects.* (138 mg/kg), from *Callopistria floridensis G.* (1655 mg/kg) and *S. neglecta* species (95 mg/kg). These results were obtained respectively by Wan *et al*. (2021), Jaffe *et al*. (2019) and Mathews *et al*. (2009). The analysis of Table 3 also shows that arsenic is more abundant in termite waste than other matrices. The Herbs outside the termite mounds in the area are the lowest in arsenic concentration. In view of these results, the decreasing order of arsenic accumulation in the different matrices of *Macrotermes termites* is as follows: Waste>External soils > Termite mounds > Termite tissues > Fungus combs > External herbs.

Table 3. Statistical Results (minimum, maximum, mean, standard deviation) of As Contents in the Different Matrices Studied.

N = 10	Termite	Waste	Fungus combs	Termite mound	External Soil	External Herbs
Min. – Max.	< LD-9.5	16.6-44.7	< LD-7.6	< LD-17.6	< LD-37.5	< LD-7.00
Avg.	3.25	27.01	2.77	11.79	16.3	2.51
Standard deviation	3.24	8.64	3.18	4.96	9.83	3.30

Min. = Minimum ; Max. = Maximum : Avg. = Average.

The arsenic concentration in our samples varies from one matrix to another. The analysis of variances (ANOVA) applied to the results obtained, followed by the multiple comparison test confirmed significant differences (at the 5% threshold) in as concentration between the different matrices studied, particularly between the arsenic contents contained in the waste and the other matrices (Table 4). The high arsenic values in termite waste can only be explained by the ability of these termites to detoxify and eliminate arsenic accumulated through the gastrointestinal tract and found in waste. Several studies have reported this ability for certain organisms living in polluted environments to eliminate trace elements either through the reproductive organs or in feces (Grelle, 1998; Gbem *et al*., 2001; Wagner & Boman, 2003) . The analysis of correlation Table 5 shows the existence of correlation between various matrices studied. A positive correlation clearly appears between the arsenic contents in termite tissues and the contents found in the waste. The more the arsenic contents increase in the tissues, the metabolism of this element is naturally triggered and eliminated in the waste, also leading to a high quantity of arsenic in the waste.

Table 4. Results of the Multiple Comparison Test

Comparison	Diff.	Lwr.	Upr.	P_adj.
External Herbs - Waste	-2.4500000	-3.1883632	-1.7116368	0.0000000
Fungus combs _ Waste	-2.4721111	-3.2307072	-1.7135151	0.0000000
External Soil - Waste	-0.9087778	-1.6673738	-0.1501817	0.0103765
Termites - Waste	-2.3760000	-3.1143632	-1.6376368	0.0000000
Termite mounds - waste	-1.3910000	-2.1495961	-0.6324039	0.0000227
Fungus combs - External Herbs	-0.0221111	-0.7807072	0.7364849	0.9999993
External Soil - External Herbs	1.5412222	0.7826262	2.2998183	0.0000028
Termites- External Herbs	0.0740000	-0.6643632	0.8123632	0.9996719
Termite mounds - External Herbs	1.0590000	0.3004039	1.8175961	0.0017665
External Soil - Fungus combs	1.5633333	0.7850302	2.3416365	0.0000036
Termites - Fungus combs	0.0961111	-0.6624849	0.8547072	0.9989740
Termite mounds - Fungus combs	1.0811111	0.3028080	1.8594142	0.0018846
Termites- External Soil	-1.4672222	-2.2258183	-0.7086262	0.0000079
Termite mounds- External Soil	0.2960809	0.4535482	1.7435961	0.0043150

Diff. = difference; Lwr. = lower; Upr. = upper; P_adj. = p_value

Table 5. Correlation Between Different Matrices Studied.

	Waste	Fungus combs	Termite mound	External soil	External Herbs	Termites
Waste	1.00	-0.21	-0.66	-0.06	-0.59	0.57
Fungus combs	-0.21	1.00	0.35	-0.51	-0.07	-0.76
Termite mound	-0.66	0.35	1.00	-0.17	-0.18	-0.51
External soil	-0.06	-0.51	-0.17	1.00	0.44	0.31
External herbs	-0.59	-0.07	-0.18	0.44	1.00	-0.20
Termites	0.57	-0.76	-0.51	0.31	-0.20	1.00

The table below presents the results from the calculation of the danger quotient (QD) and the Individual Excess Risk (ERI).

Table 6. Danger Quotient and Individual Excess Risk

Trace element	HQ		ERI	
	Adults	Children	Adults	Children
As	4.76	3.43	$9.19.\ 10^{-4}$	$6.62.\ 10^{-4}$

The correlation circle of the variables (Figure 2) indicates that all the variables were well represented in the factorial plan with sources of contamination certainly similar between the external herbs and the termites and different from those of the External soils, the termite mound and the haystacks which would also present the same source of pollution. The individuals (sampling sites) projected in the factorial plan reveal two groups. Those whose arsenic contents in waste, Fungus combs and termite mounds are relatively high (M1, M2, M5). These are the sites of Hahotoé and Goumoukopé which are located respectively in the phosphate mining and phosphate processing zone and which form the first group (GI). The second group (GII) contains the sites whose arsenic contents are relatively high in termite tissues and external soils. These are sites M6, M7 and M9 of the localities of Hahotoé and Akoumapé. These are the sites located only in the Hahotoé-Kpogamé phosphate mining zone of Togo. This is the phosphate outcrop zone and also the most exposed zone for termites. The latter, in permanent contact with the soil of the area for the search for materials for the construction/rehabilitation/maintenance of termite mounds or the search for food, are exposed to risks of accumulation of trace elements contained in the soil. The results have also shown that these three sites (M6, M7 and M9) have the highest arsenic values in the external soil compared to the other sites and whose contents are respectively 22.7 mg/kg ; 37.5 mg/kg and 19.7 mg/kg.

