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Mercure, arsenic et sélénium au Burkina Faso: bioaccumulation, transfert trophique dans les systèmes aquatiques et évaluation de bioaccessibilité chez les humains

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Cette thèse intitulée:

Mercure, arsenic et sélénium au Burkina Faso: bioaccumulation, transfert trophique dans les systèmes aquatiques et évaluation de bioaccessibilité chez les humains

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Résumé

L'extraction aurifère est l'une des activités humaines qui a fortement accru l'émission de contaminants métalliques dans l'environnement. Le mercure (Hg), l'arsenic (As) et le sélénium (Se) sont 3 polluants métalliques de grande toxicité environnementale. En milieu aquatique, ils peuvent subir des transformations menant à des composés capables de bioaccumulation et de bioamplification. Il peut en résulter des concentrations 10^6 fois celle mesurée dans l'eau chez les poissons et les organismes situés en haut des chaînes alimentaires posant de ce fait de graves menaces pour la santé de ces organismes ainsi que leurs consommateurs y compris les humains.

Cette étude a évalué les teneurs en Hg, As et Se dans les milieux aquatiques au Burkina Faso, une région d'Afrique sub-saharienne soumise à une exploitation minière intensive. Le risque potentiel pour les organismes aquatiques et les humains a été évalué en considérant les effets des interactions antagonistes Se/Hg et As/Se. La bioaccumulation et le transfert du Hg et du Se dans les réseaux trophiques sont également décrits. L'exposition au Hg de poissons par les humains a été également évalué au laboratoire par mesure de la bioaccessibilité comme équivalent de la biodisponibilité par simulation de la digestion humaine.

En général, les milieux aquatiques étudiés étaient peu affectés par ces 3 métal(loïd)s bien que certaines espèces de poisson issus des réservoirs les plus profonds indiquent des teneurs de Hg au dessus de 500 ngHg/g (poids frais) recommandé par l'OMS. Ces niveaux sont susceptibles de présenter des risques toxicologiques pour les poissons et pour leurs consommateurs. En considérant l'antagonisme Se/Hg, 99 % des échantillons de poisson seraient moins exposés à la toxicité du Hg dû à la présence simultanée du sélénium dans le milieu et pourraient être

consommés sans risque. Cependant, les effets potentiels de l'antagonisme As/Se pourraient réduire les effets bénéfiques du Se et ramener cette proportion à 83 %.

L'application des mesures de signatures en isotopes stables d'azote ($\delta^{15}N$) et de carbone ($\delta^{13}C$) des organismes aquatiques a permis le traçage des voies de transfert du Hg et du Se dans les réseaux trophiques. On y observe des chaînes trophiques très courtes (3 - 4 niveaux trophiques) et des poissons majoritairement benthiques. L'approche isotopique n'a cependant pas permis de détecter les variations saisonnières des niveaux de contamination en Hg des poissons. L'exploration des contenus stomacaux des poissons a permis de mieux expliquer la baisse des concentrations en Hg et Se observées chez certains poissons au cours de la saison sèche en lien avec la variation de la composition des proies que l'analyse isotopique n'a pas cerné. L'étude suggère que l'analyse de contenus stomacaux ainsi que l'étude de la dynamique des communautés d'invertébrés couplées à celle des métaux pourraient améliorer la compréhension du fonctionnement des écosystèmes étudiés. Enfin, l'évaluation expérimentale de l'exposition au Hg indique que les modes de traitement avant consommation ainsi que l'usage de composés alimentaires tels le thé, le café lors de repas de poisson par certaines communautés humaines ont un impact sur la bioaccessibilité du Hg de poisson. Ces résultats, sous réserve de validation par des modèles animaux, suggèrent la prise en compte des habitudes alimentaires des communautés dans l'élaboration adéquat des avis de consommation de poisson.

Mots-clés : Élément en trace métallique, mercure, arsenic, sélénium, bioaccumulation, bioamplification, réseaux trophiques, isotopes stables, évaluation de risque, bioaccessibilité, Burkina Faso, extraction aurifère, santé environnementale.

Abstract

Mining of gold is one of the human activities that have increased the inputs of trace elements into the environment. Mercury (Hg) arsenic (As) and selenium (Se) are trace elements that are of environmental importance. Under aquatic environmental conditions, they can be transformed into organic forms which can bioaccumulate through aquatic food webs to reach high concentrations in predatory fish posing harmful effects to wildlife and humans due to their toxicological properties.

This study assessed mercury (Hg), arsenic (As) and selenium (Se) levels in aquatic systems and their potential health risk for humans and wildlife in African sub-Saharan region of Burkina Faso where small scale gold mining practices are widespread. Bioaccumulation and trophic transfer of Hg and Se through food webs were also assessed. Human Hg exposure from fish consumption was also assessed *in vitro* by measure of bioaccessibility as proxy of Hg bioavailability to improve risk assessment.

Water and fish levels of these elements were relatively low and did not reveal an important impact of gold mining activities. However some fish, mainly from deepest reservoirs, exhibited Hg concentrations above the international marketing limit of 500 ngHg/g (w.w.) recommended by WHO/FAO. These levels may be harmful for these fish and their predators including human. However, when taking into account the antagonistic effect of Se on Hg toxicity, up to 99 % of all fish could be protected from Hg toxicity by their Se content. When considering both As/Se and Se/Hg antagonism, 83% instead the 99% of fish should be considered safe for consumption. Stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) allowed us to draw food webs structure and pathways of Hg and Se bioaccumulation and

biomagnification through food webs. We reported that food webs structure were similar across the three reservoirs. Many fish were found to rely on littoral habitat and were associated with short food chains (3-4 levels). However isotopic analyses were not sufficient to understand seasonal variation of Hg from fish linked to seasonal variation of main food items and subsequent analyses of gut contents suggest that stomach content analysis and invertebrate functional groups dynamics may be complementary to isotopic analysis in ecosystem dynamics studies.

Bioaccessibility assessment indicated that cooking and addition of tea or coffee led to very low levels of Hg bioaccessibility suggesting that Hg bioaccessibilty from fish can be modified by cooking and by the co-ingestion of tea and coffee. These *in vitro* results should be further validated using *in vivo* approaches with animal models, thereby for each community, risk assessment should consider the impact of dietary habits on mercury bioavailability.

Keywords : Trace metals, mercury, arsenic, selenium, bioaccumulation, biomagnification, food webs, stable isotopes, risk assessment, bioaccessibility, Burkina Faso, gold mining, environmental health.

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« L'ignorance est l'attribut primitif de l'homme brut et isolé : dans la société, elle est la plus funeste infirmité des hommes; elle y est même un crime, parce que les hommes, étant doués d'intelligence, doivent s'élever à un ordre supérieur à l'état des brutes. Elle y est un crime énorme, car l'ignorance est la cause la plus générale des malheurs du genre humain et de son indignité envers l'auteur de la nature, envers la lumière éternelle, la suprême raison et la cause première de tout bien »

(F. Quesnay)

Je dédie cette thèse à mes premiers enseignants: Mme ***OUÉDRAOGO*** *née* ***SANKARA Thérèse,*** *de l'école primaire publique mixte de Napalgué (année 1976)****,*** *Mr* ***RAMDÉ Y Albert et*** *Mr* ***SANKARA Ernest*** *de l'école de Saponé centre A (années 1980). Par des chemins non défrichés vous avez été des artisans anonymes de cette nation naissante, le Burkina Faso. Puissiez-vous trouver ici le réconfort d'un travail accompli.*

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Liste des sigles et abréviations

Adj. R^2.	Coefficient of determination adjusted (*coefficient de détermination ajusté*)
ANOVA:	Analyse de variance
As	Arsenic
BAF	Bioaccumulation factor (*facteur de bioaccumulation)*
BMF	Biomagnification factor (*facteur de bioamplification*)
CVAFS	Cold Vapour Atomic Fluorescence Spectrometry
DHg	Dissolved mercury
DO	Dissolved Oxygen (*oxygène dissous*)
DOC / COD	Dissolved Organic Carbone (carbone organique dissous)
d.w.	dry weight
GSH	Glutathion
Hg	Mercury (mercure)
HG-AFS	Hydrure Generation Atomic Fluorescence Spectrometry
kg	Kilogramme
LDA	Linear Discriminant Analysis
MeHg	Methylmercry (m*éthylmercure*)
MDL	Method detection limit (*limite de détection de la méthode*)
mL.	millilitre
ng	nanogramme
NO_3^-	nitrate
PCA (*ACP*)	principal component analysis (*analyse en composantes principales*)

PHg	particulate mercury (*mercure particulaire*)
RDA (*ACR*)	redundancy analysis (*analyse canonique de redondance*)
Se	Selenium (sélénium)
SO_4^{2-}	sulfate
T	Température
TAs (AsT)	Dissolved total arsenic (*arsenic total dissous*)
THg (HgT)	Total mercury (*mercure total*)
TMF	Trophic Magnification Factor
Tp	Trophic position
TSe (SeT)	Dissolved total selenium (sélénium total dissous)
TWI	Tolerable weekly intake
V	Volume
w.w.	wet weigth
WHO/OMS	World Health Organisation (Organisation Mondiale de la Santé)
δ	«delta» notation
$\delta^{15}N$	nitrogen stable isotopes ratios (ratios *d'isotopes stables d'azote*)
$\delta^{13}C$	carbon stable isotopes ratios (ratios *d'isotopes stables de carbone*)
µg	microgramme

Chapitre 1

Introduction Générale

Au détour de la décennie 1950, les accidents successifs d'empoisonnement humains au mercure à Minamata et à Niigata au Japon et en Irak, ont placé le sujet de la pollution environnementale comme l'un des défis majeurs de notre temps. En effet, ces drames sont vite apparus comme le corollaire du développement industriel triomphant. La révolution industrielle et technologique du 19^{e} siècle porteuse de promesses de vie meilleure s'est accompagnée d'un accroissement sans précédent de rejets par les humains de substances naturelles ou de synthèses dont les effets directs ou indirects constituent de graves menaces pour la santé humaine et environnementale.

À Minamata, par exemple, c'est le déversement dans la baie d'eaux usées contenant du méthylmercure, un sous-produit de la synthèse de l'acétaldéhyde par une usine locale de produits chimiques qui a été à l'origine de ce désastre d'intoxication chez les personnes qui consommaient de grandes quantités de poissons et de fruits de mer provenant de ladite baie. En Irak, c'est du pain à base de blé traité avec des produits organomercuriels qui était incriminé. En conséquence, la contamination mercurielle a suscité un grand intérêt de recherche en vue d'apporter des réponses appropriées aux inquiétudes soulevées par ces épisodes dramatiques. De nos jours les sources locales d'émissions de ces contaminants sont en passe d'être maîtrisées du moins dans les pays développés. Cependant, la dispersion planétaire de plusieurs de ces substances via l'atmosphère et les océans fait encore de cette problématique un impératif écologique majeur. Ainsi, même les pays où les émissions sont minimes et les écosystèmes les plus éloignés de toute activité humaine ne sont épargnés par cette pollution transcontinentale. Les récentes estimations d'émission de mercure notamment par combustion d'énergie fossiles, place le continent africain en 2^{e} rang après la Chine (Streets et al., 2009). En plus de cela

plusieurs États du continent africain connaissent un essor des activités d'extraction minière source de rejet de métaux dans l'environnement. Malgré ces constats, les eaux intérieures du continent Africain n'ont que très peu fait l'objet d'investigation quand à la contamination métallique.

La présente recherche conduite dans le cadre d'une thèse de doctorat et qui porte sur la dynamique du mercure (Hg), de l'arsenic (As) et du sélénium (Se) dans les hydrosystèmes au Burkina Faso suivie d'une évaluation de bioaccessibilité chez les humains constitue l'une des premières études portant sur des éléments en traces métalliques en Afrique sub-saharienne. Les récents problèmes de santé en lien avec l'exposition à l'arsenic de l'eau de puits rapportés chez les populations dans la zone d'étude (Smedley et al., 2007) ainsi que les interactions chimiques liants ces trois éléments largement documentées dans la littérature ont guidé notre choix de les réunir dans une même étude.

Cette introduction générale permettra avant d'exposer le contenu de ce travail, de faire une revue de littérature sur la problématique de ces trois éléments dans l'environnement notamment en milieu aquatique. La revue porte aussi sur les approches d'évaluation de suivi d'exposition humaine liée à la consommation de poisson. Elle se termine par une brève synthèse des études menées en Afrique sur la contamination métallique. La structure de la thèse et les hypothèses de recherche sont données à la suite de cette revue de littérature.

1.1 Problématique du mercure, de l'arsenic et du sélénium dans environnement

Le mercure, l'arsenic et le sélénium sont trois éléments chimiques d'importance environnementale du fait de leur potentielle toxicité pour les populations humaines et animales (A.T.S.D.R., 1999; Cai, 2003; Campbell et Couillard, 2004). Tous trois existent sous forme d'éléments en trace (0.01%) comme constituants de la croûte terrestre et ont des cycles fortement perturbés par les activités humaines. Les émissions atmosphériques du mercure, de l'arsenic et du sélénium se seraient accrues d'un facteur de 3, 1,6 et 0,6 respectivement depuis la révolution industrielle (Mason et al., 2000; PNUE, 2007), Les principales sources anthropiques ayant contribué à l'augmentation des émissions de ces éléments dans l'environnement comprennent l'utilisation des combustibles fossiles et l'incinération de déchets urbains (Streets et al., 2009), les activités d'exploitation minière (Risher et De Rosa, 2007), l'industrie chimique tels les raffineries, la manufacture et l'usage de pesticides agricoles (Oremland et Stolz, 2003; Pacyna et Pacyna, 2001; Stolz et al., 2006). En environnement aquatique, ces éléments chimiques peuvent subir des transformations biogéochimiques menant à des formes toxiques pour l'environnement.

1.1.1 Le mercure en environnement aquatique

1.1.1.1 Le cycle biogéochimique du mercure

Le mercure (Hg) est un élément chimique du groupe 12 du tableau périodique dont les propriétés physiques sont résumées à la Table S1.1. Il est présent dans toutes les composantes de la biosphère à savoir, l'atmosphère, l'hydrosphère et la lithosphère et se rencontre principalement sous trois formes ou espèces chimiques : le mercure élémentaire (Hg^0, liquide ou gazeux) et les composés du mercure monovalent Hg(I) et divalent Hg(II) (Table 1.1). Il peut subir des transformations réversibles qui le font passer d'une forme à une autre, mais aussi d'un compartiment de l'environnement à un autre. Ses formes gazeuses volatiles et persistantes peuvent être transportées et distribuées à l'échelle planétaire, ce qui en fait un polluant global (Fitzgerald et Lamborg, 2005; Morel et al., 1998). En contact avec les halogénures (B_r^-, F^-, Cl^-), le Hg^0 peut s'oxyder en Hg ionique (Hg^{2+}) et se déposer sur les sols et à la surface des eaux (océans, mers, lacs, rivières) via les courants atmosphériques et les précipitations. Dans les milieux aquatiques, la spéciation du Hg détermine sa solubilité, sa mobilité et sa toxicité pour les organismes vivants (Babiarz et al., 2001). La spéciation chimique du Hg est contrôlée par deux couples de réactions : les réactions d'oxydo - réduction et les réactions de méthylation – déméthylation. Le potentiel rédox (E_h), le pH et les ions en solution déterminent la nature des formes chimiques dominantes dans le milieu aquatique (Gabriel et Williamson, 2004). En milieu sub-oxique, le Hg(II) serait transformé principalement par des bactéries sulfato-réductrices (BSR) pour produire des formes méthylées du Hg (Barkay et al.,

2003; Compeau et Bartha, 1985; Poulain et al., 2007). Cependant de récentes études soulignent l'importance d'autres microorganismes tels les bactéries ferro-réductrices et les méthanogènes dans la méthylation du mercure. (Fleming et al., 2006; Hamelin et al., 2011). La production de méthylmercure se réaliserait aussi en condition abiotique (Barkay et al., 2003). Plusieurs variables influencent la méthylation du Hg dans les eaux douces. La température de l'eau, la concentration en sulfates (Benoit et al., 2003; Gllmour et al., 1992), la teneur en matière organique et le pH de l'eau (Barkay et al., 2003; Mason et al., 2000).

1.1.1.2 Bioaccumulation et bioamplification du Hg dans les réseaux trophiques

Le monométhylmercure (MeHg) est l'espèce de Hg la plus rapidement absorbée par les organismes aquatiques. La prise en charge du Hg par les organismes serait régie par des phénomènes de diffusion passive ou par transports facilités et/ou actifs selon l'espèce considérée (Mason et al., 1996). Le MeHg présente une forte affinité pour les ligands tels le carbone organique dissous (COD), les sulfures, les ions Cl^- et OH^-. Avec les ions Cl^-et OH^-, il forme en particulier des complexes non chargés favorisant sa diffusion au travers des membranes biologiques. Une fois à l'intérieur de l'organisme, le MeHg s'accumule dans certains organes comme le cerveau, le foie, le rein et les muscles (Gonzalez et al., 2005; Maury-Brachet et al., 2005). C'est la forte affinité du MeHg pour les protéines, notamment celles avec des fonctions thiols (glutathion, cystéine, métallothionéines, etc.), qui détermine sa distribution tissulaire et sa bioaccumulation intense (George et al., 2008; Harris et al., 2003).

La bioaccumulation est le processus par lequel une substance est absorbée et concentrée dans les organismes vivants au fil du temps à partir de sources biotiques (autres organismes) et abiotiques (sol, air et eau) (PNUE, 2007). La bioamplification est le processus par lequel le prédateur concentre une substance (ou un élément) à un niveau supérieur à celui où il se trouve dans sa proie (PNUE, 2007). La bioaccumulation et la bioamplification du MeHg ont été largement documentées dans une grande variété d'écosystèmes, en régions tempérées (Kidd et al., 2012) comme en milieu tropical (Campbell et al., 2006; Guimaraes et al., 2000; Poste et al., 2008). Il apparaît que le MeHg se bioamplifie des proies aux prédateurs avec des facteurs de concentrations élevées atteignant 10^6 voire 10^7 fois celle du milieu aquatique chez les poissons carnivores (Boudou et al., 2006; Munthe et al., 2007). Ces concentrations peuvent poser des risques pour la santé des poissons et celles des consommateurs humains (Crump and Trudeau, 2009; Scheuhammer et al., 2007). Des concentrations élevées en Hg pouvant causer des problèmes de santé chez les humains ont été rapportées chez des poissons de lacs ayant des teneurs du métal dans l'eau en dessous de la limite du seuil de détection des appareils de mesure (Chen et al., 2000). De même, des niveaux élevés de métal ont été mesurés dans l'eau de lacs où les poissons présentaient une relative faible accumulation (Boudou et al., 2005). Ainsi, en dépit des connaissances largement rapportées sur la bioaccumulation et la bioamplification des métaux dans les réseaux trophiques, il reste cependant difficile de prédire la teneur de métal dans les poissons du fait de l'implication de multiples facteurs qui déterminent une variabilité entre espèces et entre systèmes (Kidd et al., 2012; Mason et al., 2000; Trudel et Rasmussen, 2006).

1.1.1.3 Toxicité du mercure

Le méthylmercure est la forme la plus toxique du Hg. C'est un neurotoxique bien connu qui peut en particulier avoir des effets nocifs sur le développement du cerveau. Il franchit aisément les barrières placentaire et hématoencéphalique (PNUE, 2007) d'où ses effets neurologiques (Clarkson et Magos, 2006) et tératogéniques (Grandjean et al., 2005). En outre, quelques études laissent entendre que même de faibles augmentations de l'exposition au MeHg peuvent être nocives pour l'appareil cardiovasculaire (Choi et al., 2009). L'exposition humaine au méthylmercure (MeHg) à des doses de 0.1µg /kg et par jour peut affecter la santé de l'organisme (USEPA, 2010).

1.1.2. L'arsenic dans l'environnement aquatique

1.1.2.1 Le cycle biogéochimique de l'arsenic

L'arsenic de symbole As est un élément chimique appartenant au groupe 15 du tableau périodique des éléments (Table S1.1). C'est un métalloïde, qui est présent naturellement dans toutes les composantes de la biosphère (air, sol, roches, eaux naturelles et êtres vivants) (Plant et al., 2004; Smedley et Kinniburgh, 2002). Il peut exister sous quatre états d'oxydation, l'arséniate As (V), l'arsénite As (III), l'arsenic élémentaire As (0) et l'arsine As (-III). L'As comprend naturellement des formes inorganiques (As (III) et As (V)) et des formes organiques méthylées (Table 1.1). Les arséniates (As (V) tel $H_2AsO_4^-$ et $HAsO_4^{2-}$) sont les formes prédominantes dans les environnements aquatiques aérobies, par contre les arsénites (As (III) tel H_3AsO_3 et

$H_2AsO_3^-$) prévalent dans les environnements aquatiques anoxiques. Les arséniates sont généralement adsorbés à la surface de minéraux tels les hydroxydes de fer et les alumines, une propriété qui restreint leur mobilité hydrologique. Les arsénites par contre s'adsorbent moins fortement et avec très peu de minéraux, ce qui fait d'eux les oxyanions les plus mobiles. L'arsine As (-III) est la seule forme gazeuse (Francesconi et Kuehnelt, 2004; Plant et al., 2004; Watt et Le, 2003).

La toxicité, la mobilité et la biotransformation de l'As dépendent fortement de ses propriétés physiques et chimiques. En milieu aquatique, le potentiel redox (Eh) la température et le pH seraient les plus importants facteurs qui contrôlent la biométhylation de l'As inorganique (Watt et Le, 2003). Ainsi, en condition aérobie, les arséniates peuvent subir des transformations (oxydation, réduction, méthylation) par des microorganismes conduisant à la formation de composés organiques d'As tels l'acide monométhylarsonique (MMAs V), l'acide diméthylarsonique (DMAs V) et les oxydes triméthylarsine TMAO). En condition anoxique par contre, la forme ionique est susceptible de subir une réduction ou une méthylation pour produire la forme volatile et facilement oxydée en méthylarsine. La biométhylation serait plus un mécanisme de détoxification chez les microorganismes. Le cycle de l'As peut donc être compris comme des transformations entre les états d'oxydation (III) et (V) sous médiation microbiennes (Oremland et Stolz, 2003).

1.1.2.2 Bioaccumulation et bioamplification de l'As dans les réseaux trophiques

Les teneurs élevées en As observées dans les milieux aquatiques sont souvent associées aux sources naturelles géologiques, mais aussi à la pollution due aux activités anthropiques (Watt et Le, 2003). Bien que les poissons de mer présentent des concentrations en As de l'ordre de 5 à 50 µg/g poids sec dans les tissus, il a été observé que la majeure partie (85-90 %) se trouve sous forme organique (arsénobétaine, arsénocholine et acide diméthylarsonique). L'espèce inorganique As (III) et les autres formes sont généralement présentes à de faibles concentrations (Goessler et al., 1997; Rahman et Hasegawa, 2012). L'arsénobétaine est un composé inoffensif non chargé qui est rapidement excrété par l'espèce humaine (Goessler et al., 1997). Contrairement aux poissons marins, les poissons d'eau douce ont des concentrations en général inférieures à 1µg As/g et ne seraient pas des sources potentielles d'exposition puisque la plupart des guides ont des valeurs permises de 1 µg As/g (Jankong et al., 2007).

Dans les eaux marines, l'arsénobétaine a été identifiée comme la principale forme présente chez les poissons (Foster et al., 2006; Goessler et al., 1997; Grotti et al., 2010; Khokiattiwong et al., 2009). Chez les poissons d'eau douce en revanche, on connaît encore très mal les formes dominantes (Rahman et Hasegawa, 2012; Schaeffer et al., 2006). Des hautes teneurs en As inorganiques ont même été observées chez le poisson carnivore d'eau douce *Channa striata* en Thaïlande (Jankong et al., 2007). Cette observation laisse penser que les poissons d'eau douce pourraient constituer une source potentielle d'exposition des populations humaines à l'As. Les mécanismes de prise en charge et de bioaccumulation des différentes formes d'As demeurent insuffisamment compris (Rahman et Hasegawa, 2012). Des études rapportent une diminution de

l'accumulation de l'As avec le niveau trophique (Chen et Folt, 2000; Mason et al., 2000) tandis que celles portant sur la bioamplification indiquent que ce sont principalement les formes organiques qui sont impliquées (Grotti et al., 2010; Khokiattiwong et al., 2009).

1.1.2.3 La toxicité de l'As

L'arsenic est un métalloïde connu depuis l'antiquité pour sa haute toxicité et utilisé comme poison. Il serait carcinogène, mutagène et tératogène (NRC, 2000). Il induirait également des maladies cardiovasculaires, respiratoires, des désordres neurologiques et l'arriération mentale ainsi que plusieurs autres pathologies (Centeno et al., 2007). Une ingestion chronique par les humains de 200-250 µg par jour de la forme inorganique de l'arsenic pourrait conduire à l'empoisonnement à l'arsenic. Le mode de toxicité de l'As dépend des formes chimiques. L'arséniate As(IV) est un analogue du phosphate et inhibiteur de la fonction de phosphorylation oxydative, court-circuitant ainsi le système de production d'énergie de la cellule. Il entre habituellement dans la cellule par des transporteurs de phosphate. L'arsénite As(III) est 60 fois plus toxique que l'arséniate et 100 fois plus que le MMA ou le DMA (Wai et al., 2002) car il se lie au groupe sulfure altérant ainsi la fonction de plusieurs protéines. Il peut se complexer au groupe thiol de la pyruvate déshydrogénase et affecter le système respiratoire.(Oremland et Stolz, 2003).

1.1.3 Le sélénium dans l'environnement aquatique

Le sélénium de symbole (Se) est un élément chimique du groupe 16 du tableau périodique de masse atomique 34 (Table S1.1). Il existe dans l'environnement sous différentes formes, la forme élémentaire, les formes oxydées (les sélénites Se (IV) et les séléniates Se (VI)) et des formes réduites qui comprennent des formes organiques méthylées volatiles et inorganiques (Table 1.1)

1.1.3.1 Le cycle biogéochimique du selenium

Dans les milieux aquatiques, le Se existerait principalement sous deux états d'oxydation: sélénite Se (IV) et séléniate Se (VI). Il peut être soit absorbé par les organismes, soit former des complexes avec la matière particulaire et les sédiments de surface ou être dissous en solution (Simmons et Wallschläger, 2005).

Les processus qui déterminent la mobilisation ou la séquestration du Se comprennent la réduction chimique et biologique des formes oxydées (séléniates et sélénites) en Se (0) aussi bien que leur adsorption aux minéraux argileux (particulièrement ferreux) et au carbone organique dissous (COD) (Plant et al., 2004; Simmons et Wallschläger, 2005).
Dans les zones oxygénées, ce sont les séléniates $Se(IV)O_4^{2-}$ qui existent principalement tandis que les sélénites $HSe(IV)O_3^-$ et $Se(IV)O_3^-$ dominent sous conditions réductrices. La distribution des différentes espèces varie en fonction de l'environnement (Stolz et al., 2006). Le potentiel rédox et l'activité microbienne des sédiments de surface modulent les

mouvements du Se entre la colonne d'eau et les chaînes trophiques perpétuant à long terme les effets de la toxicité du Se dans le système.

1.1.3.2 Bioaccumulation et bioamplification du Se dans les réseaux trophiques

Dans les eaux de surface (lacs et rivières), le Se serait capable de s'accumuler et de produire des formes potentiellement toxiques pour les poissons et les animaux (Adams et al., 2000; Hamilton, 2004; Lemly et Ohlendorf, 2002; Simmons et Wallschläger, 2005). Avec une teneur de 0.1 µg/L de sélénium organique dans l'eau, le Se serait capable de s'incorporer dans les chaînes trophiques via les producteurs primaires et mener à des concentrations (5 - 15 µg/L) susceptibles d'altérer la santé reproductive des poissons (Lemly, 2004). Les algues et les daphnies accumulent plus largement le sélénite que le séléniate. En effet bien que la concentration de sélénium total soit plus largement réduite de l'eau au phytoplancton et au niveau trophique suivant, les études ont montré que la concentration de sélénométhionine (Se-Met) est plus consistante chez les macro-invertébrés que chez les microphytes (Hamilton, 2004; Simmons et Wallschläger, 2005). Son facteur de bioconcentration serait plus grand que ceux des espèces inorganiques (Simmons et Wallschläger, 2005).

1.1.3.3 La toxicité du Se

Le sélénium est un métalloïde essentiel pour la santé humaine et animale, mais dans une gamme de concentrations très étroite, au-delà de laquelle il devient toxique (Hamilton, 2003). La déficience de son incorporation (< 40 µg/jour) ou son excès (> 350 µg/jour) chez les humains, serait associé à la survenue de cancers, de maladies cardiaques

et musculaires, au désordre des systèmes immunitaire et reproducteur (Plant et al., 2004). Bien que le mécanisme de la toxicité du sélénium ne soit pas clarifié, on admet que c'est la production de radicaux libres ou le remplacement dans les enzymes des groupements sulfures par leurs homologues du sélénium (ex, Se-Met et sélénocystéine (Se-Cys)) altérant leurs structures et fonctions qui justifient sa toxicité (Fox et al., 2003). Il est largement admis que la toxicité des différentes espèces de Se par ordre décroissant est la suivante : Se-Met (séléno-amino-acide) > sélénite > séléniate (Simmons et Wallschläger, 2005).

Il importe de noter que notre compréhension des transformations qui affectent ces contaminants dans les milieux aquatiques s'est beaucoup améliorée grâce l'utilisation des isotopes stables de carbone et d'azote que nous rappelons brièvement ci-après.

Table 1.1. Les formes chimiques du mercure, de l'arsenic et du sélénium

Formes de l'élément et stade d'oxydation	Les formes chimiques majeures
Mercure	
Hg(0)	Mercure élémentaire (Hg)
Hg(I)	Ion mercureux (Hg^{+})
Hg(II)	Ion mercurique (Hg^{2+}) chlorocomplexes ($HgCl^{+}$; $HgCl_2$; $HgCl_3^{-}$); Hydroxocomplexes ($Hg(OH^{+})$; $Hg(OH_2)$); sulfure de mercure (HgS), séléniure de mercure (HgSe).
Hg(II) organique	Méthylmercure (monométhylmercure (CH_3HgCl), diméthylmercure (CH_3HgCH_3); Thiocomplexes (HgSR, CH_3HgSR).
Arsenic	
As(-III)	Arsine[H_3As]
As(-I)	Arsenopyrite [FeAsS], loellingite[$FeAs_2$]
As(0)	Arsenic élémentaire [As]
As(III)	Arsenite [$H_2AsO^{-}_3$, H_3AsO3]
As(V)	Arsenate[AsO_4^{3-} , $HAsO_4^{2-}$, H_2AsO^{4-} , H_3AsO4]
As(V and III) organique	Diméthylarsinate [DMA, $(CH_3)_2AsO(OH)$], Monométhylarsonate [MMA(V), [$CH_3AsO(OH)_2$], arsénobétaine [AsB, $(CH_3)_3As+ CH_2COO-$], arsénocholine [AsC, $(CH_3)_3As+ CH_2CH_2OH$)]
Sélénium	
Se(-II)	Sélénide [Se^{2-} , HSe^{-} , H_2Se]
Se(0)	Sélénium élémentaire [Se]
Se(IV)	Sélénite[SeO_3^{2-} , $HSeO_3^{-}$, H_2SeO_3]
Se(VI)	Sélénate[SeO_4^{2-} , $HSeO_4^{-}$, H_2SeO_4]
Se organique	Diméthylsélénide [DMSe, Ch_3SeCh_3]; diméthyldisélénide [DMDSe, $CH_3SeSeCH_3$], sélénométhionine [$H_3N^{+}CHCOO^{-}CH_2CH_2SeMe$], sélénocysteine [$H_2N^{+}CHCOO^{-}CHSeH$]

1.1.4 Méthodes d'étude du transfert métallique dans les réseaux trophiques

L'utilisation des rapports isotopiques des isotopes stables de l'azote ($^{15}N/^{14}N$) et du carbone ($^{13}C/^{12}C$) a permis de décrire le cheminement des contaminants tel le MeHg à travers les réseaux trophiques aquatiques. La composition isotopique d'un animal est sensiblement enrichie de 3.4 (± 1 ‰) en isotopes lourds d'azote (Cabana et Rasmussen, 1994; Peterson et Fry, 1987) et de < 1 ‰ (Vander Zanden et Rasmussen, 2001) pour celui du carbone par rapport à celle de ses aliments. Dans les lacs, il a été rapporté une différence d'enrichissement en ^{13}C entre les producteurs primaires d'origine benthique et ceux d'origine littorale (France, 1995a; Post, 2002). Ainsi, les mesures d'enrichissement isotopique d'azote ($\delta^{15}N$) sont utilisées comme indicateurs de niveau trophique et les variations du rapport $\delta^{13}C$ comme témoins des différentes sources littorale ou pélagique de carbone primaire (Cabana et al., 1994; Vander Zanden et Rasmussen, 1999).

Par ailleurs, des études toxicologiques ont montré que de multiples facteurs sont susceptibles d'interagir avec ces éléments métalliques dans l'environnement et modifier leur disponibilité, et leur toxicité pour les organismes. Parmi ces facteurs on peut citer les interactions avec d'autres éléments chimiques telles celles entre Hg/Se et As/Se.

1.2 Les interactions mercure/sélénium et arsenic/sélénium

L'étude des interactions multiples en écotoxicologie revêt une grande importance pour l'évaluation et le suivi de la contamination environnementale. L'antagonisme sélénium/mercure (Hg/Se) est probablement l'une des interactions entre deux substances chimiques la mieux connue et la plus documentée (Padros et al., 2004). Le rôle protecteur du Se contre la toxicité du Hg et particulièrement du MeHg, a été observé à la fois dans des études en laboratoire (Ganther et Sunde, 2007; Ganther et al., 1972; Parizek et Ostadalova, 1967; Parizek et al., 1971; Sørmo et al., 2011) et de terrain (Belzile et al., 2006; Chen et al., 2001; Yang et al., 2010). Le Se serait capable de séquestrer le Hg réduisant ainsi sa biodisponibilité dans les organismes. L'inverse aussi est vrai, le Hg séquestre le Se, empêchant la formation des enzymes séléno-dépendantes (Sørmo et al., 2011).

Chez les mammifères, le GSH (glutathion) serait largement impliqué dans l'antagonisme Hg/Se par la formation de complexes insolubles HgSe. Vraisemblablement, sous l'action du GSH cellulaire, le sélénium IV est réduit en Se II et les anions séléniures (Se^{2-}) sont expulsés et réagissent avec le Hg plasmatique pour former des complexes avec les protéines où le GSH tient un rôle de ligand. Cet ensemble serait en mesure de se lier à une sélénoprotéine P grâce probablement au groupement libre du GSH (Gailer, 2007; Suzuki et al., 1998).

L'effet antagonique Hg/Se se produirait lorsque le rapport molaire Se:Hg est proche de 1. En conséquence, il a été proposé d'utiliser ce critère dans l'évaluation de risque de

toxicité du Hg pour les poissons lesquels seraient protégés contre la toxicité du Hg lorsque ce rapport est supérieur à 1 (Peterson et al., 2009; Raymond et Ralston, 2006; Yang et al., 2010).

L'effet antagoniste de l'As contre la toxicité de plusieurs formes de Se est également bien connu et documenté depuis les années 1940 (Gailer, 2007; Hamilton, 2004). Comme dans le cas de l'antagonisme Se/Hg, les effets potentiels de l'As sur le Se se produiraient lorsque le rapport molaire As:Se est supérieur à 1 (Gailer, 2007). La prise en compte des interactions entre éléments dans l'évaluation du risque de toxicité des substances suppose la connaissance de la fraction biodisponible à même d'engendrer une réponse de l'organisme.

1.3 Évaluation de risque d'exposition au Hg de poissons chez les humains.

La connaissance de la persistance, la bioaccumulation et la toxicité d'une substance est une étape préalable dans l'évaluation du danger que présente cette substance en vue de l'évaluation plus globale du risque. Ainsi les agences de régulation en tenant compte de ces critères fixent des doses d'ingestion journalières maximales tolérables qui traduisent mieux les risques d'exposition. La dose de 1,6 µgMeHg/kg de masse corporelle et par semaine (Provisional Tolerable Weekly intake, PTWI) est considérée comme une dose de référence admissible en deçà de laquelle l'exposition au MeHg serait sans danger sur la santé du fœtus chez les humains (FAO/WHO, 2004). La plupart des pays et des instances internationales se sont dotés de valeurs guides (US-EPA,

Santé Canada, Union Européenne). Par ailleurs des marqueurs d'imprégnation tels la teneur du Hg dans les cheveux ou dans le sang permettent un suivi de l'exposition de communautés à risque (Canuel et al., 2006; Lebel et al., 1997). Dans les démarches courantes d'évaluation de risque d'exposition, la dose orale, calculée à partir de la teneur en Hg du muscle du poisson et de la quantité ingérée, est considérée comme la fraction biodisponible. Cependant, de nombreux travaux ont rapporté des écarts significatifs entre l'exposition mesurée chez les populations et les niveaux d'imprégnation attendus (Canuel et al., 2006; Passos et al., 2003; Passos et al., 2007). Ces observations traduisent, toutes, la nécessité d'une évaluation adéquate de la biodisponibilité du Hg si l'on veut améliorer l'évaluation de risques d'exposition.

Les approches d'évaluation de la biodisponibilité de métaux chez les humains sont basées sur une évaluation de leur bioaccessibilité. La bioaccessibilité d'une composante alimentaire se définit comme la quantité chimique de cet élément qui est solubilisée dans le fluide gastro-intestinal après simulation de digestion (Oomen et al., 2003). De ce fait elle représente la fraction disponible à l'absorption intestinale. Plusieurs modèles de simulation de digestion ont été mis au point afin d'évaluer la bioaccessibilité des métaux. Ils simulent le transit gastro-intestinal de l'échantillon en reproduisant séquentiellement les conditions physiologiques des compartiments du tractus gastro-intestinal (bouche, estomac et intestin grêle) (Rodriguez et al., 1999; Zagury et al., 2009).

Enfin, il importe de donner un aperçu des recherches effectuées dans le cadre de la contamination métallique en Afrique pour servir de base de comparaison avec la présente étude.

1.4 Revue de littérature sur la contamination métallique aquatique en Afrique

Les études portant sur la contamination des eaux africaines par les métaux remontent à seulement une trentaine d'années. Les premières études ont été menées sous l'impulsion de la FAO dans les années 1970. Par la suite, ces études ont été renforcées par la mise en place de trois grands programmes de recherche conduits par plusieurs scientifiques universitaires et patronnés par le Programme des Nations Unies pour l'environnement (PNUE) à partir des années 1980. Cela a permis d'avoir une première évaluation de la pollution métallique en Afrique. Une revue de ces études pionnières a été faite par (Biney et al., 1994). Ces études pionnières ont conclu toutes à l'absence de graves problèmes de pollution métallique même si elles soulignent la nécessité d'accroître la surveillance, notamment du fait de l'urbanisation accélérée et la croissance de la population.

L'essor de l'exploitation minière dans de nombreux pays africains a suscité de grandes inquiétudes dues aux rejets de métaux qu'elle engendre et qui peuvent être préjudiciables à la santé humaine et environnementale.

A partir de 1990, des recherches focalisées sur le Hg ont été menées en régions minières (Donkor et al., 2006; Ikingura et Akagi, 2003; Ikingura et al., 2006; Taylor et al., 2005; Van Straaten, 2000) et dans les Grands Lacs de l'Est Africain (Campbell et al., 2006; 2005; 2007; Desta et al., 2008; Ikingura et al., 2006; 2004; Kidd et al., 2003; Machiwa, 2005; Tadiso et al., 2011). Cependant peu d'études ont porté sur la contamination métallique des rivières et réservoirs artificiels (barrages hydroélectriques) en Afrique

(Agorku et al., 2009; Black et al., 2011; Kwaansa-Ansah et al., 2011). On ignore aussi l'incidence des variations saisonnières sur la dynamique des contaminants dans les réseaux trophiques. Une synthèse non exhaustive des récentes études sur la contamination de l'eau et des poissons par le Hg est donnée dans les Tables 1.2 et 1.3, respectivement. Peu de données existent sur l'état de la contamination aquatique en Afrique par l'arsenic et le sélénium.

De façon générale, des niveaux relativement élevés en HgT ont souvent été observés dans l'eau dans les localités sous influence minière (Table 1.2) sans que les poissons n'en soient significativement contaminés (Campbell et al., 2003a). Plusieurs hypothèses sont avancées pour tenter d'expliquer ce faible niveau de contamination des poissons malgré des teneurs en Hg élevées dans l'eau. Le piégeage du Hg avec les oxyhydroxides de Fe, d'Al et de Mn abondants dans les sols tropicaux constitue l'une des explications (Ikingura et Akagi, 1999; Taylor et al., 2005).

Quelques études ont rapporté des niveaux de contamination au Hg de poissons, légèrement au-dessus du seuil de 500 µg Hg / kg (poids frais) recommandé pour le commerce international par l'OMS comme rapporté à la Table 1.3. Ce sont entre autres, les espèces *Lates microlepis* et *Polypterus congicus* dans le lac Tanganyika (Campbell et al., 2008); l'espèce *Barbus paludinosus* dans le lac Awassa en Éthiopie (Desta et al., 2007; 2008); *Hepsetus odoe, Synodontis sp* et *Clarias sp* dans la rivière Pra au Ghana (Donkor et al., 2006). Ces observations indiquent une tendance à la contamination des systèmes aquatiques en Afrique et soulignent la nécessité d'une surveillance accrue de ces écosystèmes.

Par ailleurs, si les études en Afrique corroborent souvent les observations classiques sur la bioaccumulation et la bioamplification du Hg rapportées par les études menées en régions tempérées, il a été observé diverses relations (corrélations positives, négatives et nulles) entre la charge en Hg de certaines espèces de poissons et leurs tailles (Campbell et al., 2006; Desta et al., 2008; Kidd et al., 2004; Tadiso et al., 2011). Sampaio Da Silva et al. (2009; 2005) ont rapporté des résultats similaires sur la relation Hg- taille des poissons dans le bassin de l'Amazone. Ces observations semblent indiquer que la bioaccumulation serait sous l'influence de plusieurs facteurs à déterminer selon la nature du poisson et le milieu.

Table 1.2. Concentrations de mercure total (HgT) de MeHg et d'arsenic (TAs) dans l'eau de milieux aquatiques en Afrique.

Localités	Système aquatique	HgT (gamme, ng/L)	MeHg (ng/L)	TAs (gamme) (ng/L)	Référence
Afrique de l'Ouest et du centre					
Ghana	Region minière (Rivière Pra)	28.7 – 420.3	0.00 - 19.640		Donkor et al. (2006)
Afrique de l'Est					
Tanzanie	Rivière (Isingile)de Malagarasi	70		970 (mean)	Taylor et al.(2005)
	Bassins d'amalgamation de Hg (Rwamagasa)	430-450		540 - 1500	Taylor et al. (2005)
	Region minière	10- 6780			Ikingura et al. (1997)
Ouganda	Lac Victoria (Golf Napoleon)	1.8 -15.5	1		Campbell et al. (2003b)
Afrique du Sud	Rivière	0.02 - 26.65 ± 3.53	0.02 - 0.89 ± 0.02		Walters et al. (2011)
					Williams et al. (2010)
	Region minière		2.73 ± 0.10		Walter et al. (2011)
	Rivière Limpopo			< 1000	Ogola et al. (2011)
Afrique du Nord					
Egypt	Lake Mariout	1.6 - 12.1			Campbell et al. (2003a)

Table 1.3. Concentrations de mercure total (HgT) et de méthymercure (MeHg) dans les poissons d'eau douce en Afrique. Les chiffres en gras représentent des concentrations supérieures à la limite de 0.5 µg/g de HgT recommandé par l'OMS pour la commercialisation.

Localités	**Système aquatique**	**Espèce de poisson**	**HgT µg/g (moyenne/gamme)**	**MeHg µg/g (moyenne/gamme)**	**Référence**
Afrique du Nord					
Egypte	**Rivière (**Nil)	*Bagrus sp*	0.026 – 0.391	_	Campbell et al. (2003a)
Afrique de l'Ouest					
Ghana	**Lacs**	*Tilapia sp*	< 0.001 − 0.070	_	Agorku et al. (2009)
	Bosomtwi	*S. membranaceus*	0.011 - 0.076		Kwaansa-Ansah et al. (2011)
	Akosombo	*Tilapia zilli*	< 0.001 − 0.076		Agorku et al. (2009)
	Rivière (Pra)	*Tilapia sp*	0.013 - 0.163		Oppong et al. (2010)
		Synodontis. sp	**0.69 ± 0.57**	0.0035 ± 0.001	Donkor et al. (2006)
		Hepsetus odoe	**4.473 ± 0.42**	0.0072 ± 0.0014	
		Clarias sp	**1.04 ± 0.05**	0.0019 ± 0.0001	
Afrique de l'Est	**Lac**				
Ethiopie	Awassa	*Barbus intermedius*	**0.1086 - 0.6280**		Desta et al. (2008)
		Clarias gariepinus	0.002–0.154		Tadiso et al. (2011)
Ouganda	Victoria	*Clarias gariepinus*	**0.005 - 0.630**		Campbell et al. (2003a)
	Nabugabo	*Lates niloticus*	**10.6 -42.2**		
		Tilapia zilli	**1.9 -10.6**		

Table 1.3. (continued)

Localités	Système aquatique	Espèce de poisson	HgT µg/g (moyenne/gamme)	MeHg µg/g (moyenne/gamme)	Référence
Afrique de l'Est	**Lake**				
Kenya	Turkana	*C. gariepinus*	0.0435 - 0.1113		Campbell et al. (2003c)
	Naivasha	*L. niloticus*	0.0461 - 0.132		
	Baringo	*H. forskahlii*	**0.0312 - 0.636**		
Tanzanie -Burundi	Tanganyika	*O. tanganyikae*	0.015		Campbell et al. (2008)
		Polypterus congicus	**3.23**		
	Mtera- Kidatu	*C. mossambicus*	0.005 - 0.062		Ikingura et Akagi (2003)
	Centre aurifère de Rwamagasa	*O. niloticus*	0.002–0.031		Taylor et al. (2005)
		Haplochromis spp.	**0.145 -2.65**		
Malawi	Malawi	*O. lidole*	0.007 ± 0.0014		Kidd et al. (2003)
Afrique centrale	**Lake**				
Tchad	Tchad	*O. niloticus*	0.007 ± 0.004		Kidd et al. (2004)
		C. anguillaris	0.033 ± 0.005		
Afrique du Sud					
Afrique du Sud	**Rivière**	*Amphilius spp.*	_	0.020 - 0.085	Williams et al. (2010)
	Olifan- Upper Vaal	*G. affinis*	_	0.010 -0.034	Walters et al. (2011)
	Inkomati WMAS	*Labeobarbus sp.*	_	0.014 - 0.218	
Botswana	**Okavango Delta**	*O. macrochir*	0.0027 - 0.0232		Black et al. (2011)
		C. gariepinus	0.0057 - 0.216.6		
		S. intermedius	0.0082 - 0.2062		

1.5 Objectifs et hypothèses de recherche

L'objectif général de cette étude était d'évaluer le niveau de la contamination de l'environnement aquatique par le mercure l'arsenic et le sélénium au Burkina Faso et l'impact de la consommation de poissons contaminés sur les risques d'exposition humaine au Hg.

Cette dissertation comprend 6 sections structurées comme suit: un premier chapitre (Introduction générale) fait une revue de littérature sur les trois contaminants avec une emphase sur les études menées en Afrique. Ce chapitre se termine par les objectifs et hypothèses de recherche. Cette section est suivie de quatre chapitres présentés sous forme d'articles scientifiques, chacun abordant un aspect des objectifs spécifiques poursuivis dans la thèse. Ainsi les résultats d'une première investigation sur les teneurs en Hg, As et Se dans les milieux d'eau douce au Burkina sont rapportés en chapitre 2. Les chapitres 3 et 4 s'intéressent aux facteurs environnementaux et biologiques qui modulent la bioaccumulation et le transfert des contaminants au niveau des réseaux trophiques aquatiques. Le chapitre 5 est un essai expérimental de l'impact des modes alimentaires sur l'exposition des populations humaines au mercure. Enfin, le chapitre 6 discute les principaux résultats de cette étude, souligne l'importance de leurs apports à l'avancement des connaissances scientifiques et dégage des perspectives.