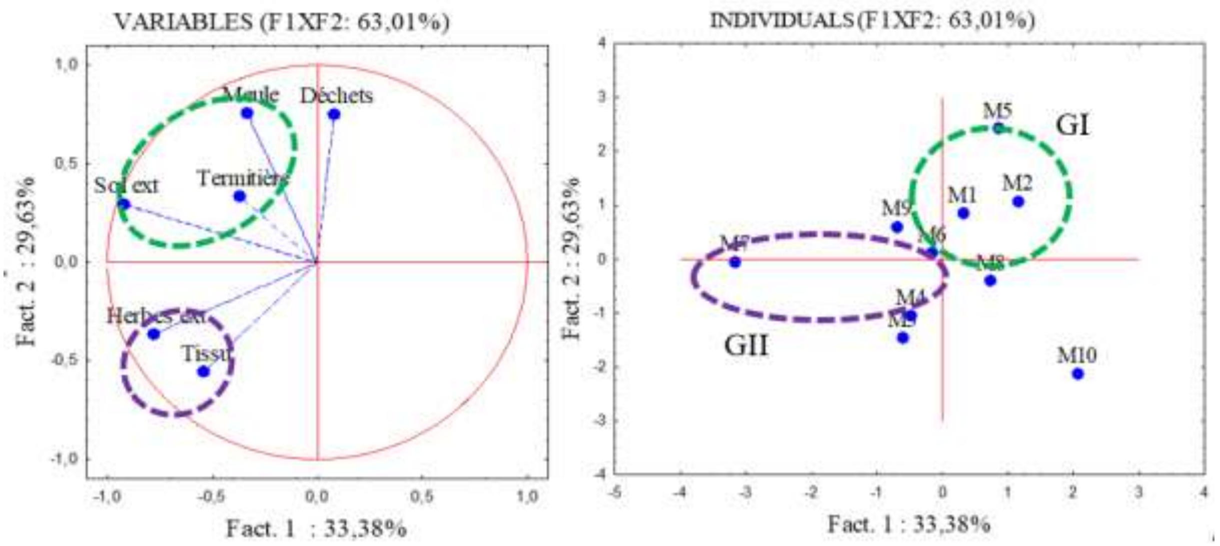

Figure 2. FIXF2 Factorial Plan of (a) Variables and (b) Individuals.

As accumulated in termite tissues can cause adverse health effects in human populations that regularly consume these termites. To assess health risks, the calculation of the hazard quotient is generally used in toxicological studies (Ju *et al*., 2017; Diop *et al*., 2019 ; Maurya *et al*., 2019 ; Ramos-Miras *et al*., 2019 ; Soltani *et al*., 2019 ; Badassan, 2020 ; Ouro-Sama *et al*., 2022) . The analysis of the table shows arsenic hazard quotients greater than 1 (HQ > 1). For non-threshold effects, the assessment of the excess individual risk (ERI) gives ERI > 10^{-4} in both adults and children. These results show that the consumption of termites in the study area presents a risk of health hazard for both adults and children with risks of carcinogenic effects.

The results also showed that children would be more exposed to toxicological risks than adults. This was the case during the assessment of health risks related to the consumption of *Metapenaeus affinis* shrimp. Soltani *et al*. (2019) had noted an exposure dose in children of 14.98 µg/kg/week, higher than that of adults. This predisposition could be explained by their low body weight, ease of absorption of contaminants and less easy detoxification and excretion than in adults (RCAP, 1996) . Although the hazard quotients and the individual excess risk calculated in this study are above the recommended values, it is also important to emphasize that the amount of arsenic measured in this study takes into account both organic and inorganic arsenic. However, the toxicological reference values were calculated based on inorganic arsenic, which presents more danger. In the literature, authors such as Li *et al*. (2015) and Tran *et al*. (2018) agree that the inorganic As fraction represents 10% of total As. On this basis, the daily exposure dose values for adults and children would represent 1.40.10^{-4} mg/kg/day and 1.02.10^{-4} mg/kg/day respectively. These doses would then become lower than the TRV with the consequence of HQ lower than 1, resulting in low risks of carcinogenic effects occurring.

CONCLUSION

The objective of this study was to assess the level of as pollution in termite tissues and their immediate environment then to assess the health risk linked to the consumption of these termites by populations. The characteristics in terms of circumference and height of termite habitats were found to be very variable from one termite mound to another. The As contents found in the different matrices analyzed (termite tissues, termite mound, Fungus combs, external soil, external herbs) are moderately high and vary from one matrix to another. As is more abundant in termite waste than other matrices; this allows us to conclude that termites have the capacity to excrete the pollutant studied. The external herbs of termite mounds in the area are the lowest in arsenic. The principal component analysis identified two distinct major groups in terms of As accumulation. The sites of Hahotoé and Goumoukopé located respectively in the phosphate mining and phosphate processing area have relatively high arsenic levels in waste, Fungus combs and termite mounds and those located only in the phosphate mining area (Hahotoé and Akoumapé) have relatively high levels in termite tissues and external soils. The health risk assessment carried out revealed that the consumption of termites of the genus *Macrotermes* from the Hahotoé-Kpogamé phosphate mining and processing area would not present a risk of carcinogenic effects for arsenic. However, it is necessary to extend this study to other trace elements from the mining area and especially in terms of perspective, to seek to characterize the fraction of the inorganic form in the tissues of termites of the genus *Macrotermes.*

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