1.5.1 Le mercure, l'arsenic et le sélénium en milieu aquatique au Burkina Faso.

Dans cette section, les objectifs étaient: 1) de mesurer les concentrations de Hg, d'As et de Se dans l'eau et les poissons pour évaluer le niveau de contamination des milieux aquatiques 2) déterminer le rôle des variables physicochimiques des sites sur la variation des teneurs de Hg, As et Se d'un site à l'autre 3) estimer si les concentrations en Hg, As et Se dans l'eau et les poissons peuvent constituer des menaces pour la faune aquatique et les humains.

En dépit de l'accroissement des activités anthropiques (agriculture, exploitation minière) au Burkina Faso, très peu de données de leur impact sur l'environnement et plus encore sur la santé humaine existent. En 2003, une étude révélait l'usage du Hg dans le processus de récupération de l'or par certains exploitants artisanaux. La même étude évaluait les rejets de polluants dans un site minier au cours de ladite année à 142 kg de mercure, 5680 kg de détergent et 2860 tonnes de CO_2 pour 71kg d'or (Ouattara, 2003). Par la suite, d'autres études ont rapporté des manifestations d'une contamination des aquifères par l'arsenic en région minière (Barro-Traoré et al., 2008; Smedley et al., 2007). Dans ces conditions, nous avons émis l'hypothèse que les teneurs de Hg, d'As et de Se dans les milieux aquatiques au Burkina Faso seraient plus élevées que celles recommandées dans les guides de l'OMS et d'autres normes de références internationales tels U.S.E.P.A. et Santé Canada. Nous avons espéré que des variables physicochimiques telles la température et le pH de l'eau, les sulfates ou le carbone organique dissous expliquent la variation des teneurs de ces métaux d'un site à l'autre comme l'ont suggéré

plusieurs études (Benoit et al., 2003; Gllmour et al., 1992; Heyes et al., 2000). Nous avions aussi prédit que les concentrations de ces métaux traces dans les poissons seraient potentiellement dangereuses pour la santé des poissons et leurs consommateurs incluant les humains, mais en considérant leurs antagonismes, nous anticipions que ce potentiel de risque serait amoindri.

1.5.2 Bioaccumulation et bioamplification du mercure et du sélénium dans les réseaux trophiques aquatiques au Burkina Faso

Dans cette section, trois objectifs étaient poursuivis: 1) décrire les réseaux trophiques de milieux aquatiques au Burkina Faso en utilisant les outils d'isotopes stables d'azote et de carbone, 2) déterminer les taux de bioaccumulation du Hg et du Se dans les poissons de ces sites et identifier les facteurs biologiques qui affectent leur variation, 3) estimer les taux de bioamplification du Hg et du Se dans les chaînes trophiques. L'As ayant été peu détecté dans les poissons dans l'étude précédente n'a pas été pris en compte dans cette étude.

De nombreux travaux ont montré en milieux tempérées et froids que la teneur en Hg augmente généralement avec la taille et l'âge des poissons (Simoneau et al., 2005; Tremblay et al., 1998) ainsi que le niveau trophique (Cabana et Rasmussen, 1994; France, 1995b). Si des facteurs biologiques tels la taille, et écologiques tels la position trophique et la source du carbone des poissons déterminent leurs teneurs en Hg ou en Se comme l'ont rapporté plusieurs auteurs, cela donnera des outils accessibles de gestion et permettra d'asseoir des mesures de suivis environnementaux. Des relations très variées

entre les teneurs en Hg et la taille des poissons sont rapporté au chapitre 2. Ces observations également rapportées par de précédentes études conduites notamment en milieux tropicaux et subtropicaux (Sampaio Da Silva et al., 2005; Tadiso et al., 2011) semblent nuancer le rôle de la taille dans la prédiction de la concentration en Hg. chez les poissons. Du moins, elles suggèrent l'implication de multiples facteurs dans la bioaccumulation du Hg selon les espèces et les systèmes considérés (Mason et al., 2000; Trudel et Rasmussen, 2006). D'où la nécessité d'investiguer au sein de chaque écosystème les variables clés qui pourraient contrôler la bioaccumulation et le transfert des contaminants métalliques au sein du réseau trophique.

Nous pensons que les systèmes aquatiques au Burkina Faso seront caractérisés par de courtes chaînes trophiques étant donné l'omnivorie observée chez plusieurs espèces de poissons tropicaux (N'guessan et al., 2010; Ouéda, 2009). Nous prévoyons que les taux de bioaccumulation du Hg et du Se des poissons d'eau douce au Burkina Faso seraient plus élevés que 5000, prévu par les agences de régulation comme seuil déterminant le caractère bioaccumulatif de substance chimique. En plus, nous pensons que la bioaccumulation sera influencée par la taille des poissons mais aussi par leur condition physiologique définie ici par l'indice de masse corporelle (W_r). Enfin, une corrélation entre les teneurs en métal(loïd) des organismes aquatiques (poissons et invertébrés) et leur signature en isotope stable d'azote ($\delta^{15}N$) est attendue comme manifestation de la bioamplification dans les réseaux trophiques.

1.5.3 Influence de la saison sur la dynamique de la bioaccumulation et du transfert du Hg et du Se dans les réseaux trophiques aquatiques au Burkina Faso.

Le troisième objectif général de cette recherche était d'évaluer l'effet du changement de saison sur la dynamique de la bioaccumulation et du transfert trophique du Hg et du Se au sein des réseaux aquatiques au Burkina Faso.

La saison pluvieuse étant une période de grande abondance de nourriture pour les organismes aquatiques, nous avons émis l'hypothèse que le passage à la saison sèche (période de moindre abondance de proies) devait se traduire par une baisse de niveau trophique notamment pour les poissons en haut de chaînes trophiques. La rareté des proies en saison sèche accentuerait l'omnivorie et la flexibilité alimentaire des poissons. En conséquence, une baisse des teneurs en Hg et Se est attendue étant donné la relation entre teneurs en métal et le niveau trophique largement documenté (Cabana et Rasmussen, 1994; Chen et Folt, 2000; Kidd et al., 1995).

1.5.4 Effets des modes de cuisson et des habitudes alimentaires sur la bioaccessibilité du Hg de poisson chez les humains.

Le quatrième et dernier objectif de cette étude était de déterminer l'effet de la cuisson et de composantes alimentaires tels le thé, le café et l'amidon utilisés par certaines communautés humaines (y compris celles habitant les zones de notre étude) sur la bioaccessibilité du Hg de poissons chez les humains afin d'en évaluer le risque d'exposition au Hg lié à la consommation de poissons contaminés. Cette section concerne uniquement le Hg du fait que les concentrations des autres éléments observées sont très loin de constituer une menace à la santé environnementale et humaine.

Dans l'évaluation actuelle de risque, la dose orale d'un produit spécifique est considérée comme égale à ce qui est biodisponible. La relation dose-réponse entre dose orale du produit et l'accumulation dans l'organisme est donc largement utilisée dans l'élaboration des guides et avis de consommation de poissons par les humains. Cependant des études provenant des populations humaines spécifiques ont rapporté de très faibles teneurs en Hg par rapport à ce qui était attendu (Canuel et al., 2006; Dolbec et al., 2001). L'usage très répandu du thé par certaines de ces communautés serait entre autres une explication à de tels écarts observés (Canuel et al., 2006). Nous avons formulé l'hypothèse que la quantité de Hg bioaccessible de poisson consommé cru serait plus élevée en comparaison à celles de poissons ayant subi la cuisson (bouilli ou frit) avant le repas dû à la perte de métaux durant la cuisson (Gokoglu et al., 2004). Nous avons en plus, espéré que la prise simultanée de certains composés, tels le thé, le café et la farine de maïs riches en phytates (Kumar et al., 2010) lors d'un repas de poisson, réduise significativement la

bioaccessibilité du Hg du poisson. Les effets du type de traitement en cuisson et de l'utilisation de composantes alimentaires ont été évalués dans l'optique d'amélioration des approches d'évaluation du risque d'exposition au Hg par la consommation de poisson par les humains.

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Chapitre 2

Mercury, arsenic and selenium concentrations in water and fish from sub-Saharan semi-arid freshwater reservoirs (Burkina Faso)

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2.1 Abstract

Despite intensive mining activities in Burkina Faso, little is known on the environmental impacts of metals and metalloids potentially released from these activities. Water samples and 334 fish from 10 reservoirs were taken in order to evaluate the extent of mercury (Hg), selenium (Se) and arsenic (As) contamination in aquatic systems and their potential health risk for humans and wildlife, taking into account their antagonistic interactions. Water and fish levels of these elements were relatively low and did not reveal an important impact of gold mining activities. Water temperature and conductivity were the key factors associated with higher levels of MeHg. Higher sulfate content was reported in sites with more particulate Hg, As and Se, suggesting anthropogenic origin of metal(loid) inputs in water reservoirs. Metal(loid) concentrations in fish were low and ranged from 0.002 to 0.607 μg/g wet weight (w.w.) for Hg, 0.023 to 0.672 for Se and 0.039 to 0.42 for As. These levels are similar or slightly higher than those reported in many other studies from Africa. Nevertheless, more than 70% of piscivore fish exceeded the threshold for wildlife protection for MeHg. Further, a traditional risk analysis performed ignoring Se antagonism indicated that these piscivores should be consumed by humans with caution. However, when taking into account the antagonistic effect of Se on Hg toxicity, up to 99% of all fish could be protected from Hg toxicity by their Se content. When considering both As/Se and Se/Hg antagonism, 83% instead of 99% of the fish should be considered safe for consumption. Fish Se and As concentrations did not pose potential risk for both animals and humans. Overall, these reservoirs were relatively unaffected by As, Se and Hg contamination despite the rising gold mining activities.

Further, considering antagonistic effects of As, Se and Hg may help refine consumption advisories.

Keywords: Reservoirs, Trace elements, antagonistic interactions, risk assessment, arsenic, mercury, selenium.

2.2 Introduction

Mercury, arsenic and selenium are trace elements that are of environmental importance due to their high toxicity (Cai, 2003). They occur naturally in the earth's crust, and are released in the environment from natural and anthropogenic sources. Gold mining is one of the key human activities that have increased the concentration of these three trace elements in the environment (Plant et al., 2004). In aquatic systems, these elements can be converted into toxic forms which accumulate in food webs posing a potential threat to wildlife and human health (Hamilton, 2004; Khokiattiwong et al., 2009; Munthe et al., 2007; Scheuhammer et al., 2007). High levels of contaminants in fish have resulted in the adoption of fish consumption advisories and guidelines in many countries or by international organizations to protect people at risk.

In recent studies, it was suggested that trace metal interactive effects must be considered to improve environmental monitoring and risk assessment (Peterson et al., 2009; Yang et al., 2010). It is well established that Se shows protective effects against Hg bioaccumulation and toxicity (Belzile et al., 2006; Gailer, 2007). Based on experimental studies, MeHg toxicity appears to occur when Se:Hg molar ratios are lower than 1 (Gailer, 2007; Peterson et al., 2009; Ralston, 2008; Ralston and Raymond, 2010; Yang et al., 2008; Yang et al., 2010). The importance of biological interactions between selenium and arsenic has also been reported (Plant et al., 2004; Zeng et al., 2005). Similar to Se/Hg interactions, As protective effects on Se appears to occur when molar ratios As:Se are

greater than 1 (Gailer, 2007; Gailer et al., 2000; Zeng et al., 2005). There are increasing field studies which assess potential toxicity and bioaccumulation of Hg from aquatic biota using the Se:Hg molar ratio approach (Belzile et al., 2006; Burger et al., 2001; Chen et al., 2001; Peterson et al., 2009; Yang et al., 2010).

In Africa, most recent studies on environmental trace metal contamination have focused on Hg (Campbell et al., 2006; 2005; Ikingura et al., 2006; Kidd et al., 2004; Machiwa, 2005; Tadiso et al., 2011). These studies were centered mainly on great lakes systems in Eastern Africa. Very few studies on man-made reservoirs and rivers from Western Africa have been reported except in Ghana (Agorku et al., 2009; Donkor et al., 2006; Kwaansa-Ansah et al., 2011). Interactive effects of As on Se, or of As and Se on Hg have not yet been reported in African field studies to our knowledge. Despite intensive mining activities in Burkina Faso since the early 1990's, environmental impacts of these activities are poorly documented. Symptoms of arsenicosis have been reported from people living near mining areas (Barro-Traoré et al., 2008; Smedley et al., 2007). In this article, we assessed mercury (Hg) as well as selenium (Se) and arsenic (As) concentrations in water and fish since high levels of these contaminants in aquatic systems may represent a significant environmental issue in this country.

The aims of this study were to (1) evaluate the extent of environmental mercury, selenium and arsenic contamination in aquatic freshwater systems from Burkina Faso. These aquatic systems were man-made reservoirs providing water to people and livestock, and supporting important fisheries (2), assess the influence of water quality on Hg, Se and As concentrations in water from these reservoirs and (3) determine the

potential health hazard of these contaminants for both humans and fish from these systems with regard to the interactive effects of these contaminants.

2.3 Material and methods

2.3.1 Study area

Burkina Faso is located in the heart of West Africa in the Sub-Saharan region ($12^{o}16$'N, $2^{o}4$'W). The climate is tropical semi-arid with temperatures varying between 24 and 40^{o}C. Evapotranspiration is in the range of 1700-2400 mm/year exceeding annual precipitation which ranges from 400 to 1200 mm (MEC, 2007). Soils types are dominated by Fe-rich laterite soils (alfisol and oxisols) and the regional geology, by the Birimian (low proterozoic) and Tarkwaian formations, of volcano-sedimentary origins (Castaing et al., 2003). Such formations are well known for their high auriferous mineralization (Castaing et al., 2003). Gold mining activities (exploration and extraction) currently occur in two thirds of the territory with more than 600 small-scale gold mines, some of which use Hg amalgamation process for Au extraction (Ouattara, 2003).

In Burkina Faso, around 1,700 small dams have been constructed, most of them during the last 30 years, to provide water to people and livestock in drought-prone areas (Boelee et al., 2009). Most of them are built on seasonal rivers and are used for agriculture, hydroelectric generation and fishing. The study area (Fig. 2.1) is the Nakambe watershed, a central part of Burkina Faso where most dams (including

hydroelectric reservoirs) were constructed and where most of the gold mines are located, representing an area of 70,000 km^2 (Gueye, 2001).

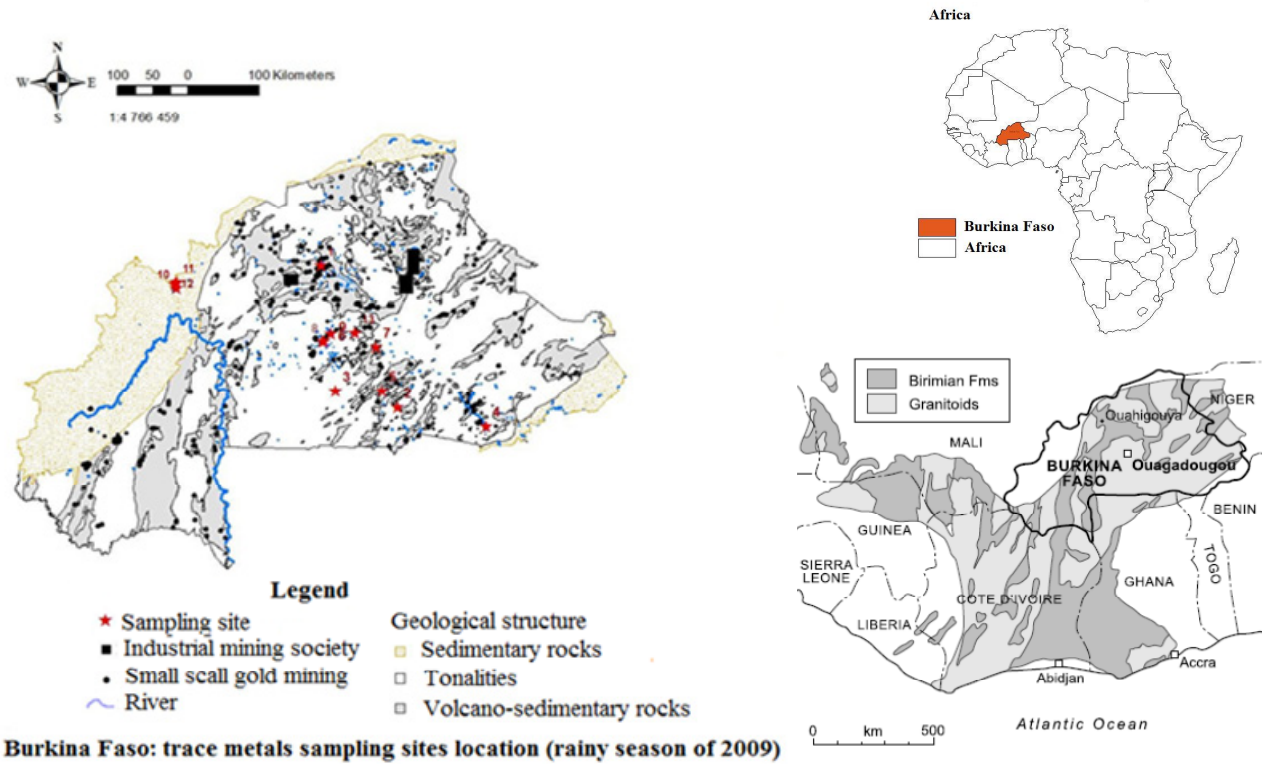
2.3.2 Field sampling

During the 2009 rainy season (July - August) in Burkina Faso, water and fish were collected once in 10 Nakambe watershed reservoirs (Fig.2.1). 13 sampling sites were visited across these 10 reservoirs (8 reservoirs with 1 station; one reservoir with 2 stations and one reservoir with 3 stations).

2.3.2.1 Water quality

Samples were taken to assess water quality, as defined by physiochemical (such as major cations and anions) and geochemical (Hg, As and Se) variables.

Water collection was done from the pelagic zone at 0.5 m from the surface at each site using a 1 L Teflon bottle. Samples were then transferred to acid-washed collection vessels. Triplicates of filtered and unfiltered samples for total mercury (THg) and methylmercury (MeHg) were collected from each site and stored in 125 mL amber glass bottles that had been pre-washed with acid and thoroughly rinsed with ultrapure water. Filtration was done onboard using a Whatman syringe filter of 0.45 μm pore size. All bottles were rinsed three times with dam water prior to water collection. All aqueous mercury samples were preserved at pH 2 with ultra high purity hydrochloric acid (VWR) (0.5%, v/v) and kept in a field cooler and refrigerated (+ 4 °C) upon return to laboratory until analysis.



Burkina Faso: trace metals sampling sites location (rainy season of 2009)

Sampling sites

1. Bam (natural lake)
2. Bazèga (dam)
3. Bagré (hydroelectric reservoir)
4. Koubri (dam)
5. Kompienga (hydroelectric reservoir)
6. Loumbila (dam)
7. Mogtedo (dam)
8. Ouagadougou 2 (dam)
9. Ouagadougou 3 (dam)
10. Sourou 1 (flood plain)
11. Sourou 2 (flood plain)
12. Sourou 3 (flood plain)
13. Ziga (dam)

Figure 2.1: Map of study areas showing the location of Burkina Faso in Africa, distribution of mining sites and of sample stations, and geological structure.

Samples for ancillary chemical analyses were also collected at the same depth. Filtered water was collected in two separate 30 mL Nalgene HDPE bottles. One bottle was acidified using hydrochloric acid (0.5%, v/v) for major cations [potassium (K^+), calcium (Ca^{2+}), sodium (Na^+) and magnesium (Mg^{2+})]; the second bottle was left unacidified for analysis of anions [chloride (Cl^-), nitrite-nitrate (NO_3^-), sulfate (SO_4^{2-})]. For dissolved organic carbon (DOC) analyses, water samples were filtered on 0.45 µm Whatman filters and collected in glass bottle (pre-combusted at 550 °C during 1h).

30 mL Nalgene HDPE bottles samples of filtered water preserved with 1% v/v EDTA (Sigma) were collected for the determination of arsenic and selenium. All Nalgene HDPE bottles were pre-washed thoroughly with ultrapure water.

2.3.2.2 Fish sampling

Fish samples were bought from local fishermen in the ten studied reservoirs. Manipulation of the fish specimens was done in the following manner: (a) sex identification, measurements of total and standard length (cm) and mass (g); (b) removal of a section of dorsal muscle tissue devoid of skin and bones for analyses of Hg. Portions of dorsal muscles were kept in polyethylene bag, were frozen at -20 °C, then freeze-dried and shipped to Montreal (Canada) for laboratory analysis. A total of 334 individuals were analysed. Fish species were categorized as non piscivores (with plankton and insects as main food items), omnivores (fish feeding on variety of food items including fish, according to food availability) or piscivores (fish as main food items) according to their gut content (Ouéda et al., 2008; Ouédraogo and Amyot, unpublished). Collected species

included the four fish families most consumed by local populations, namely: *Oreochromis niloticus*, (Cichlidae, detritivore), *Clarias anguillaris* (Clariidae, omnivore), *Bagrus bajad* (Bagridae, piscivore), *Auchenoglanis occidentalis* (Claroteide, invertebrates-feeders). Additional species such as the Nile perch, *Lates niloticus* (Centropomidae, piscivore), *Synodontis membranaceus* (Mochokidae, planktivore), *Heterotis niloticus* (Osteoglossidae, detritivore), *Gymnarchus niloticus* (Gymnarchidae) *Hydrocynus forskalii* (Alestidae, piscivore), *Hemichromis fasciatus* (Cichlidae, piscivore) and *Synodontis schall* (Mochokidae, invertebrates-feeders) were also collected.

2.3.3 Total mercury analysis

THg analysis in water samples (filtered and unfiltered) was performed by cold vapor atomic fluorescence spectrometer (CVAFS) by Tekran 2600 (Tekran Instruments Corporation, Knoxville, TN, USA) following U.S. Environmental Protection Agency (U.S. EPA) method 1631. Briefly, 50 mL of sample was digested with 200 mL of BrCl, and excess of BrCl was neutralized with 50 μL of hydroxylamine. Samples were then reduced with stannous chloride ($SnCl_2$, 3% w/v) prior to analysis. Laboratory control spike (0.5 mL of a Hg solution of 250 ng/L) were analyzed with every sample batch. The detection limit for this analysis was 0.06 ng THg/L and the mean relative recoveries were 99.3 ± 5.1 % (n = 42). The coefficient of variation (standard deviation/mean) for field triplicate determinations was 11%. Quality assurance and quality control (QA/QC) also included a proficiency test for Hg in water from the Canadian Association for Laboratory Accreditation which was successfully passed.

Fish tissues were analyzed for THg using a direct mercury analyzer (DMA 80, Milestone inc., Pittsburgh, PA), in which samples were combusted at 750°C and mercury vapors were retained on a gold trap for analysis by cold vapor atomic absorption spectrometry. DMA threshold analysis was between 0.12 and 600 ng of THg and detection limit was 0.05 ng THg/sample, with average analytical variance of 5 %. Certified reference materials (CRM), TORT-2 (lobster hepatopancreas, National Research Council, Canada) and DORM-2 (National Research Council, Canada) were used for quality control, and recoveries ranged from 101 ± 8% for DORM-2 to 104 ± 9% for TORT-2 (Annexe 2, Table S2.1).

2.3.4 Methylmercury analysis.

Water samples (50 mL) for MeHg were acid-distilled to remove matrix interferences, then derivatized by aqueous-phase ethylation with $NaB(C_2H_5)_4$, purged on Tenax (Tenax Corporation, Baltimore, MD, USA), separated by gas chromatography and quantified with a Tekran 2500 CVAFS (Tekran Instruments Corporation) based on the method of Bloom (1989). Field and procedural blanks contained less than 1 ± 1 pg MeHg and revealed no contamination during sampling, filtration, distillation, and analysis. Method detection limit (MDL) based on three times the standard deviation of 10 blanks was 0.02 ng L^{-1}.

For MeHg analysis in fish, 10 to 50 mg of dried tissues were digested in 5 mL of 4 M HNO_3 at 55 ˚C for 16 h. Digested samples then underwent aqueous-phase ethylation

followed by gas chromatography separation with CVAFS detection (Tekran 2500). Analytical accuracy was checked by analysis of TORT-2 at each 10 samples (Annexe 2, Table S2.1). Recoveries using TORT-2 averaged 95 ± 13%. The average coefficient of variation for field triplicate determinations was 12%.

2.3.5 Selenium and arsenic determination

Prior to analysis of aqueous total selenium (TSe) and total arsenic (TAs), samples were acid-digested to allow the reduction of Se (VI) to Se (IV) and As (V) to As (III), the forms which will produce a hydride with ($NaBH_4$). Detailed digestion protocol is given in Annexe 2, Supporting information (SI). 10 mL of digested sample solution were treated with $NaBH_4$ to generate covalent gaseous hydrides. Se (IV) and As (III) levels were then determined by hydride generation atomic fluorescence spectrometry (HG-AFS; model PSA 10.055, Millenium Excalibur; PS Analytical, Orpington, Kent, UK). The method detection limit (MDL) was 22 ng/L (Se) and 9 ng/L (As) for water analysis. During the reduction step, 99 % ± 12 of Se (VI) was converted to Se (IV), and 107 % ± 5 of As (V) was converted to As (III). Procedural blanks values were respectively 21 ng/L ± 8 (n = 8) and 67 ng/L ± 22 (n = 9) for selenium and arsenic analysis. QA/QC also included a proficiency test for As and Se analyses in water from the Canadian Association for Laboratory Accreditation which was successfully passed.

For the determination of selenium and arsenic concentrations in fish tissues, a microwave digestion was performed in a mixture of HNO_3 and H_2O_2 in order to extract

elements from the solid matrix. After microwave digestion, an aliquot was taken and underwent the same steps as for aqueous samples. The method detection limit (MDL) was 0.022 µg/g dry weight (d.w.) (Se) and 0.4 µg/g d.w. (As) for fish analysis. Detailed fish samples preparation protocol is given in SI. The analytical quality of fish sample analysis was controlled by using the certified reference materials DORM-3 and TORT-2 with recoveries of 91 ± 7 for TSe and 88 ± 11 for TAs (Annexe 2, Table S2.1).

2.3.6 Physico-chemical analyses

Anions (Cl^-, SO_4^{2-}, NO_3^-) were analyzed by ion chromatography using a DIONEX-DX500 (MDLs: 1 µmol/L). Cations (Ca^{2+}, Mg^{2+}, K^+ and Na+) were analyzed by atomic absorption spectrometry with MDLs of 0.5, 0.1, 0,5 and 0.5 µmol/L, respectively. Water color was measured by spectrophotometric determination at 440 nm wavelength on filtered water. Water column profiles of temperature, dissolved oxygen concentration, pH, and specific conductivity were obtained from each site using a YSI-650 DMS multiprobe. Oxygen and pH calibrations were completed every sampling day.

2.3.7 Data statistical analysis

All statistical analyses were performed with the R software (version R-2.11.1) (http://www.r-project.org/). The typology of site according to their metal(loids) concentration was assessed by cluster analysis. Relationship between site typology and physicochemical descriptors was assessed by Linear Discriminant Analysis (LDA). A classical ANOVA or a non-parametric Kruskal–Wallis test allowed selection of variables which differed significantly among the clustering groups.

Trace element contamination in fish among trophic guilds was assessed by one way ANOVA to explore biomagnification processes. Before computing analysis, principal component analysis (PCA) was used to identify the main descriptors. LDA was performed on the normalized data (all log (x +1) transformation, except SO_4^{2-} which was fourth-root transformed). Shapiro-Wilk test of normality and multinormality was used to assess normality of variables. Homoscedasticity conditions of within-group covariance matrices of the explanatory variables were further examined before computing LDA. For mean calculation, sample values under the MDLs were assigned the method detection limit divided by 2.

2.3.8 Risk assessment linked to human consumption of fish

Risk estimates are based on a WHO/FAO provisional tolerable weekly intake (TWI) for metals (MeHg, Se, or As). TWI is an estimated weekly chemical intake that appears to be without risk if ingested over a lifetime. MeHg TWI of the WHO/FAO has been set at 1.6 μg/kg of body mass to protect vulnerable populations from neurotoxic effects. We assumed that fish consumption was the only source of human exposure to MeHg. Mean MeHg concentrations and mean weights of fish species (Annexe 2, Table S2.4) were used to determine how much fish can be safely consumed weekly. Fish amount A (g) can be computed as follow:

A (g) = W(kg)×I (μg/kg body weight)/C (μg/g fish).

Where W = average body weight (65 or 70 kg for adults woman or man respectively),
I = tolerable weekly intake of fish (μg/kg body weight) and C = metal concentration in fish (μg/g). For example, if a species of fish is known to have 0.3 μg/g of MeHg, an adult man of 70 kg could safely consume 224 g of this fish each week (70 kg body weight × (1.6 μg/kg body weight)/0.3 μg/g fish). Fish amount A (g) can be converted to mean number of fish that could be safely consumed weekly by dividing "A" by the mean weight (g) of each fish species.

2.4 Results

2.4.1 Study sites

Most of the study sites had warm waters (mean of 29 °C), were shallow (<5 m) and had a pH close to neutral (mean of 6.8), with high conductivity (≥100 μScm^{-1}) (Table 2.1). Previous studies did not report eutrophication in this area (Cecchi et al., 2005; Ouéda et al., 2008; Woch, 2006). Two sites located in Ouagadougou exhibited high SO_4^{2-} and NO_3^- concentrations, suggesting anthropogenic pollution. Ouagadougou is the capital city of Burkina Faso and sampling sites located in this area received municipal wastes and agricultural releases from water runoff. Temperature, oxygen and conductivity varied by a factor of 1.2, 2.9 and 7.1 among sites, respectively (Table 1).

The water columns were not well stratified, and bottom waters were well oxygenated (Annexe 2, Fig. S2.1).

2.4.2 Metal(loid) levels in water.

Statistics on trace element concentrations in water are given in Table 2.1. Unfiltered total mercury (THg), and dissolved Hg (DHg) fluctuated by a factor of 53 and 80 times over the study sites, respectively (Table 2.1). In contrast, min:max ratio for particulate mercury (PHg) concentrations in water was 475. PHg represented 43 – 85 % of the THg. The ratio of max:min for MeHg concentration was 21, whereas the range of

total selenium (TSe) and total arsenic (TAs) was four-fold and two-fold respectively. Site typology based on Hg, As and Se levels in water, are reported in Figure 2.2. Sites were partitioned by cluster analysis in three main distinct groups. The best number of groups, K = 3 (that should minimize total error sum of squares (E^2k)) was given by K-means partitions (Annexe 2, Fig.S2.2). The group 1 had relatively high levels of MeHg and lower levels of TSe and TAs (Fig.2.2; annexe 2, Table S2.2). Six sites (Bagré, Bazéga, Kompienga, Koubri, Loumbila and Ziga) composed this group. The group 2 included four study sites (Bam, Mogtedo and Ouagadougou 2, and 3). This group had higher particulate Hg concentrations, and were enriched in TSe and TAs. Group 3 (3 sites) (Sourou 1, Sourou 2 and Sourou 3) had the lowest MeHg concentrations, with levels below the detection limit (0.020 ng/L) and the highest total As levels (Annexe 2, Table S2.2).

Linear discriminant analysis (LDA) between clustering results (response variable) and water physicochemistry indicated that 67% of group dispersion was explained by water quality (Fig.2.3; annexe2, Table S2.3). Boxplots showing the clustering group mean of the nine physicochemical variables are given in annexe 2, Fig.S2.3. Four variables were significantly different among the three clustering groups, namely water color (ANOVA, $p < 0.01$), water temperature (ANOVA, $p < 0.01$), conductivity (ANOVA, $p < 0.05$) and NO_3^- (Kruskal-Wallis test, $p < 0.01$).

Table 2.1. Summary statistics of physico-chemical and metal(loid) levels measured in water for the 13 sampling sites. Abbreviation: DOC= Dissolved Organic Carbon, DO= Dissolved Oxygen, DHg = dissolved total mercury, PHg= Hg in the particulate phase of water, TSe = total dissolved selenium and TAs = total dissolved arsenic

Physico-chemical descriptors	**All sites (N=13)**				
	Mean	sd	min	max	Max/min
Depth (m)	4.23	2.54	1.5	11.0	7.3
Ca^{2+} (mg/L)	7.46	5.06	0.25	16.24	65
Mg^{2+} (mg/L)	4.00	3.94	1.20	12.26	10.2
K^{+} (mg/L)	6.67	3.23	1.13	12.80	11.3
Na^{+} (mg/L)	5.76	3.60	2.74	13.18	4.8
Cl^{-} (mg/L)	3.84	5.57	0.85	16.48	19.4
NO_3^{2-} (mg/L)	1.55	2.03	0.001	5.93	5930
SO_4^{2-} (mg/L)	2.04	3.30	0.001	9.88	9880
DOC (mg/L)	7.37	4.93	2.66	19.80	7.4
DO (%)	81.24	22.15	41.05	117.60	2.86
Color (pt,mg/L)	27.96	22.87	0.28	62.32	222.6
T (°C)	29.17	1.53	27.50	32.39	1.17
Cond (µS/cm)	189.00	122.40	57	406	7.12
pH	6.87	0.89	5.60	8.52	1.52
Metal(loid) variables					
THg (ng/L)	5.3	6	0.40	21.38	53.45
DHg (ng/L)	1.40	1.14	0.05	4	80
PHg (ng/L)	3.90	5.47	0.04	19	475
MeHg (ng/L)	0.03	0.02	0.01	0.21	21
TSe (ng/L)	77	37.57	36.5	170.6	4.67
TAs (ng/L)	512.44	156.86	306.8	742.6	2.42

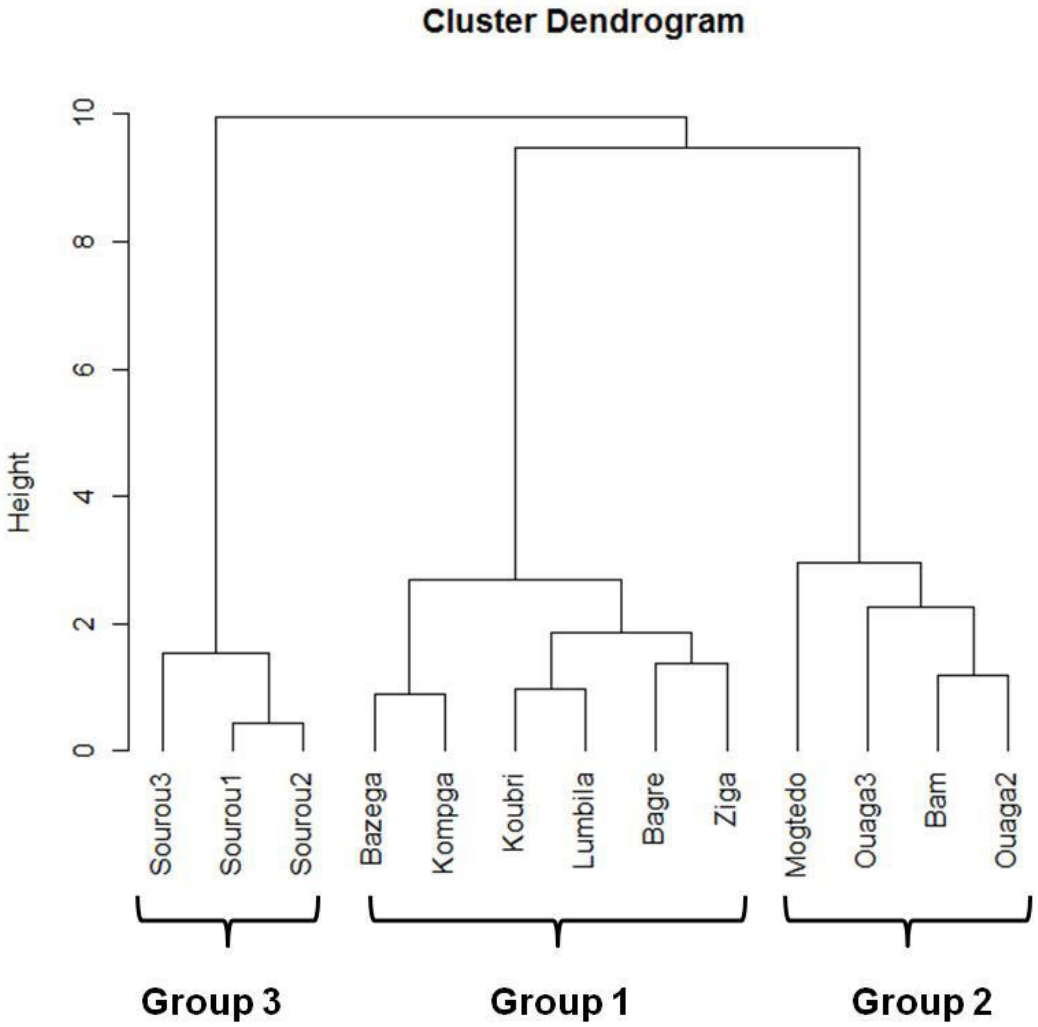


Figure 2.2. Hierarchical classification of study sites related to their trace element concentrations. Three main groups were shown by dendrogram which was confirmed by K-means partitions (Annexe 2, Fig. S2.2). Abbreviations: Ouaga 2, 3 refers to Ouagadougou dams 2 and 3, Lumbila refers to Loumbila and Kompga refers to Kompienga.

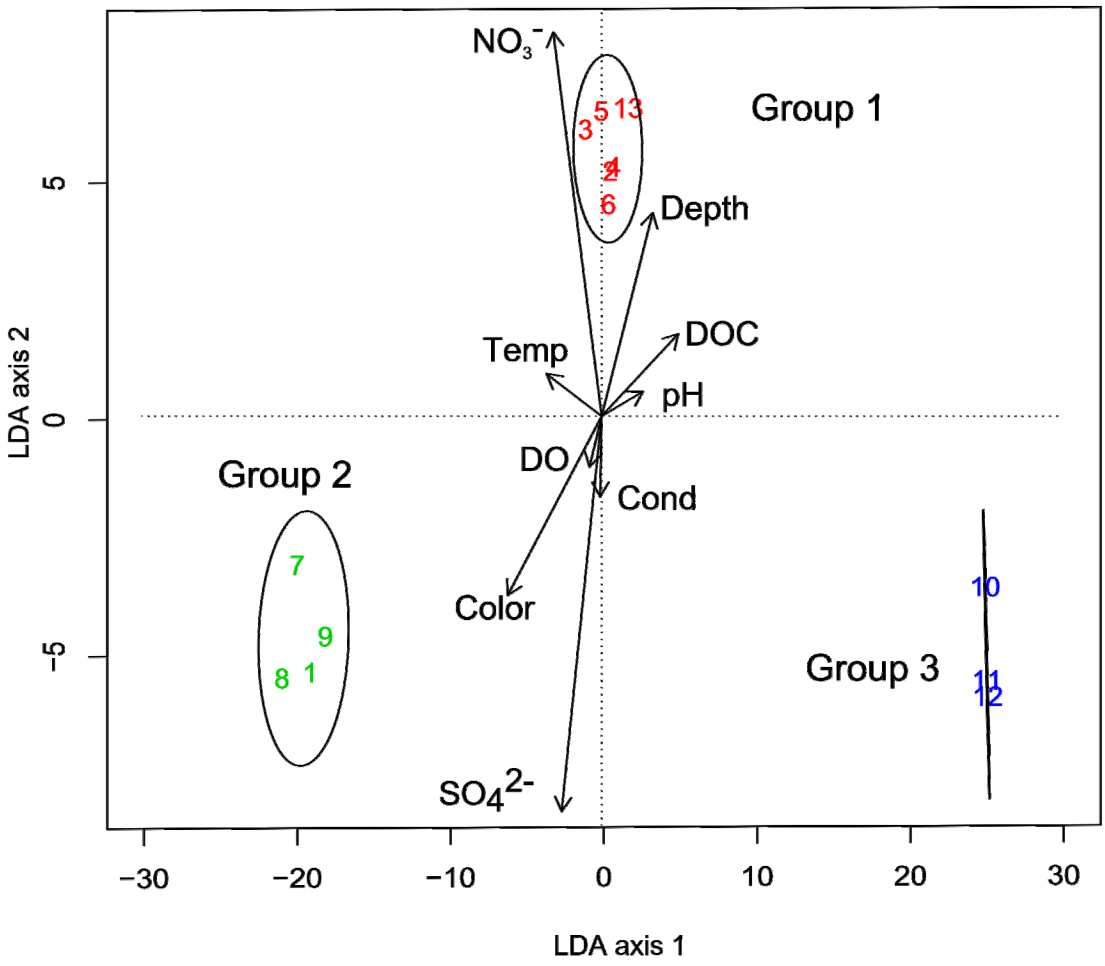


Figure 2.3. Relationship between site typology (based on metal(loid) levels) and water physicochemical variables by Linear Discriminant Analysis (LDA).The two axes accounted for more than 67% of among groups dispersion. DO, color, Temp and cond represent the descriptors dissolved oxygen, water color, temperature, and water conductivity, respectively. Numbers in each group ellipse represent sampling sites as defined in figure 2.1.

2.4.3 Metal(loid) levels in fish

A total of 11 fish species were represented in the samples collected and analyzed across the 10 reservoirs. Means and ranges of THg, MeHg, TSe and TAs concentrations in fish muscles from each reservoir are fully reported in annexe 2, Table S2.3 and Table S2.4. Scatter plot of fish metal(loid) concentrations versus their total length was used to appreciate the pattern of trace metal contamination of the four most common fish species through study sites (Fig.2.4). Horizontal dotted lines in Fig.2.4 represent the Hg threshold used to assess the extent of site contamination. The first line set at 0.1 µg/g w.w. refers to U.S.E.P.A. wildlife Hg threshold. MeHg concentration in fish above this limit may pose toxicity risk to fish themselves. The other one represents U.S.E.P.A. Water Quality Criterion (WQC) of MeHg set at 0.3 µg/g w.w. and represents the limit of safe fish consumption by humans in order to avoid neurotoxicity risk. A bottom feeder fish *O. niloticus* (n=138; Fig. 2.4A) had the lowest mercury concentration (under the wildlife Hg threshold) with ranges of 0.003 – 0.045 µg/g THg and 0.002 – 0.04 µg/g MeHg (Table S2.3). MeHg concentration in *A. occidentalis* (n = 52, Fig 2.4B) had similar contamination patterns than O. *niloticus,* with some fish exceeding the wildlife threshold. However, *C. anguillaris* (n = 39, Fig 2.4G) and *B. bajad* (n=33, Fig 2.4H) had high MeHg concentrations. Some fish exceeded the WQC limit of 0.3 µg/g w.w., particularly the piscivore *B. bajad* from the deeper reservoirs such as Kompienga and Bagré.

When the fish feeding habits (nonpiscivore, omnivore and piscivore) were considered (Fig.2.5), an increase of THg concentration with trophic level was observed, with mean THg concentrations of 0.06 µg/g for nonpiscivores, 0.128 µg/g for omnivores

and 0.166 µg/g for piscivores. Therefore, omnivores were two times more contaminated than nonpiscivores, and piscivores 1.3 times more than omnivores. These differences were statistically significant (ANOVA, $F = 76.2$, Tukey's post-hoc test, $p < 0.001$). For MeHg, mean concentrations in omnivores (0.091 µg/g) and piscivores (0.135 µg/g) were significantly higher than for non-piscivores (0.044 µg/g) (ANOVA, Tukey's post-hoc test, $p > 0.05$) (Fig.2.5).

Similar to Hg, the highest total selenium concentrations were recorded in the piscivores (*B. Bajad*; 0.189 – 0.672 µg/g, mean: 0.33 µg/g, n = 33; Annexe 2, Table S2.3; Fig. 2.4J). The bottom feeder *A. occidentalis* showed the lowest concentration of Se (0.057– 0.286 µg/g, mean: 0.11 µg/g, n = 54; Fig 2. 4D). Total selenium concentration in fish showed a weak increase with trophic level (Fig.2.5), however, significant difference was only found between TSe concentration of piscivorous fish and the two other trophic levels (ANOVA, Tukey's post-hoc test, $p < 0.001$) which were not different from each other.

TAs concentrations in fish ranged from 0.04 to 0.42 µg/g (mean: 0.07 µg/g) for *C. anguillaris* (Fig. 2.4K), from 0.05 to 0.32 µg/g (mean: 0.08 µg/g) for *O. niloticus* (Fig. 2.4E) and from 0.05 to 0.26 µg/g (mean: 0.07 µg/g; Fig. 2.4L) for the piscivore fish *B. bajad*. Many fish samples (74%) had TAs concentrations under detection limit of 0.4 µg/g (d.w.) corresponding to 0.08 µg/g (w.w). For this reason, TAs data were discarded for multivariate analysis.

Molar ratios of Se:Hg and As:Se per fish are reported in Figure 2.6. Most fish had Se:Hg molar ratios higher than 1 regardless of their guilds, with only 0.6% below this limit (Fig. 2.6, top panel). Further, 89% of all fish had As:Se molar ratios lower than 1

(Fig. 2.6, bottom panel). The only statistical difference in molar ratios between guilds was observed for Se:Hg ratios between nonpiscivores and piscivores with the piscivores displaying mean ratios three times lower than their counterparts (ANOVA, Tamhane test, $p < 0.001$).

Relationships with metal(loid) levels in the most common fish species and fish size were assessed by linear regression. Only *B. bajad*, a piscivore fish yielded a clear relationship between metal(loid) levels and fish length ($p < 0.001$) (Table 2.2). MeHg and THg were strongly correlated ($p < 0.001$) for all fish species examined.

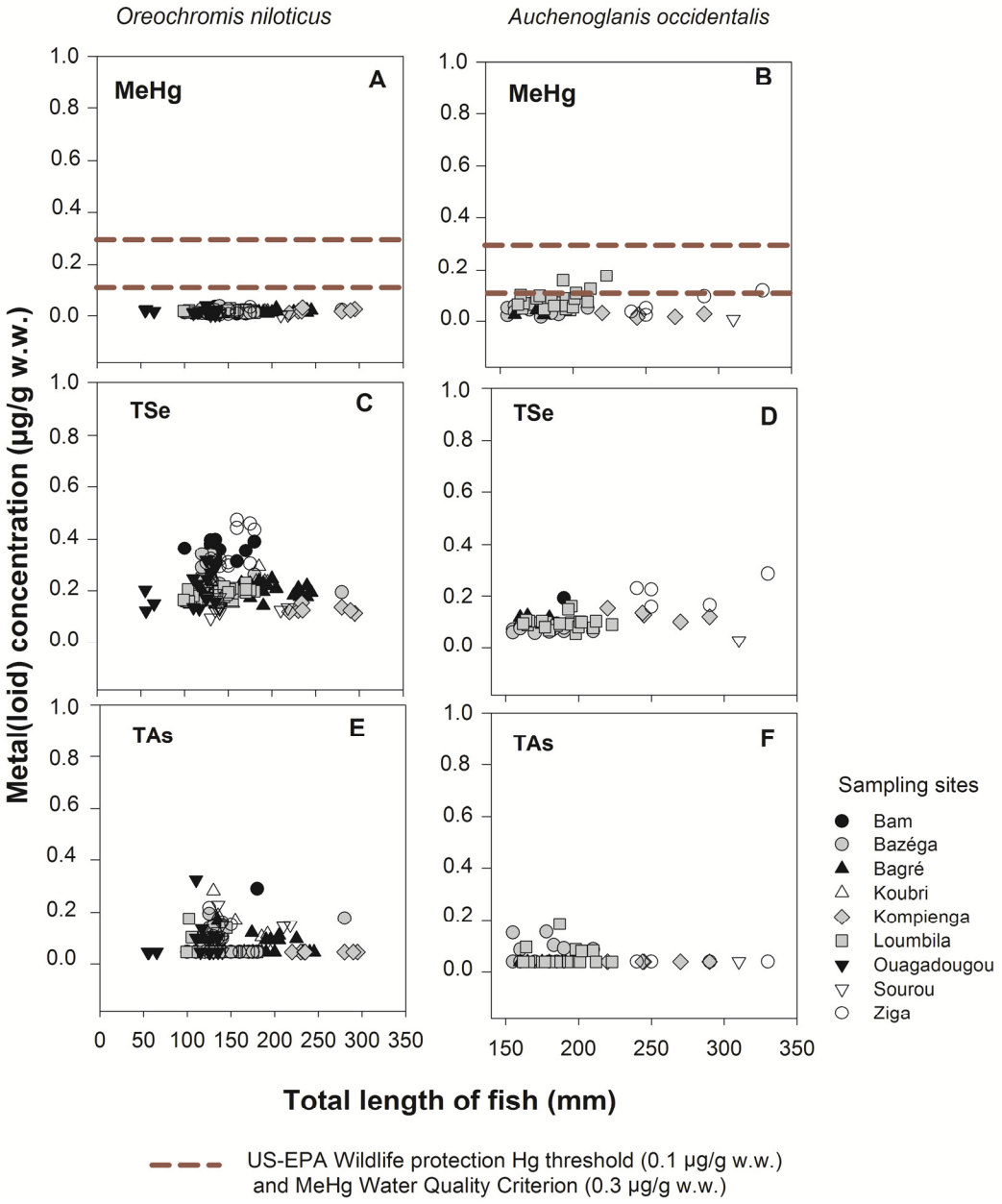


Figure. 2.4. Scatter plots of MeHg, TSe and TAs concentrations and total length of fish for the four most common fish species across study sites. Figures A, B, C, D, E and F represent scatter plots of MeHg, TSe and TAs for *Oreochromis niloticus* and *Auchenoglanis occidentalis*, and figures G, H, I, J, K and L),the scatter plots of the same metal(loid)s for *Clarias anguillaris* and *Bagrus bajad.*

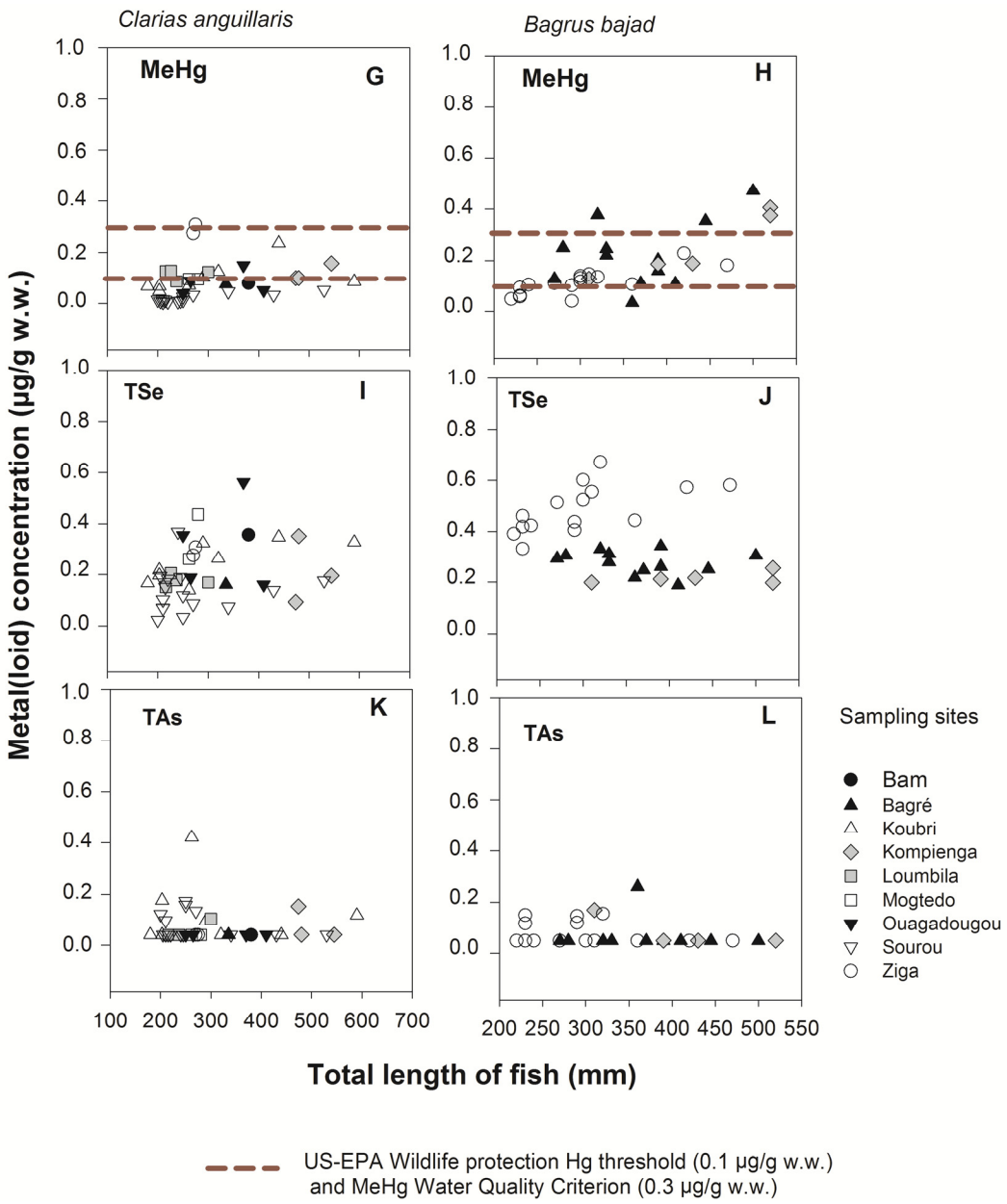


Figure 2.4. (continued)

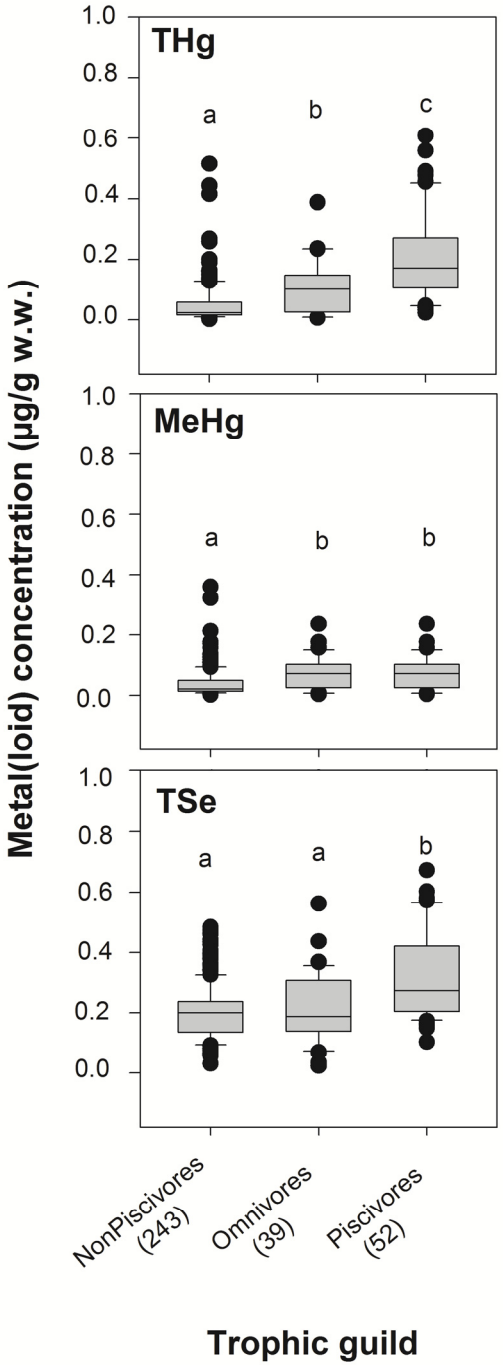


Figure 2.5. Box plots showing median values and 10th, 25th, 75th, and 90th percentiles of metal concentrations (THg, MeHg and TSe) in nonpiscivores fish (n = 243), omnivores fish (n = 39) and piscivore fish (n=51). The letter a, b and c showed significant level after ANOVA analysis followed by Tukey's test ($p < 0.05$).

2.4.4 Potential toxicological risk to biota

Fish Se and As levels did not pose a risk for both animals and humans. None of the fish had Se concentrations above the limit of 3 µg/g (w.w.) (Lemly, 1993) and As concentration close to 1 µg/g (w.w.) (Jankong et al., 2007) considered damaging for fish and other aquatic organisms. Therefore, we only assessed the potential toxicological risk to biota posed by Hg levels in fish (Table 2.3). Considering the total number of fish in our sample (n = 334), 21% exceeded the wildlife Hg threshold (0.1 μgHg/g w.w.) (U.S.E.P.A., 2001; Yeardley et al., 1998). Specifically, 71% of the total number of piscivores, 26% of omnivores and 9% of nonpiscivores exceeded this limit. There was only 2% of all fish (10% of piscivore) which exceeded the MeHg Water Quality Criterion (0.3 μg MeHg/g w.w.) (USEPA, 2001) for human health protection corresponding to World Health Organization guideline of 0.5 μg THg/g to protect groups vulnerable to mercury toxicity (FAO/WHO, 2004). When considering Se-Hg interactions only, 2 individuals had molar ratio of Se:Hg $\leq$ 1 (0.6% of the total sample) (Fig.2.6, Table 2.3). Consequently 0.6% of the total number of fish sampled will present health risks when considering the protective effect of Se on Hg.

Further, since As can potentially decreases the availability of Se as a protective element against Hg toxicity (Gailer, 2007), we calculated the difference between As and Se molar concentrations for each fish, and considered this difference as representing the minimal Se molar concentrations available for preventing Hg toxicity. We then recalculated the Se:Hg ratios, corrected for As (Table 2.3). When using this corrected

Se:Hg ratio, the number of fish that could present health risk related to Hg toxicity increased to 56 individuals (16.7% of the total exhibiting molar ratio of Se:Hg under 1) (Table 2.3). Note that an important caveat for the use of the proposed As correction factor is that most As species in fish are probably organic species, and there is a lack of study on the antagonistic role of each organic As species on Se cycling.

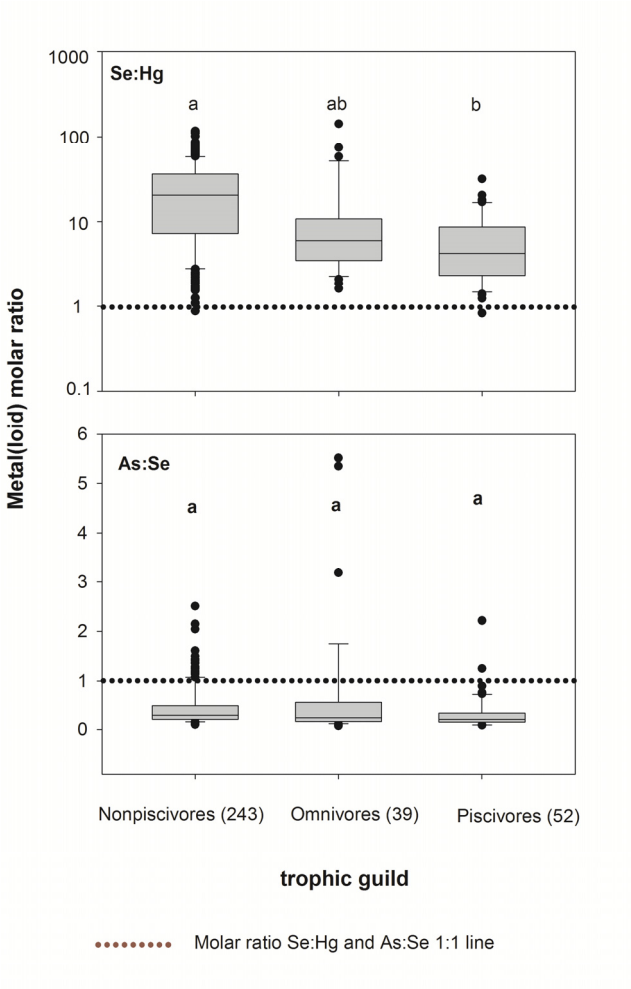


Figure 2.6. Box plots showing median values of molar ratio of Se:Hg and As:Se in collected fish from Burkina Faso according to their trophic guild. Top panel represents the Box plots of molar ratio of Se:Hg. and molar ratio of As:Se was in.bottom panel. Horizontal dotted line indicates the molar ratio 1:1 line. The letter a, b and c showed significant level after ANOVA analysis followed by Tamhane post test.

Table 2.2. Linear regression models predicting transformed metal concentrations in muscles of four fish species with other factors such as co-occurring metal(loid) and fish size (TL = Total length, "n" was the sample number used in analysis. $p < 0.05$ was the significant level, and bold figures correspond to significant results).

Fish species	Regression	n	intercept	slope	R^2	p-value
B. bajad	THg vs. MeHg	33	-0.01	1.33	0.96	**< 0.001**
	THg vs. TL		-0.22	0.0013	0.53	**< 0.001**
	TSe vs. TL		0.58	-0.0005	0.12	> 0.05
	MeHg vs. TSe		0.26	-0.233	0.08	> 0.05
C. anguillaris	log(THg+1) vs. log(MeHg+1)	39	-0.008	1.45	0.95	**< 0.001**
	log (THg+1) vs. TL		3.89	1.84	0.08	> 0.05
	log(TSe+1) vs. TL		0.13	0.0002	0.05	> 0.05
	log(MeHg+1) vs. log(TSe+1)		0.12	0.93	0.25	**< 0.01**
A. occidentalis	THg vs. MeHg	52	-0.007	1.42	0.91	**< 0.001**
	THg vs. TL		7.35	$2.13*10^{-5}$	0.0002	> 0.05
	TSe vs. TL		-0.006	0.0006	0.25	**< 0.001**
	MeHg vs. TSe		0.097	0.177	0.047	> 0.05
O. niloticus	log(MeHg) vs. log(THg)	138	-0.2	0.91	0.84	**< 0.001**
	log(THg) vs. log(TL)		-4.8	0.138	0.006	> 0.05
	log(TSe) vs. log(TL)		-1.07	0.096	0.006	> 0.05
	log(MeHg) vs. log(TSe)		-4.11	0.116	0.005	> 0.05

2.4.5 Allowable fish consumption estimates

Although the protective effect of Se on Hg toxicity has been well established for aquatic wildlife, there is currently no clear evidence that human dietary Se can modulate the toxicity of CH_3Hg^+ (Choi et al., 2008; Mergler et al., 2007; Saint-Amour et al., 2006). Consequently, conservative allowable fish consumption advisories were estimated here and given as recommendation for protecting local people at risk

Allowable consumption estimates are summarized in Table 2.4. Results show that, except for bottom feeders (*O. niloticus* and *A. occidentalis*), the other fish species should be consumed moderately. For instance *B. bajad* which showed MeHg concentrations above the WQC of 0.3 μg/g was hazardous to human health. Consumption of *B. bajad* with mean concentration of 0.2 μg/g w.w. Hg (mean concentration for *B. bajad* found in this study) should be limited to one fish per week both for men and women.

Table 2.3. Proportion of fish with mercury concentration potentially causing toxicological threat to wildlife and human. "n" represents the number of fish from each trophic guild above the threshold limits of each risk criterion and "%" represents the percentage of this number to the total number in the group. MeHg levels are those of USEPA WQC, 2001 for wildlife ($\leq 0.1 \mu g.g^{-1}$) and human (≤ 0.3 $\mu g.g^{-1}$) protection).

Potential risk for	**MeHg criteria**	**Nonpiscivores (243)**		**Omnivores (39)**		**Piscivores (52)**		**Total (334)**	
		n	%	n	%	n	%	n	%
wildlife	≥ 0.1 µg/g	22	9	10	26	37	71	69	21
	Molar ratio Se:Hg ≤ 1	1	0.3	0	0	1	2	2	0.6
humans	≥ 0.3 µg/g	2	1	0	0	5	10	7	2
	Molar ratio Se:Hg ≤ 1	1	0.4	0	0	1	2	2	0.6
	(Molar Se – Molar As)/Molar Hg ≤ 1	37	15	9	23	10	19	56	16.7

Table 2.4. Allowable fish consumption estimates. Mean weight, mean MeHg concentration of each fish species was used in calculation. Average body weight of 65 and 70 kg for woman and man respectively was considered."(n)" = sample number of fish species, range weight (min., max) = minimum weight and maximum weight,. O.= *Oreochromis,* A. = *Auchenoglanis*, C. = *Clarias*, L. = *Lates*, B.= *Bagrus.*

Fish species	**Number of fish sampled**	**Mean weight (g)**	**Mean [MeHg] (µg/g w.w.)**	**Allowable fish consumption per week (g) and indicative individual number for humans**			
				Amount per week(g)		Number per week	
				woman	man	woman	Man
O. niloticus	138	92 ± 84	0.01 ± 0.01	6933	7466	75	81
A. occidentalis	52	104 ± 75	0.06 ± 0.03	1762	1898	17	18
C. anguillaris	38	274 ± 350	0.09 ± 0.05	1143	1230	4	5
L. niloticus	10	468 ± 423	0.10 ± 0.05	1040	1120	2	2
B. bajad	33	321 ± 270	0.20 ± 0.10	520	560	1	1

2.5 Discussion

2.5.1 Metal(loid) levels in water

This study is one of the few published reports on the occurrence of mercury, selenium and arsenic in freshwater systems from African Sub-Saharan countries. Mercury concentrations in freshwaters from Burkina Faso are in the same order of magnitude than those reported by previous studies from similar ecosystems in Africa and from other tropical regions (Table 2.5). There was no evidence of significant Hg contamination of aquatic systems, despite the rising use and release of Hg from amalgamation process in small scale gold mining near our sampling sites (Ouattara, 2003). This result suggests that freshwater systems in Burkina Faso were not significantly affected by gold processing.

Aqueous MeHg concentrations in Burkina Faso ranged from 0.02 to 0.21 ng/L (mean = 0.03 ng/L), accounting for approximately 2% of total mercury concentrations in water (mean = 1.40 ng/L). These concentrations were in the range of those in similar tropical systems (Table 2.5). % MeHg/HgT in surface water was reported to be in the range of 0.1 - 5% (Mason et al., 2000) but, in anoxic freshwaters, higher % MeHg/THg are often encountered, with values up to 50 % (Balogh et al., 2006; He et al., 2007; Roulet et al., 2001; Zhang et al., 2010).

Table 2.5. Mercury, selenium and arsenic levels in freshwaters from previous studies in Africa and other parts of the world.

Locations	Type of system	THg range (ng/L)	MeHg (ng/L)	TSe range (ng/L)	TAs range (ng/L)	Reference
Review	Lake	0.14 – 15.1				Petrisor. (2006)
	Surface water	0.23 – 7.2				Petrisor. (2006)
	River	2 – 45				Petrisor. (2006)
Western Africa						
Burkina Faso	Dam, reservoirs	0.38 to 21.38	0.02- 0.21	36.2 - 170.5	306 - 742	This study
Burkina Faso	Dam, reservoirs	0.05 - 4				This study
Ghana	Mining area (Pra river)	28.7 – 420.3	0.0 - 19.6			Donkor et al. (2006)
	Uncontaminated site	3.22 (mean)				Bonzongo et al. (2004)
Eastern Africa						
Tanzania	Rwamagasa background	40-50			100 -500	Taylor et al. (2005)
Tanzania	Malagarasi River (Isingile)	70			970 (mean)	Taylor et al.(2005)
	Rwamagasa drainage system	10-70			130 -2420	Taylor et al.(2005)
	Rwamagasa Hg amalgamation ponds	430-450			540 -1500	Taylor et al. (2005)
Zimbabwe	River	20 -				Van straaten (2000)
Zimbabwe	Mining area (Tafuna Hill)	20 to 650				Van straaten (2000)
Uganda	Lake Victoria	0.7 - 200				Campbell et al. (2003a),
	Lake Victoria (Napoleon Gulf)	1.8 -15.5	1			Campbell et al. (2003b)
	Lake Victoria (Winam stream)	4.5				Campbell et al. (2003b)
	Lake Victoria (prison gulf)	3.2	0.7			Campbell et al. (2003a)
	Lake Victoria (Bugaia Island)	_	0.14			

Table 5 (continued**).**

Locations	Type of system	THg range (ng/L)	MeHg (ng/L)	TSe range (ng/L)	TAs range (ng/L)	Reference
Uganda	Lake Victoria	4	0.7			Campbell et al. (2003a)
South Africa	River	0.02 - 26.65 ± 3.53	0.02 - 0.89 ± 0.02			Walters et al. (2011)
						Williams et al. (2010)
	Mining area		2.73 ± 0.10			Walter et al. (2011)
	Limpopo				< 1000	Ogola et al. (2011)
Northern Africa						
Egypt	Lake Mariout	1.6 - 12.1				Campbell et al. (2003a)
Other locations						
Northern and Southern America	Florida Everglades	2.6 - 8.3				Liu et al. (2008)
	Everglades	0.9 – 27.1	0.08 -0.86			Babiarz et al. (2001)
	Wisconsin lakes	0.5 – 4.4	0.04 – 0.8			Watras et al. (1998)
	Solomon River			6750 ± 5560		May et al. (2008)
	Madeira River	9.51 (Mean)				Lechler et al. (2000)
	Tapajos River	4 (mean)				Lechler et al. (2000)
	Tapajos River	0.6 -2.6	0.02 – 0.24			Roulet et al. (2000 ; 2001)
China	Hongfeng Reservoir, Guizhou		0.062 – 0.24			He et al. (2008)
	Guizhou		0.031 - 25			Zangh et al. (2010)

Geochemistry of mercury is poorly documented in the water bodies of Africa; therefore, we can only speculate about causes for the low methylmercury concentration found in this study. Demethylation and reduction are two important sets of reactions in the biogeochemical cycling of mercury that could decrease MeHg levels in freshwaters. It is well-known that methylation is mostly due to sulfate-reducing bacteria. These bacteria are likely less active in shallow, well-oxygenated waters (Mason et al., 2000), such as those studied here (Annexe 2, Fig. S2.1). Under warm temperature as those encountered in these reservoirs, various microbial organisms are known to metabolically mediate demethylation and reduction of mercury (Barkay et al., 2003; Poulain et al., 2007). Reduction of mercury may be due to biotic and abiotic processes. Abiotic reduction of mercury under high UV irradiation is often the main process leading to volatile elemental mercury (Hg^0) formation hence, to high Hg evasion rates from lakes (Amyot et al., 1997a; 1997b; 2000). In the context of Burkina Faso, a country which receives high irradiance fluxes, photodegradation of MeHg processes could also lead to low net MeHg production in the water. Moreover, as suggested by Ikingura and Akagi (2003), semi-arid environment characterized by very low organic matter, and high mercury binding capacity of Fe-rich laterite soils in the reservoir catchments did not favor high levels of MeHg production and bioaccumulation. There is a need to assess biogeochemical cycling in water bodies of the countries from Africa in order to enhance our understanding on the fate of Hg in these aquatic systems.

Selenium concentrations in freshwater sampled from Burkina Faso were far lower compared to those reported by studies from similar tropical regions (Burger et al., 2001; May et al., 2008). For instance, May et al. (2008) reported mean selenium in water of 6.75 ± 5.56

µg/L over the entire Solomon River Basin (USA), an irrigated area. Our results were comparable to background levels reported in North America (Mailman and Bodaly, 2006). Our data on freshwater dissolved total arsenic (mean: 512 ± 157 ng/L) are close to those reported from uncontaminated areas from Africa (Taylor et al., 2005; Table 2.5). Comprehensive reviews on arsenic in natural waters have reported As concentration in freshwater less than 10 µg/L and frequently less than 1 µg/L (Reimann et al., 2009; Smedley and Kinniburgh, 2002). Lower As and Se concentrations in freshwater systems from Burkina Faso suggest that these aquatic ecosystems were not contaminated.

Multivariate analysis revealed that four key physicochemical variables played an important role in the co-occurrence of metal(loid)s in Burkina Faso. Study sites with the highest unfiltered Hg concentrations in water (sites with higher anthropogenic activities like those of Ouagadougou) had the highest SO_4^{2-} concentrations which are often associated with anthropogenic acidification. Anthropogenic activities include municipal sewage, manufacture, artisanal gold mining, fuel combustion and agriculture. These findings support the hypothesis that anthropogenic perturbations near city centers (Group 2 on Fig. 2.2; annexe 2, Table S2.2) are important sources of total and particulate Hg inputs to aquatic systems in Burkina Faso. Further, sites with relatively high MeHg concentrations (Group 1 on Fig. 2.2; annexe 2, Table S2.2) differed from others by their elevated water temperature, conductivity and lower nitrate (Annexe 2, Fig S2.3). High water temperature may have modified the balance between bacterial methylation and demethylation. High concentration of nitrate was reported in Group 2 is an agreement with anthropogenic source of pollution to theses reservoirs. Water color (which is influenced by the humic fraction of dissolved organic carbon) was reported to be

relatively higher in group 3 than in group 1 (Annexe 2, Fig S2.3). This fraction may affects availability of contaminants and may partly explain the lower MeHg levels, often under the detection limit, reported in these sites.

Overall, water temperature, conductivity, color and nitrate could be considered as the key factors which explained metal(loid) concentrations in the study reservoirs.

2.5.2 Metal(loid) levels in fish

Hg concentrations in fish species from Burkina Faso were similar or slightly higher than those of previous studies from similar ecosystems in Africa (Annexe 2, Table S2.6). They were in agreement with levels found in systems from nearby Ghana (Agorku et al., 2009; Donkor et al., 2006; Kwaansa-Ansah et al., 2011; Oppong et al., 2010); this can be explained by the resemblance of study sites (river systems) and by the fact that Ghana's reservoirs partly receive their waters from Burkina Faso rivers (Kwaansa-Ansah et al., 2011). The relatively low levels of Hg in African fish could be explained by different factors, including (1) short food-chain lengths, (2) increased growth dilution in fast-growing fish including higher tissue turnover in tropical biota; (3) biomass dilution in eutrophic systems. For instance, these mechanisms have been invoked to explain low Hg levels in Lake Victoria despite the presence of relatively high aqueous total mercury (Campbell et al., 2003a). Low MeHg levels in waters reported in some studies and in ours (Walters et al., 2011; this study) could also partly explain low Hg concentrations in fish.

Selenium concentration in fish from Burkina Faso reservoirs were low and similar to those reported from other river systems (Burger and Gochfeld, 2005; Burger et al., 2001). The low arsenic concentrations in fish observed in this study were in agreement with data reported for other freshwater fish species which were generally under 1 µg/g. (Jankong et al., 2007; Schaeffer et al., 2006). Low levels of As in fish samples may be explained both by low As levels in water and by biological metabolism of this element. In fish, inorganic arsenic species are metabolized by methylation leading to organic arsenic as the main arsenic forms. These forms are excreted much faster than inorganic ones (Mandal and Suzuki, 2002) thereby yielding to observed low level.

Fish Hg and Se concentrations increased from bottom feeders to piscivores suggesting that these elements are biomagnified through food webs. These observations are consistent with other studies from tropical (Campbell et al., 2008; Kidd et al., 2004; Kidd et al., 2003) and temperate zones (Cabana and Rasmussen, 1994; Chen and Folt, 2000; Chen et al., 2000).

Furthermore, from temperate studies, a positive correlation with size and MeHg concentrations in fish has been widely reported as the result of continual accumulation and slow depuration of mercury (Kidd et al., 2003). As indicated in Table 2.2, with the exception of the piscivore *B. bajad*, fish size did not explain metal(loid) fish concentrations. Other studies from tropical areas have sometimes also shown no correlation between fish Hg concentration and total length (Campbell et al., 2006; Kidd et al., 2003; Sampaio da Silva et al., 2009; Tadiso et al., 2011). *A. occidentalis* and *C. anguillaris* which were respectively bottom and benthic feeders, could accumulate metal in relation with habitat use rather than age, length or ontogenic diet shift. Fish foraging in habitat with high metal(loid) inputs will

have higher contaminant intake. The absence of significant relationships between Hg concentration and fish size has been previously reported for nonpiscivores fish in tropical studies (Campbell et al., 2003c; Desta et al., 2008; Kidd et al., 2004). This suggests that change in dietary habits, possibly as a result of seasonal food availability, may explain this phenomenon specifically observed in tropical regions. Therefore, knowledge of seasonal influence on metal(loid) dynamics is needed to further understand their bioaccumulation pattern in fish from tropical freshwater systems.

2.5.3 Potential health hazard assessment

21% of total fish collected from Burkina Faso exceeded the WQC criterion for aquatic wildlife protection suggesting hazardous effect for fish, since MeHg exposure may induce reproductive impairment (Crump and Trudeau, 2009). Early 1960's experimental studies have shown the antagonistic effects of selenium on mercury (Ganther et al., 1972; Parizek and Ostadalova, 1967). Recently, it has been proposed to use the molar ratio of Se:Hg as a criterion for fish health, with values of 1 and more being safe (Peterson et al., 2009; Ralston and Raymond, 2010; Yang et al., 2010). When applying the Se:Hg molar ratio approach, 99% of freshwater fish samples collected in Burkina Faso were safe for human consumption. However, when correcting Se:Hg ratios for the presence of As, an antagonist of Se, 83% instead the 99% of fish samples from Burkina Faso should be considered as safe for consumption by humans and wildlife. However, since most fish (66%) with As-corrected

Se:Hg ratios below 1 are nonpiscivores fish with low MeHg levels (Table 2.3), the toxicological risk associated with their consumption should be low.

There is still a controversy over how much Se (based on Se:Hg molar ratio) is needed to protect against Hg toxicity in humans (Burger and Gochfeld, 2012; Gochfeld et al., 2012). Indeed, Burger and Gochfeld (2012) suggest caution before integrating Se:Hg ratios in risk assessments for mercury toxicity. We therefore also calculated allowable fish consumption advisories in the traditional manner, excluding information on Se and As antagonism. Using this conservative approach, we conclude that piscivore fish like *B. bajad* inhabiting deeper reservoirs (Kompienga and Bagré) should be consumed moderately. The relationship between MeHg and fish size reported with *B. bajad* lead us to advise caution when consuming large individuals. These larger piscivores often exceed the WHO and US-EPA WQC set at 0.3 µg/g w.w. to protect vulnerable populations for Hg toxicity.

2.6 Conclusion

Metal(loid) water concentrations in Burkina Faso was mainly driven by water temperature, color, conductivity, SO_4^{2-} and NO_3^- content. Mercury and selenium levels in fish were mostly influenced by water DHg. The overall low concentrations of metals in water and fish species reported in this study suggest that the aquatic system have not been significantly impacted by trace metal contamination despite the rising mining activities and reservoirs

impoundment. Further investigations on Hg geochemical transformation are needed to provide better understanding on the fate of Hg in these ecosystems.

When considering Se:Hg ratios, 99% of Burkina Faso fish sample had sufficient Se concentration to potentially protect themselves and their consumers against Hg toxicity. However, when taking into account potential As/Se interactions, 83% of these sample should be considered as potentially protected by their selenium content. Se and As levels in fish were low and should not pose health hazard to wildlife and human. Since Se:Hg molar ratio is not yet a widely accepted tool in Hg risk assessment, local populations most at risk (women of child-bearing age and young children) should consider restricting their fish consumption of larger piscivores such as *B. bajad* and *L. niloticus*. Further investigation is needed to monitor piscivores fish contamination from the deeper reservoirs such as Kompienga and Bagré to provide safe consumption advisories for local inhabitants.

This study indicates that different advisories for fish consumption may be reached depending on the integration of information on Se/Hg and As/Se antagonistic interactions. Since fish is an important protein source for many populations and such elemental interactions are too poorly known to be routinely used in risk assessments, it is important to focus future research on the impact of mixtures of metalloids on Hg toxicity.

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Chapitre 3

Mercury and selenium bioaccumulation and trophic transfer in the food webs of African sub-tropical fluvial reservoirs (Burkina Faso)

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Article en préparation pour soumission à Ecotoxicology and Environmental Safety

3.1 Abstract

Little is known about the mechanisms controlling mercury (Hg) and selenium (Se) bioaccumulation and trophic transfer in sub-tropical African freshwater systems located near mining activities. During the 2010 rainy season, samples of water, sediment, fish, zooplankton and mollusks were collected from three reservoirs in Burkina Faso and analysed for total Hg (THg), methylmercury (MeHg), and total selenium (TSe). Ratios of $\delta^{13}C$ and $\delta^{15}N$ were measured to determine food web structures and patterns of contaminant accumulation and transfer to fish. Metal(loid) concentrations in aqueous and sediment compartments were relative low but overall higher in the profundal zone compared to littoral areas. Concentrations of THg, MeHg and TSe in fish were low and did not pose a health risk for humans. Food web structures were similar in the three reservoirs. Many fish were found to rely on littoral habitat and were associated with short food chains (3-4 levels). Biomagnification of Hg occurred, with a trophic magnification factor (TMF) for total mercury and methylmercury greater than 1 for each reservoir. There was little or no evidence of selenium biomagnification in food webs. Fish relative weight (W_r), which describes variation in fish body condition, was found to better predict Hg bioaccumulation than total length in some fish species, suggesting that Hg bioaccumulation in some fish may be due to effect of body conditions on food intake. Overall, food webs from western sub-Saharan reservoirs in gold mining areas were relatively short, relied mainly on littoral sources and were not highly affected by Hg and Se.

Keywords: Food web structure, mercury, selenium, biomagnification, stable isotopes.

3.2 Introduction

Aquatic environments are sinks for most contaminants including trace metals and metalloids. The sources, fate, and bioaccumulation of trace metals in aquatic systems have received greater attention since the Minamata and Niigata poisonings in the mid-twentieth century. Trace metal and metalloid contamination are a concern because they are toxic to aquatic organisms and humans and are persistent in the environment. The West-African landscape is today characterized by the presence of many small water reservoirs used for multiple purposes including livestock watering, irrigation, flood protection, groundwater recharge, and human drinking water (Boelee et al., 2009). Several countries from this region have also been subjected to increasing mining activities. After coal and municipal waste burning (Fitzgerald and Lamborg, 2005), mining activities are reported to be the most important anthropogenic source of trace metal inputs into the environment (Risher and De Rosa, 2007).

In a previous survey in Burkina Faso (Ouedraogo and Amyot, 2012), we reported relative low levels of Hg and TSe in fresh water and fish. In the present study, we focus on characterizing the little known processes controlling the bioaccumulation and subsequent biomagnification of trace metals and metalloids deposited into these aquatic systems. Bioaccumulation occurs when an organism absorbs a substance at a greater rate than it is excreted (Zhou et al., 2008). Biomagnification is the process by which the tissue concentration of a bioaccumulated substance increases as it transfered up the food-chain (Regoli et al.,

2012). Bioaccumulation and biomagnification of contaminants such as methylmercury (MeHg) has been shown to affect fish behavior and to induce reproductive impairment (Crump and Trudeau, 2009). Bioaccumulation and biomagnification potential is been important component in hazard assessment leading to a global evaluation of the risks that chemical substances may pose to humans and environment Metals and metalloids such as Hg and Se can also have antagonistic interactions that result in lower toxicity.We reported in our previous survey that up to 99% of the fish sampled could be protected from Hg toxicity because of adequate Se content in their tissues (Ouedraogo and Amyot 2012). An understanding of trace metal bioaccumulation and its movement through food webs is critical for conservation and management. Biomagnification of mercury was reported in large lakes from Africa (Campbell et al., 2006; France, 1995a; Kidd et al., 2003; Tadiso et al., 2011), but little is known about Hg biomagnification in smaller systems such as reservoirs and rivers in this locality.

Carbon and nitrogen stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) have been successfully used to analyze trophic relationships and food web structures in lake ecosystems from temperate zones (Cabana and Rasmussen, 1994; Peterson and Fry, 1987) and from tropical zone (Campbell et al., 2006; Jardine et al., 2012; Sampaio Da Silva et al., 2005). In aquatic environments, pelagic and benthic algae often show distinctive carbon signatures as a result of differential fractionation during carbon fixation (France, 1995b; Power et al., 2002) . Benthic algae generally exhibit less ^{13}C fractionation during carbon fixation than phytoplankton resulting in enriched $\delta^{13}C$ ratios. Further, $\delta^{13}C$ values are relatively unaffected by trophic transfer (< 1‰ fractionation between a predator and its prey) (Hecky and Hesslein, 1995).

Therefore, thereby it is commonly used to provide information about the sources of energy to food webs (Vander Zanden and Rasmussen, 2001). In addition, a stepwise trophic level enrichment in $\delta^{15}N$ by 3 - 4‰ (mean = 3.4 ‰) has been reported (Cabana and Rasmussen, 1994; Peterson and Fry, 1987), allowing the use of $\delta^{15}N$ ratios to trace contaminant biomagnification (Kidd et al., 1995; Post, 2002; Power et al., 2002).

High variability has been documented within and among systems in the $\delta^{15}N$ at the base of the food web from which organisms draw their nitrogen and carbon (Cabana and Rasmussen, 1996; Vander Zanden and Rasmussen, 1999). As a result, it has been suggested for among system comparisons to use $\delta^{15}N$ signatures of primary consumers (rather than primary producers) as baseline indicators for estimating trophic position because their large body size and greater longevity result in less seasonal changes in $\delta^{15}N$ (Cabana and Rasmussen, 1996; O'Reilly et al., 2002; Vander Zanden and Rasmussen, 1999).

The first objective of this study was to describe the structure of freshwater food webs in Burkina Faso reservoirs using carbon and nitrogen stable isotopes. The second objective was to characterize Hg and Se bioaccumulation rates in fish and to determine biological factors responsible for Hg and Se concentrations, taking into account their antagonistic interactions (Belzile et al., 2006). The third objective was to assess Hg and Se biomagnification in these food webs. We hypothesized that the food webs are short because many fish in the study region are opportunistic or omnivorous feeders (N'guessan et al., 2010; Ouéda, 2009). Nevertheless, we anticipated positive significant relationships between log-transformed concentrations of Hg or Se and adjusted $\delta^{15}N$ signatures reflecting food web biomagnification.

3.3 Material and Methods

3.3.1 Study sites

The three reservoirs were located in the central part of Burkina Faso in the Nakambe catchment area (Fig.3.1). The first one, Loumbila dam (12° 29'N, 1° 24' W) is a small man-made reservoir used to provide water for people, livestock and agriculture. It has an average surface area of 1500 ha and a mean depth of 6.5 m. The second system, Ziga dam (12° 30' N, 1° 4' W) is a 8,000 ha reservoir that was built twenty years ago for a drinking water supply. The third reservoir, Kompienga (11° 5' N, 0° 41' W), is one of the two largest hydroelectric reservoirs in the country with a surface area ranging from 16,000 to 20,000 ha. All sites had warm waters (26 – 30°C), low levels of dissolved organic carbon (DOC) (average of 2 mg/L), were not thermally stratified during the sampling period (Annexe 3, Figure S3.1) and had a circumneutral pH (Annexe 3,Table S3.1). Trace element levels in water were found to be similar in these three reservoirs in a previous field survey (Ouedraogo and Amyot, 2012). Differences in watershed use and reservoir size (depth, area) as well as the age of the reservoir led to the choice of these sites to investigate bioaccumulation and biomagnification patterns, since they cover the range of aquatic systems found in Burkina Faso.

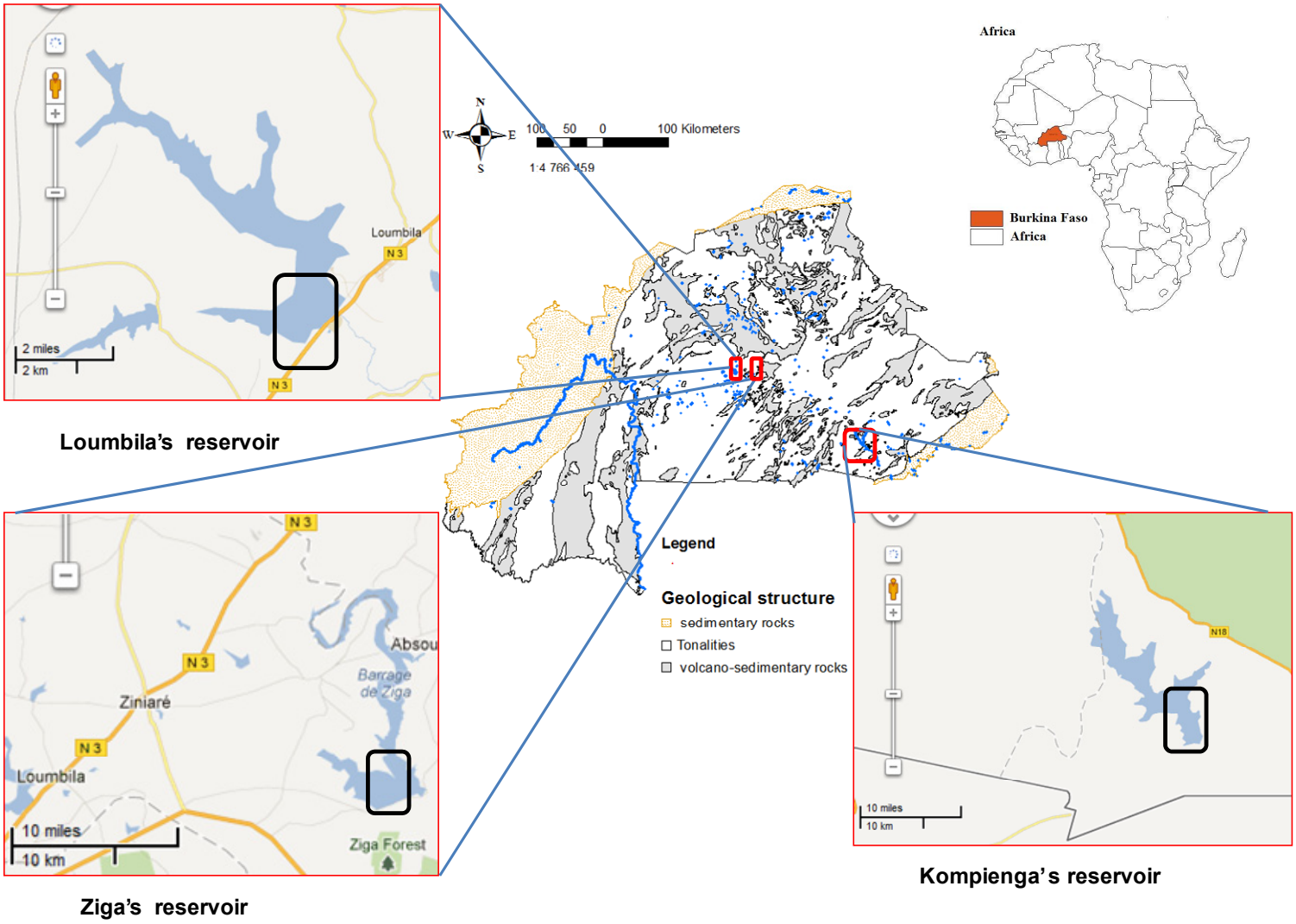

Figure 3.1. Map of Burkina Faso showing the locations of the three sampling sites.

3.3.2 Field sampling

During the 2010 rainy season (July-August) in Burkina Faso, water, sediment, zooplankton and fish were collected from the three Nakambe catchment reservoirs.

3.3.2.1 Water collection

Ultra clean protocols for trace metals (U.S.E.P.A., 1996) were employed to collect water. In each reservoir, water was collected on one occasion at the near shore station (littoral zone) and open water station (pelagic zone) at a depth of 0.5 m from the sediment surface where THg and MeHg concentrations were potentially higher (Morel et al., 1998). Water was collected with a peristaltic pump and acid-washed Teflon tubing. Triplicates of filtered and unfiltered water samples for THg and MeHg were collected and stored in 125 mL amber glass bottles that had been pre-washed with acid, thoroughly rinsed with ultrapure water (Milli-Q: > 18 Mohm cm^{-1}), and placed in double ziplock bags for transport to the field. Filtration was done on site using a Whatman syringe filter of 0.45 μm pore size. Bottles were rinsed three times with the sample water prior to collection. All aqueous mercury samples were preserved at pH 2 with ultra high purity hydrochloric acid (VWR) (0.5%, v/v), kept in a field cooler, and refrigerated (+ 4 $^{\circ}C$) upon return to the laboratory until analysis.

Samples for ancillary chemical analyses were also collected at the same depth. Filtered water was collected in two separate 30 mL Nalgene HDPE bottles. One was acidified using hydrochloric acid (0.5%, v/v) for major cations [potassium (K^+), calcium (Ca^{2+}), sodium (Na^+)

and magnesium (Mg^{2+})], while the second was left unacidified for analysis of anions [chloride (Cl^-), nitrite-nitrate (NO_2^-, NO_3^-), sulphate (SO_4^{2-})]. For dissolved organic carbon (DOC) analyses, water samples were filtered with 0.45 μm Whatman filters and stored in glass bottles (pre-heated at 550 °C for 1 hour). Filtered water samples for Se analysis were taken in 30 mL Nalgene HDPE bottles samples and preserved with 1% v/v EDTA (Bednar et al., 2002).

3.3.2.2 Sediment sampling

Sediments were sampled by Ekman grab from littoral and pelagic sites. Duplicate samples of sediments were taken from the surficial 5 cm layer, avoiding sediment directly in contact with the Ekman, freeze dried in clean polyethylene vials, and stored in double Ziploc bags at 4°C until analysis.

3.3.2.3 Zooplankton sampling

Zooplankton were sampled for trace metal and stable isotope analyses with a 0.25 m diameter net of 100 μm mesh size in the pelagic zone of each lake by vertical hauls. A large volume of water was filtered to collect enough biomass for analyses. Samples were transferred into clean Teflon containers, placed in double Ziploc bags at -20°C, freeze-dried and stored in double Ziploc bags at 4°C until analysis.

3.3.2.4 Benthos and fish sampling

Fish samples were bought from local fishermen. Twenty to thirty fish from three size classes, small (generally small: < 150 mm), medium: (151 - 400 mm) and large: > 400 mm. (see annexe 3, Table S3.2 for species-specific classes) were obtained for each fish species. After sex identification and body measurements, sections of dorsal muscle tissue were taken for analyses of trace element and stable isotope ratios. Dorsal muscle samples were kept in polyethylene bags, frozen at -20°C, freeze-dried and shipped to Canada for laboratory analysis. Approximately 350 individual fish were collected and included the five fish species most consumed by the local population, namely *Oreochromis niloticus*, (Nile tilapia, Cichlidae, detritivore), *Clarias anguillaris* (Catfish, Clariidae, omnivore), *Bagrus bajad* (Bagridae, piscivore), *Auchenoglanis occidentalis* (Claroteidae, invertebrates feeders), and *Schilbe intermedius* (Schilbeidae, invertebrates feeders-piscivore). Additional species such as *Lates niloticus* (Nile perch, Centropomidae, piscivore), *Synodontis membranaceus* (Mochokidae, planktivore), and *Hydrocynus forskalii* (Alestidae, piscivore) were also collected. Profundal unionids were sampled using Ekman grab and gastropods were hand removed by fishermen from their nets. All invertebrates were prepared in the same manner as fish for trace metal and isotopic analyses.

3.3.3 Laboratory analysis

3.3.3.1 Total mercury analysis

THg in water samples (filtered and unfiltered) was measured by cold vapor atomic fluorescence spectrometer (CVAFS, Tekran 2600, Tekran Instruments Corporation, Knoxville, TN, USA) following U.S. Environmental Protection Agency (U.S. EPA) method 1631. Briefly, 50 mL of sample was digested with 200 µL of BrCl, and excess of BrCl was neutralized with 50 μL of hydroxylamine. Samples were then reduced with stannous chloride ($SnCl_2$, 3% w/v), purged and trapped by two-stage gold amalgamation prior to analysis. The detection limit for this analysis was 0.13 ng THg/L and the mean relative recovery was 104 ± 5% (n = 5). The coefficient of variation (standard deviation/mean) for field triplicate determinations was 2%.

Solid tissues from aquatic organisms (fish, zooplankton, bivalves, gastropods) and sediments were analyzed for THg using a direct mercury analyzer (DMA 80, Milestone Inc., Pittsburgh, PA), in which samples were combusted at 750°C and mercury vapors were retained on a gold trap for analysis by cold vapor atomic absorption spectrometry. DMA threshold analysis was between 0.12 and 600 ng and detection limit was 0.05 ng THg/sample, with average analytical variance of 5%. The certified reference materials TORT-2 (lobster hepatopancreas, National Research Council, Canada) and DORM-2 (National Research Council, Canada) were used for quality control (Annexe 3, Table S3.3).

3.3.3.2 Methylmercury analysis.

Water (50 mL) for MeHg analysis was acid-distilled to remove matrix interferences, then derivatized by aqueous-phase ethylation with $NaB(C_2H_5)_4$, purged on Tenax (Tenax Corporation, Baltimore, MD, USA), separated by gas chromatography and quantified with a Tekran 2500 CVAFS (Tekran Instruments Corporation) based on the method of Bloom (1989). Field and procedural blanks contained less than 1 ± 1 pg MeHg and indicated no contamination during sampling, filtration, distillation, and analysis. Analytical accuracy was checked by analysis of TORT-2 (Annexe 3, Table S3.3).

For MeHg analysis in solid tissues (fish, zooplankton, gastropods and bivalves), 10 to 50 mg of dried homogenized tissue was digested in 5 mL of 4M HNO_3 at 55 ˚C for 16 h. Digested samples then underwent aqueous-phase ethylation followed by gas chromatography separation with CVAFS detection (Tekran 2500). Analytical accuracy was checked by analysis of TORT-2 after each 10 samples (Annexe 3, Table S3.3). The method detection limit (MDL) based on three times the standard deviation of 10 blanks was 0.02 ng/L. The average coefficient of variation (standard deviation/mean) for field triplicate determinations was 13%.

3.3.3.3 Selenium analysis

The protocol for Se determination was the same as in Ouedraogo and Amyot, (2012). For TSe analysis of water, 4 ml of water sample was digested in an acid mixture of HCl (4 mL) and HNO_3 (0.48 mL) to reduce Se (VI) to Se (IV). $NaBH_4$ (1.1 % m/v in 0.1M NaOH v/v) was added to digested sample to produce hydrides and the concentration of TSe was determined by Hydride Generation Atomic Fluorescence Spectrometry (HG-AFS, PSA

10.055, Millenium Excalibur; PS Analytical, Orpington, Kent, UK). For TSe determination in solid samples (fish, zooplankton, gastropods and bivalves), 20 to 50 mg of solid tissue was digested in a microwave with a mixture of HNO_3 and H_2O_2 based on a method developed by Corns et al. (1993) in order to extract elements from a solid matrix. An aliquot was then taken and treated using the same protocol for aqueous samples.

The analytical quality for TSe was controlled using certified reference materials DORM-3 and TORT-2 from the National Research Council of Canada (Annexe 3, Table S3.3). Efficacy of Se (VI) conversion to Se (IV) was checked by using a solution of Se (VI) that was analyzed together with the samples. Procedural blanks contained 21 ± 8 ngTSe L^{-1} (n=8). The MDLwas 22 ng L^{-1}(aqueous Se) and 0.022 µg/g dry weight (d.w.) for solids samples. Conversion of 200 ngL^{-1}Se (VI) to Se (IV) averaged 109 % ± 9.

3.3.3.4 Other physico-chemical measurements

Anions (Cl^-, SO_4^{2-}, NO_3^-) were analysed by ion chromatography using a DIONEX-DX500 (MDLs: 1 µmol L^{-1} for the three ions). Cations (Ca^{2+}, Mg^{2+}, K^+ and Na+) were measured by atomic absorption spectrometry with MDLs of 0.5, 0.1, 0,5 and 0.5 µmol L^{-1}, respectively.

Vertical profiles of water temperature (^oC), dissolved oxygen concentration (%), pH, and conductivity (µS cm^{-1}) were measured at 0.5 m intervals at each site using a YSI-650 DMS multiprobe. Dissolved oxygen and pH calibrations were completed every sampling day.

3.3.3.5 Stable isotope analyses

Stable isotope analyses were conducted at the GEOTOP research centre of the Université du Québec à Montréal (UQÀM). Prior to analysis, freeze-dried fish and invertebrate tissue samples were homogenized into a powder. Zooplankton samples were analyzed in bulk due to their small size. Small sub-samples of ground tissues were weighed in tin cups and analyzed on a Micromass Isoprime^{TM} isotope ratio mass spectrometer in continuous flow mode coupled to an Elementar Vario Micro Cube^{TM} elemental analyzer. $\delta^{15}N$ and $\delta^{13}C$ values were normalized using three internal reference materials and calibrated against nitrogen gas (N_2) in ambient air, and a PeeDee belemnite (CO_2) respectively as follows:

$$\delta X = [(R_{sample}/R_{standard})-1]\times 1000$$

where: X = ^{13}C or ^{15}N, R_{sample} and $R_{standard}$ are the molar ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample and in the international standard, respectively. Analytical precisions for $\delta^{13}C$ and $\delta^{15}N$ measurements were 0.1‰ and 0.2‰ respectively.

3.3.4 Data analysis

3.3.4.1 Fish body condition

Fish relative weight (W_r) was used as index of fish body condition and calculated according to Anderson and Neumann, (1996) reported by Ditteman and Driscoll, (2009), using the following equation:

$$W_r = W/W_s \times 100,$$

where W is the actual weight of fish and W_s is the length-specific standard weight of fish population. W_s can be predicted by the relation $W_s = a \times L^b$, where L is the total length of fish**.** The $\log_{10}$ – transformation allowed calculation of W_s by the linear weight-length regression: $\log_{10}(W_s) = \log_{10}(a) + b \times \log_{10}(L)$. Coefficients *a* and *b* were determined by the linear weight-length regression between actual weight and standard length (L_s) of fish (Annexe 3, Table S3.4). For a given fish population, when *b* (the slope of the linear regression between actual weight and standard length of fish) ranged from 2.5 to 3.5, the growth of this population was considered isometric. In this case W_s was calculated using coefficient *a* and *b.* When b is out of this range, the growth of the fish population is allometric and W_s was not calculated (Pauly, 1997).

According to Ditteman and Driscoll, (2009), mean W_r of 100 for a population is an indicator of optimal ecological and physiological conditions for fish, whereas, mean W_r of fish population under 100 may reflect food scarcity or poor feeding conditions, and a W_r value above 100 may indicate that fish are not making the best use of abundant prey.

3.3.4.2 Metal(loids) bioaccumulation factor

The degree to which bioaccumulation occurs can be expressed as a bioaccumulation factor (BAF) and at steady state the BAF can be calculated as the concentration of the substance in the organism (CB; $g.kg^{-1}$ w.w basis) divided by the concentration of the same element in the dissolved water phase (CW; g/L), where BAF (L/kg) = CB/CW. In hazard

assessment, regulatory agencies such as Environment Canada categorise chemicals with a BAF higher than 5000 (w.w. basis, log-value ≥ 3.7) as bioaccumulative (Arnot and Gobas, 2006; Borga et al., 2005). Bioaccumulation factors (BAFs) were reported here in $\log_{10}$-transformed basis to allow comparisons and to approximate normal distribution in multivariate analyses.

3.3.4.3 Biomagnification and trophic magnification factor

Within each reservoir, log-transformed metal(loid) concentrations were regressed against adjusted $\delta^{15}N$ ($\delta^{15}N_{adj}$) ratios to assess biomagnification rates. Biomagnification rate, referred to as a biomagnification factor (BMF), is represented by the slope of log-metal(loid) concentration versus $\delta^{15}N_{adj}$ (Kidd et al., 1995). It estimates the average increase in metal(loid) concentration per unit $\delta^{15}N$. A trophic magnification factor (TMF) is the average factor change in metal(loïd) concentration between two trophic levels (Hallanger et al., 2011; Jardine et al., 2012; Kidd et al., 2012). TMFs were calculated as the antilogarithm of *m* (TMF = 10^m), where *m* is the slope of the regression log-transformed-metal(loïd) vs. $\delta^{15}N$ multiplied by 3.4 (the average increase in $\delta^{15}N$ per trophic level, (Minagawa and Wada, 1984). A TMF above 1 indicates an increase in metal(loïd) concentration with increasing trophic position (i.e. food web biomagnification) whereas, a TMF < 1 indicates trophic dilution (Arnot and Gobas, 2006; Hallanger et al., 2011).

3.3.4.4 Food web analysis

To assess the significance of pelagic versus littoral carbon sources in the diets of fish, $\delta^{13}C$ ratios of a littoral primary consumer (gastropod) and a pelagic primary consumer (Unionidae bivalve or zooplankton in the case of Kompienga reservoir where bivalves were not collected) were used to calculated the percentage contribution of each source (Post, 2002) with the following equation:

$$\alpha = (\delta^{13}C_{organism} - \delta^{13}C_{(gastropod)})/(\delta^{13}C_{(unionid)} - \delta^{13}C_{(gastropod)} \times 100)$$

where α is the percentage contribution to the consumer signature from pelagic carbon. The contribution (%) of littoral carbon to the consumer signature was given by 1- α.

Classification of organism into those relying upon pelagic carbon (α > 70%) and those relaying mainly upon littoral carbon (α < 70%) was done according to Kidd et al., (2001). Because α indicated the predominance of littoral carbon contributions to fish $\delta^{13}C$ signatures (Table 1), the $\delta^{15}N$ signature of gastropods, a littoral primary consumer common to all study reservoirs, was chosen as baseline to standardize the $\delta^{15}N$ values of the other consumers collected in each reservoir. $\delta^{15}N_{adj}$ values were calculated using the approach of Cabana and Rasmussen (1996).

$$\delta^{15}N_{adj} = \delta^{15}N_{organism} - \delta^{15}N_{gastropod}$$

Corrected trophic position of consumers (TP) was then calculated using $\delta^{15}N_{adj}$, as follows:

$$TP = \lambda + \delta^{15}N_{adj}/\Delta_n$$

where Δ_n represents the isotopic enrichment per trophic level assumed to be 3.4‰ (Minagawa and Wada, 1984; Post, 2002), λ is the trophic position of the baseline indicator. The littoral primary consumer gastropod was assumed to be at a trophic position = 2.

3.3.4.5 Statistical analysis

All the results are expressed as a mean ± standard error. Prior to analysis, normality and homoscedasticity were checked using the Shapiro-Wilk test and *F* ratios, respectively. Total length, relative weight (body condition), and metal(loïd) concentrations were standardized (log transformation, square root transformation) to reduce data skewness and to approximate normal distribution.

Within each reservoir, multiple linear regression analysis between the $\log_{10}$-transformed BAFs and biological variables (total length, relative weight) and co-occurring metal concentrations for each fish species and biological variables (total length, relative weight) and co-occuring metal(loid) concentration for each fish species was used to identify key factors influencing metal(loid) accumulation. Prior to this regression analysis, forward selection analyses allowed the removal of descriptors with $p > 0.05$. Within each reservoir, log-metal and metal(loid) concentrations were regressed against trophic position to evaluate the potential for contaminant biomagnification in food webs. Site differences in BAFs, $\delta^{13}C$ and $\delta^{15}N_{adj}$ ratios and mean trophic position of each fish species were investigated by one-way ANOVAs and non parametric tests. Statistical analyses were performed with R software (version R-2.11.1) (http://www.r-project.org/).

3.4 Results

3.4.1 Mercury and selenium levels in water and sediment

During the rainy season of 2010, unfiltered THg and dissolved mercury (DHg) concentrations ranged from 2.43 ± 0.04 to 5.18 ± 1.05 and 1.52 ± 0.2 to 2.5 ± 0.1 ng/L, respectively (Fig. 3.2). On average, DHg account for 57% ± 12% of unfiltered THg. MeHg levels ranged from 0.037 ± 0.04 to 0.196 ± 0.08 ng/L and represented 2.6 - 6.6% of DHg. Within each reservoir, no statistical differences were found for unfiltered THg and DHg levels between littoral and pelagic zones (Wilcoxon rank test, $p > 0.05$) (Fig. 3.2). However, MeHg concentrations in the pelagic zone were 3 times higher than those in the littoral zone (Wilcoxon rank test, $p < 0.05$) in Loumbila and Ziga, but were similar in Kompienga (Fig. 3.2). THg levels in Ziga reservoir were significantly higher than at the two other sites (ANOVA, $F = 61.406$, $df_{2.15}$; $p < 0.001$). However, levels of DHg were higher in Loumbila than those of Ziga and Kompienga (ANOVA, $F = 34.279$, $df_{2.15}$; $p < 0.001$). Average MeHg concentration was not significantly different among the three study sites ($p > 0.05$). Average TSe concentrations ranged between 55.8 ± 12.7 to 72.7 ± 15 ng/L and did not differ significantly among the three study sites ($p > 0.05$; Fig. 3.2).

THg concentrations were 2.5, 2.0 and 1.4 times higher in profundal sediments than littoral ones for Loumbila, Ziga and Kompienga reservoirs, respectively (Fig. 3.3; annexe 3, Table S3.1). These differences were statistically significant (Wilcoxon rank test, $p < 0.05$).

The same trend was found for TSe except in Kompienga sediments where differences were not significant (Fig.3.3, Wilcoxon rank test, $p > 0.05$).

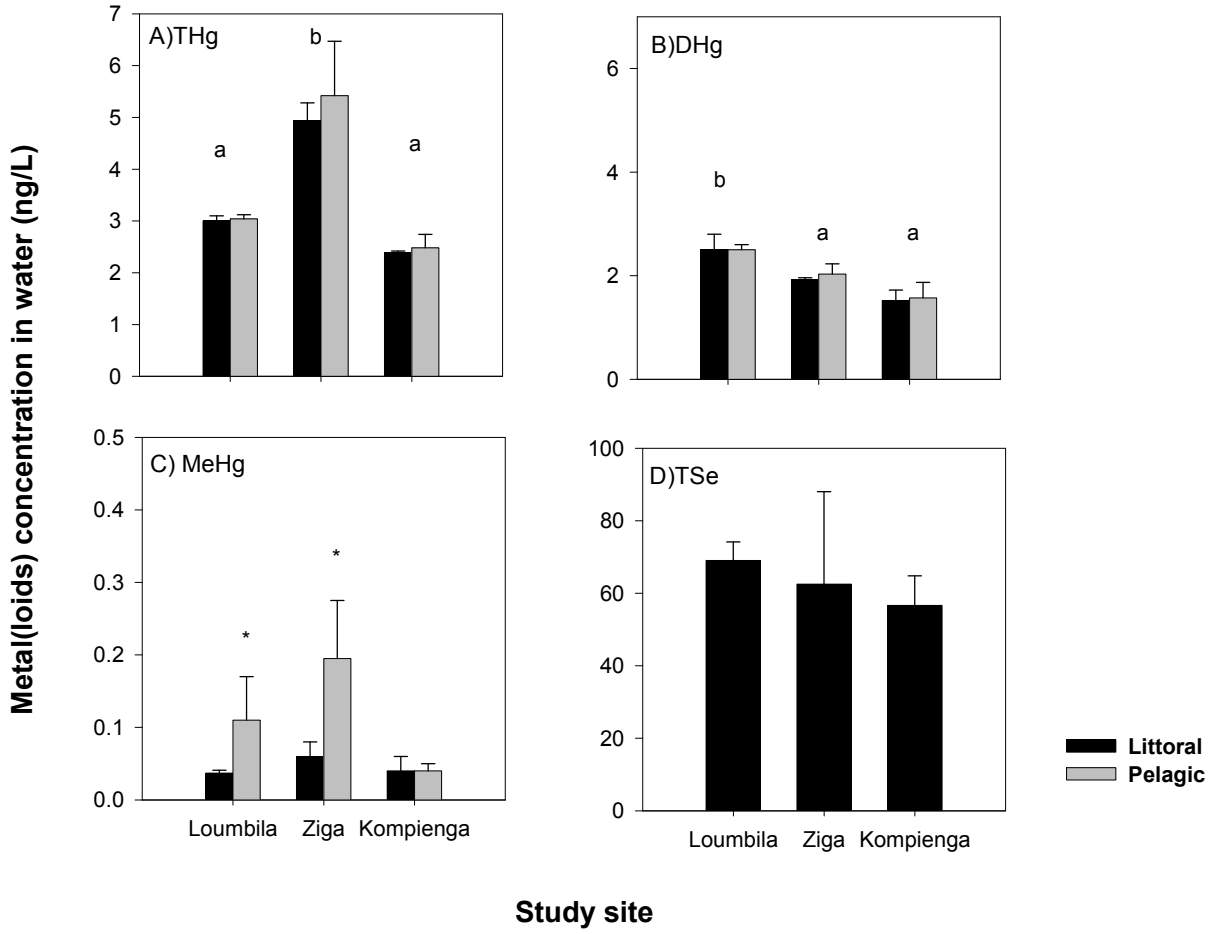

Figure 3.2: Distribution of mercury species and selenium in fresh waters from Burkina Faso during 2010 rainy season. Significant differences between littoral and pelagic samples are shown by an asterisk (*) according to a Wilcoxon rank test ($p < 0.05$). Significant difference between study sites are shown by letter a and b (ANOVA, $p < 0.05$).

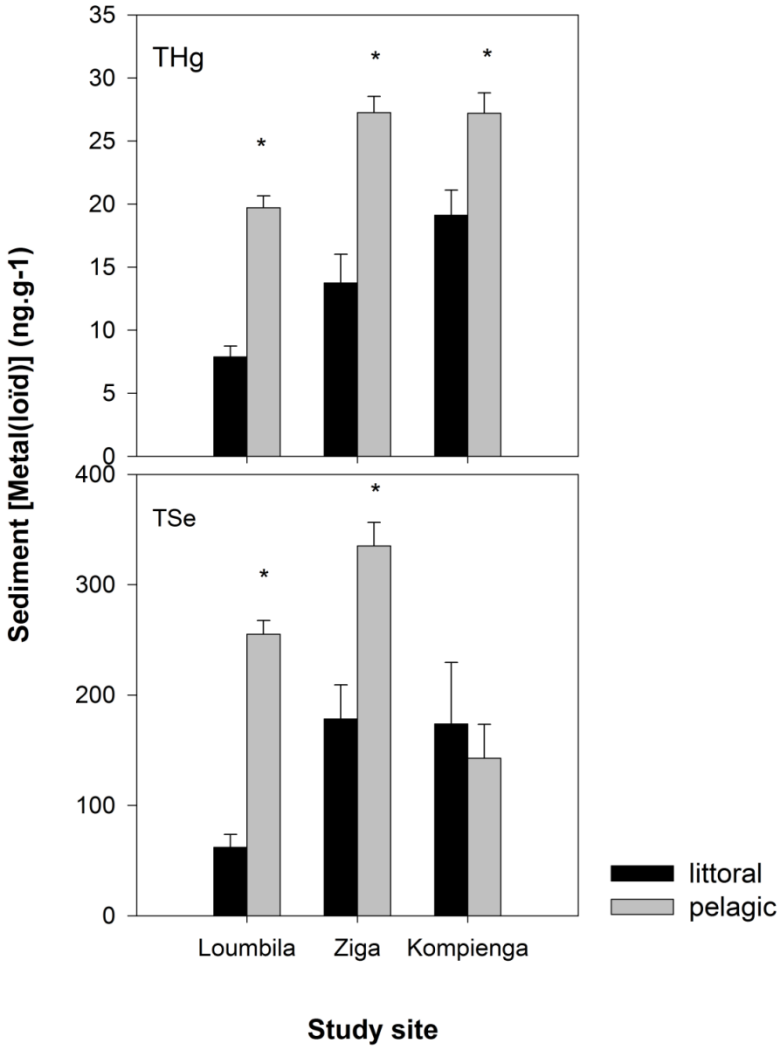

Figure 3.3 Mercury and selenium concentrations in sediment (d.w) from three study sites during 2010 rainy saison. Significant difference between littoral and pelagic samples was shown by an asterisk (Wilcoxon - test, $p < 0.05$).

3.4.2 Food webs structure and pathways of metal(loïds) transfer

3.4.2.1 Food webs structure

Carbon stable isotope ratios indicated that across the study reservoirs, mean use of littoral carbon for each fish species was greater than 70% suggesting that most fish rely mainly on littoral diet sources, except for *A. occidentalis* from Kompienga (65% ± 39%) and *O. niloticus* from Loumbila (35% ± 27) (Table 3.1).

Scatterplots of corrected $\delta^{15}N$ against $\delta^{13}C$ ratios of all biota show both the carbon sources and trophic position for all fish species (Fig. 3.4). For Loumbila reservoir (Fig. 3.4A), the $\delta^{13}C$ values of biota were in the range of -22.87 ± 1.4 (*C. anguillaris*, small size) to -28.3 ± 0.13‰ (bivalve Unionid). The littoral gastropod had a mean $\delta^{13}C$ of -25.69 ± 2.4 ‰. Fish $\delta^{13}C$ ratios (mean $\delta^{13}C$ = –24.8 ± 2.26‰) were more related to littoral carbon (-22.25 ± 0.9‰) than that of profundal unionids.

With respect to $\delta^{15}N$ enrichment in this reservoir, *C. anguillaris* (small size) followed by *S. intermedius* (size 1) had the greatest $\delta^{15}N$ ratios (8.15 ± 3.18‰ and 6.10 ± 2.83‰ respectively). The detritivore *O. niloticus* had the lowest $\delta^{15}N$ ratio (3.41 ± 0.18‰) of all fish species and had a depleted $\delta^{13}C$ ratio (mean $\delta^{13}C$ = –27.39‰), suggesting reliance on pelagic carbon sources. Therefore, *C. anguillaris* and *S. intermedius* were the fish species at the top of the food webs in this reservoir. Wide variation of $\delta^{13}C$ (-26.08 to -21.30‰) was recorded for *C. anguillaris* which may indicate different sources of carbon for this species and suggests opportunistic feeding on available prey.

The analysis of food web structure from Ziga reservoir (Fig.3.4B) indicated the same trends as from Loumbila. The highest $\delta^{13}C$ ratio was found for *O. niloticus* (-18.29 ± 4.22‰) and the most depleted $\delta^{13}C$ was found in pelagic unionids (-29.42 ± 0.9‰). The mean fish $\delta^{13}C$ ratio (-21.58 ± 3) was near that of littoral gastropods (-24.93 ± 0.1). The range of $\delta^{15}N$ enrichment in this reservoir was 2.29 ± 0.64‰ (*O. niloticus,* size 1) to 7.13 ± 1.52‰ (*S. membranaceus*). The planktivorous *S. membranaceus* followed by the piscivorous *B. bajad* (7.05 ± 0.86) were top predators in the food web. *S. membranaceus* had depleted $\delta^{13}C$ values (-25.67 ± 0.6‰) and exhibited a wide range of $\delta^{15}N_{adj}$ ratios (6.23 – 10.27‰).

For the Kompienga reservoir (Fig. 3.4C), we documented the most enriched $\delta^{13}C$ ratios in *O. niloticus* species (-17.86 ± 0.78‰). Zooplankton had the most depleted mean $\delta^{13}C$ ratio of -25.60‰. The mean $\delta^{13}C$ ratio of fish (21.46 ± 3.75‰) was comparable to that of littoral herbivorous gastropods (-21.19 ± 0.3‰). The greatest ^{15}N enrichment was observed with the piscivorous fish *L. niloticus* (7.37 ± 0.7‰) and *B. bajad* (7.18 ± 0.46‰). The lowest was reported for *O. niloticus* (large size) (2.76‰). The piscivorous fish *L. niloticus* followed by *B. bajad* were top predators in the food web.

Overall, the analysis of $\delta^{13}C$ ratios in biota from the three reservoirs indicated that zooplankton (mean $\delta^{13}C$ = -25.4‰) and unionids (mean $\delta^{13}C$ = -28.3‰) were more depleted in $\delta^{13}C$ compared to littoral gastropods (mean $\delta^{13}C$ = -23.9‰). The fish fauna in Kompienga and Ziga were generally more $\delta^{13}C$ enriched (mean $\delta^{13}C$ = –21.5‰) than those of Loumbila (mean $\delta^{13}C$ = –24.8‰) (ANOVA, Tukey's test, $p < 0.05$). There was no difference in the average trophic position of fish (mean $\delta^{15}N_{adj}$ = 3.0‰) between the three reservoirs ($p > 0.05$).

Across the study reservoirs, $\delta^{15}N_{adj}$-derived trophic position of fish ranged from 1 to 2 levels above primary consumer gastropods in all study sites (Table 3.1). Within species mean trophic position did not change significantly across reservoirs (Table 3.1; Kruskal-Wallis test, $p > 0.05$), however, mean carbon signature varied significantly across reservoir for *O. niloticus* (Kruskal-Wallis test; $\chi^2 = 10.80$; $df = 2$; $p < 0.01$) and for *C. anguillaris* (Kruskal-Wallis test; $\chi^2 = 12.79$; $df = 2$; $p < 0.01$).

Table 3.1 The mean (SD) of $\delta^{13}C$, $\delta^{15}N$ (‰), percentage of littoral carbon consumption (%) and trophic position of fish species and invertebrates collected in three reservoirs (fluvial dams) in Burkina Faso. Sample sizes (n) are in parentheses. Abbreviations: C is carbon, TP is trophic position and. $\delta^{15}N_{adj}$ is the adjusted ratio of nitrogen stable isotopes.

Organism	Size class (n)	$\delta^{15}N$ (‰)	$\delta^{13}C$(‰)	%C littoral	Adj. $\delta^{15}N$ (‰)	Tp
Loumbila						
O. niloticus	1 (3)	8.90 ± 0.2	- 27.86 ± 0.046	35 ± 27	3.41 ± 0.18	3.00 ±0.05
A. occidentalis	2 (5)	10.93 ± 1	- 25.86 ± 1.40	76 ± 19	5.44 ± 1.06	3.6 ± 0.31
C. anguillaris	1 (3)	13.64 ± 3.2	- 22.87 ± 1.4	98 ± 5	8.15 ± 3.18	4.39 ± 0.93
C. anguillaris	2 (6)	11.40 ± 1.33	- 23.04 ± 2.19		5.91 ± 2.83	3.74 ± 0.39
S. intermedius	1 (3)	12.55 ± 0.07	- 24.16 ±0.91		6.10 ± 2.83	4.07 ± 0.02
Zooplankton	(bulk) (2)	10.9 ± 0.18	-25.43 ± 0.23	0	2	2.6
Unionidae (2)		10.02 ± 0.25	- 28.3 ± 0.13	100	4.5 ± 0.25	3.3 ±0.07
Gastropod (3)		5.49 ± 0.98	- 25.69 ± 2.4	0	0	2
Sediment		4.89 ±1.25	- 22.25 ± 0.9			
Ziga						
O. niloticus	1 (3)	9.6 ± 0.6	- 18.29 ± 4.22	100	2.29 ±0.64	2.67 ±0.2
S. membranaceus	(6)	14.93	- 25.91	83 ± 10	7.13 ± 1.52	4.09 ± 0.45
B. bajad	2 (6)	14.35 ± 0.86	- 20.01 ± 0.75	100	7.05 ± 0.86	4.07 ±0.25
C. anguillaris	2 (4)	12.17 ± 0.5	- 21.55 ± 1.84	100	4.87 ± 0.48	3.43 ± 0.144

Table 3.1 (continued).

Organism	Size class (n)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	% C littoral	Adj. $\delta^{15}N$ (‰)	Tp
Ziga						
S. intermedius	1 (4)	12.40	- 21.62 ± 0.77	100	5.10	3.50
Zooplankton	(bulk) (2)	11.03	- 25.26		3.73	3.09
Unionidae (2)		11.93 ± 0.5	- 29.55 ± 0.8	0	4.6 ± 0.5	3.4 ± 0.15
Gastropoda (3)		7.3 ± 0.13	- 24.93 ± 0.09	100	0	2
Sediment		5.72 ± 1.01	- 19.42 ± 0.9			
Kompienga						
O. niloticus	2 (5)	9.29 ± 0.46	- 17.86 ± 0.78	100	3.00 ± 0.46	2.88 ± 0.13
O. niloticus	3 (1)	9.07	- 19.79 (1)		2.76	2.81
A. occidentalis	3 (8)	10.85 ± 0.42	- 23.87 ± 3.45	65 ±39	4.55 ± 0.92	3.34 ± 0.27
S. membranaceus	(3)	10.56 ±0.38	- 24.43 ± 1	74 ±14	4.26 ± 0.4	3.25 ± 0.11
B. bajad	3 (8)	12.55 ± 1.26	- 20.97 ± 1.16	97 ± 7	7.18 ± 0.46	4.11 ± 0.13
C. anguillaris	2 (5)	10.98 ± 0.8	- 19.12 ± 1.14	99 ± 3	5.18 ± 1.2	3.52 ± 0.36
C. anguillaris	3 (7)	11.80 ± 0.4	- 20.40 ± 0.68		5.50 ± 0.41	3.62 ± 0.12
L. niloticus	1 (5)	13.67 ± 0.7	- 20.11 ± 0.74	100	7.37 ± 0.7	4.16 ± 0.20
S. intermedius	1 (8)	11.76 ± 1.30	- 21.53 ± 1.52	90 ± 16	5.46 ± 1.30	3.60 ± 0.4
Zooplankton	(bulk) (2)	10.78	- 25.60	0	4.5	3.3
Gastropoda (3)		6.3 ± 0.23	- 21.19 ± 0.3	100	0	2
Sediment		5.4 ± 0.2	- 21.02 ± 2.32			

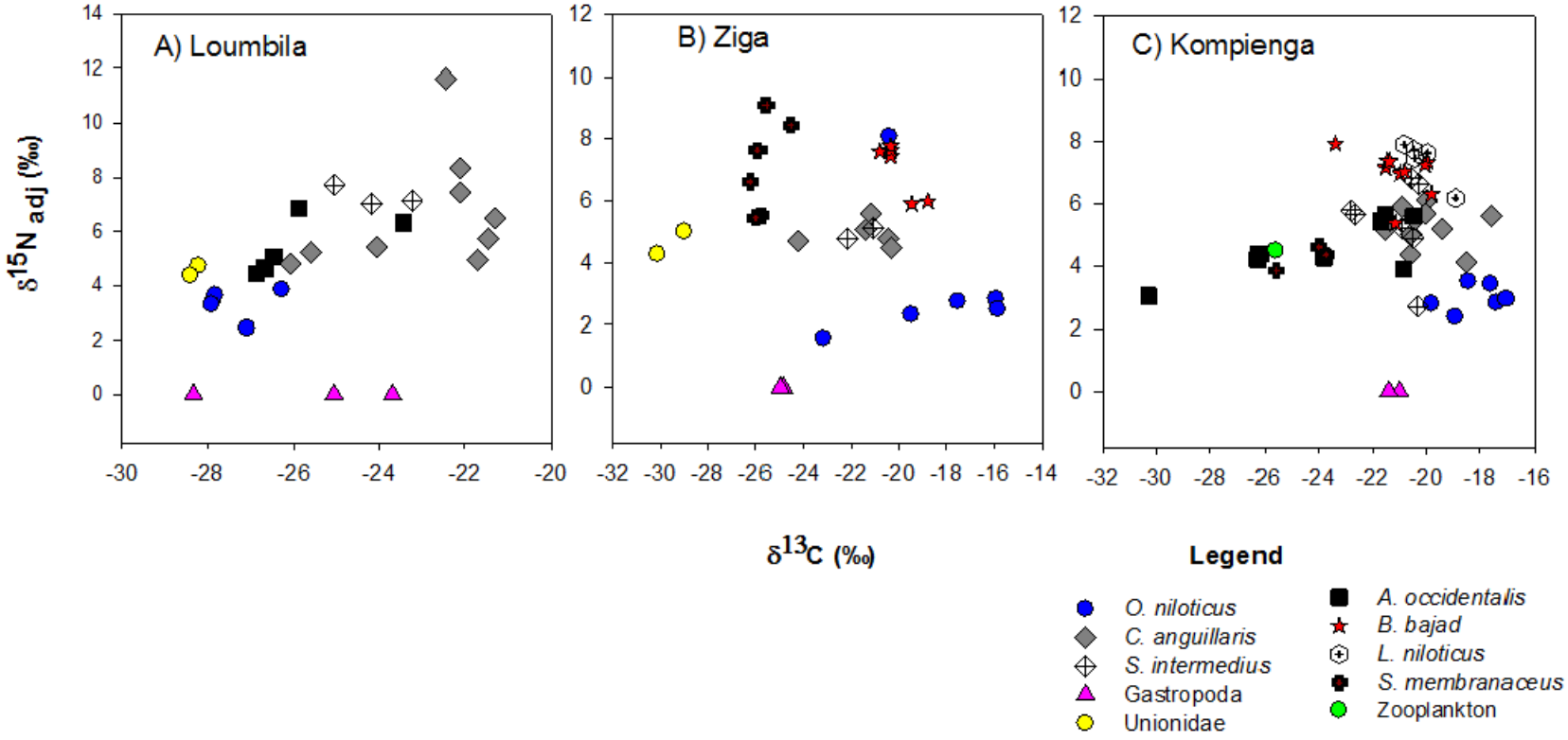

Figure 3.4 Food webs structure represented by adjusted $\delta^{15}N$, indicating trophic position, and $\delta^{13}C$, indicating dietary carbon source, in three freshwater reservoirs from Burkina Faso (Loumbila, Ziga and Kompienga).

3.4.2.2 Metal(loïd) bioaccumulation in food webs

All log BAFs values for Hg were higher than the contaminant bioaccumulation criterion of 3.7 except for *O. niloticus* from Loumbila (Table 3.2). In each reservoir, higher BAFs (Hg) were recorded from top predator fish. For instance, from Loumbila reservoir (Fig. 3.5A) $\log_{10}$BAF (Hg) of *Schilbe intermedius* (mean logBAF (Hg) = 4.92 ± 0.15) was 1.46 times higher than that of *O. niloticus* (3.3 ± 0.06) at a lower trophic level in the food webs. Inter-specific differences in log BAF (Hg) were clear and statistically significant (Kruskall-Wallis test, $p < 0.05$). The efficiency of Hg accumulation in fish increased as follow: *O. niloticus* < *A. occidentalis* < *C. anguillaris* < *S. intermedius.* However, in the other reservoirs (Ziga, Kompienga) many fish showed similar Hg accumulation efficiency. In Ziga (Fig 3.5B), a ratio of 1.3 was found between BAF (Hg) from *S. membranaceus* (4.90) and for *O. niloticus* (3.73). The BAFs of intermediate size fish did not differ significantly ($p < 0.05$). From Kompienga reservoir (Fig.3.5C) only *O. niloticus* at the base of the food web had lower a BAF(Hg) (3.75) compared to the other fish, which were not significantly different (Kruskall-Wallis test, $p > 0.05$).

The Figure 3.6 summarizes the inter-specific comparisons of total selenium bioaccumulation efficiency in fish through the three freshwater reservoirs. For Loumbila, only logBAF (Se) of *A. occidentalis* was significantly lower (3.06 ± 0.18) than those of the other ones (ANOVA, $p < 0.001$).

B. bajad at the top of Ziga food web had slightly higher BAF (Se) than the other fish species which did not differed in their BAF (Se) values (ANOVA, $p > 0.05$). In Kompienga Mean BAF for Se did not show significant difference between fish (ANOVA, $p > 0.05$).

Table 3.2 Selenium and mercury concentrations (mean ±SD) in fish and BAF for Hg and Se with fish relative weight (W_r) from three freshwater reservoirs (Burkina Faso), n is the sample size, TL refers to total length of fish.

Organism	**n**	**TL mean ± SD (mm)**	**THg (µg/g w.w)**	**MeHg (µg/g w.w)**	**TSe (µg/g w.w)**	**log_{10}BAF(Hg) (L/Kg w.w)**	**log_{10} BAF(Se) (L/Kg w.w)**	**Relative weigth (W_r)**
Loumbila								
O. niloticus	32	147 ± 14	0.0064 ± 0.003	0.006 ±0.003	0.139 ± 0.02	3.35 ± 0.24	3.3 ± 0.06	47 ± 9
A. occidentalis	28	193 ± 17	0.0234 ± 0.01	0.016 ± 0.005	0.09 ± 0.06	3.94 ± 0.18	3.06 ± 0.18	47 ± 8
C. anguillaris	28	264 ± 80	0.064 ± 0.06	0.04± 0.03	0.180 ± 0.06	4.26 ± 0.35	3.4 ± 0.14	68 ± 12
S. intermedius	14	145 ± 10	0.230 ± 0.07	0.185 ± 0.064	0.197 ± 0.034	4.92 ± 0.15	3.46 ± 0.07	70 ±14
Zooplankton			0.02					
Unionidae	4		0.024 ± 0.005	0.025±0.001	0.389 ± 0.005			
Gastropoda	5		0.026 ± 0.015	0.013 ± 0.004	0.191 ± 0.04			
sediment			0.014 ± 0.008		0.158 ± 0.14			
Ziga								
O. niloticus	30	156 ± 28	0.012 ± 0.007	0.009 ± 0.003	0.270 ± 0.04	3.73 ± 0.2	3.63 ± 0.06	45 ± 6
S. membranaceus	32	228 ± 16	0.164 ± 0.04	0.142 ± 0.046	0.216 ± 0.06	4.90 ± 0.12	3.52 ± 0.12	53 ± 7
B. bajad	34	293 ± 37	0.101 ± 0.06	0.094 ± 0.05	0.354 ± 0.06	4.66 ± 0.2	3.74 ± 0.07	51 ± 9
C. anguillaris	31	302 ± 50	0.117 ± 0.09	0.164 ± 0.09	0.245 ± 0.08	4.62 ± 0.38	3.57 ± 0.12	72 ± 10
S. intermedius	17	146 ± 13	0.102 ± 0.06	0.082 ± 0.07	0.199 ± 0.05	4.65 ± 0.24	3.5 ± 0.11	60 ± 10

Table 3.2 (continued)**.**

Organism	**n**	**TL mean ± SD (mm)**	**THg (µg/g w.w)**	**MeHg (µg/g w.w)**	**TSe (µg/g w.w)**	**log_{10}BAF(Hg) (L/Kg w.w)**	**log_{10} BAF(Se) (L/Kg w.w)**	**Relative weigth (W_r)**
Ziga								
Zooplankton			0.024					
Unionidae	2							
Gastropoda	3		0.1 ± 0.072	0.032 ± 0.006	0.3 ± 0.08			
sediment			0.02 ± 0.009		0.256 ± 0.11			
Kompienga								
O. niloticus	20	226 ± 57	0.011 ± 0.01	0.004 ± 0.001	0.097 ± 0.02	3.75 ± 0.32	3.22 ± 0.08	54 ± 5
A. occidentalis	13	298 ± 67	0.074 ± 0.05	0.066± 0.04	0.234 ± 0.43	4.6 ± 0.3	3.38 ± 0.35	38 ± 3
S. membranaceus	3	295 ± 85	0.051 ± 0.007	0.044 ± 0.004	0.187 ± 0.02	4.9 ± 0.7	3.77 ± 0.4	59 ± 29
B. bajad	10	438 ± 45	0.213 ± 0.06	0.176 ± 0.05	0.176 ± 0.03	5.06 ± 0.22	3.48 ±0.08	78 ± 11
C. anguillaris	13	439 ± 12	0.197 ± 0.08	0.111 ± 0.07	0.203 ± 0.06	5.04 ± 0.25	3.53 ± 0.16	70 ± 7
L. Niloticus	5	364 ± 60	0.190 ± 0.08	0.148 ± 0.06	0.197 ± 0.02	5.73 ± 0.22	3.53 ± 0.05	61 ± 3
S. intermedius	17	115 ± 5	0.111 ± 0.07	0.146 ± 0.043	0.151 ± 0.05	4.78 ± 0.24	3.4 ± 0.13	67 ± 13
Zooplankton			0.0078	0.006				
Gastropoda	3		0.1 ± 0.072	0.032 ± 0.006	0.3 ± 0.08			
sediment			0.023 ± 0.006		0.158 ± 0.022			

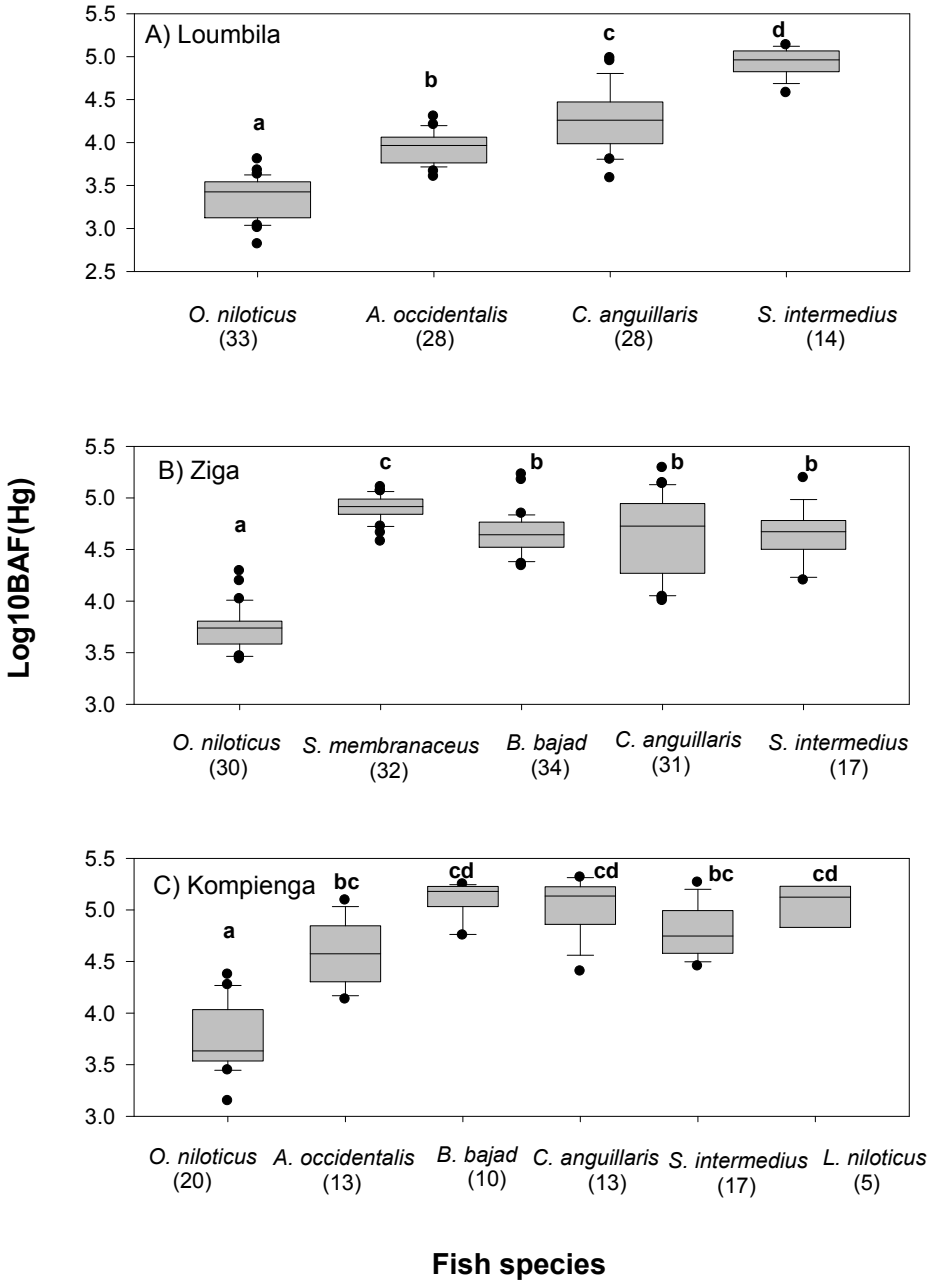

Figure 3.5 Boxplot showing median values and 10th, 25th, 75th, and 90th percentiles of logBAFs for Hg from fish species in the Loumbila (A), Ziga (B) and Kompienga (C) reservoirs. Sample numbers are indicated below each fish species. The letter a, b and c showed significant differences after ANOVA analysis followed by Tukey's test ($p < 0.05$).

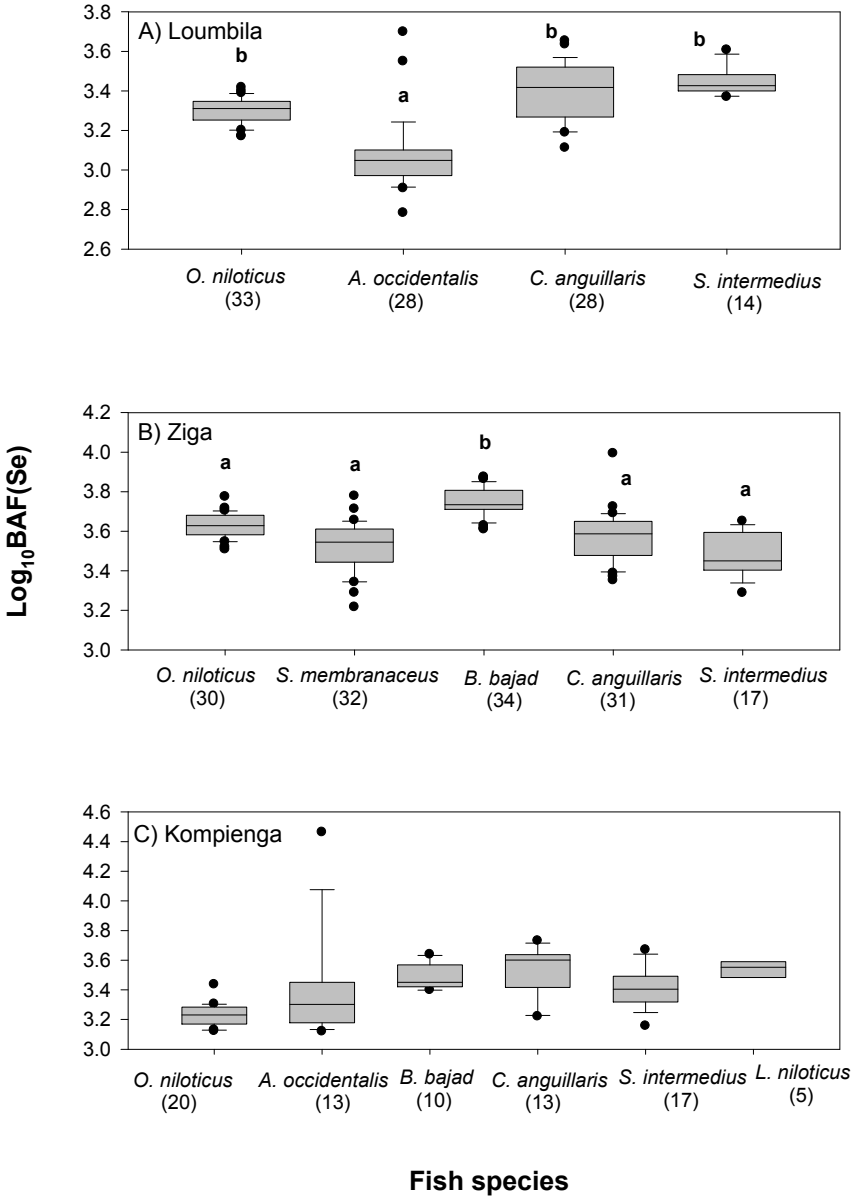

Figure 3.6 Boxplot showing median values and 10th, 25th, 75th, and 90th percentiles of logBAFs for Se in fish species in the Loumbila (A), Ziga (B) and Kompienga (C) reservoirs. Sample numbers are indicated below each fish species. The letter a, b and c showed significant differences after ANOVA analysis followed by Tukey's test ($p<0.05$).

In order to assess the influence of ecological and biological factors on metal(loïds) accumulation, fish relative weight (W_r) were calculated. The results (Table 3.2) indicated that all fish populations were in poor condition, with mean value W_r under 100.

Within each reservoir, multiple linear regression analyses were run to determine the relation between log BAF (metalloid) and three variables: fish relative weight (W_r), fish size (total length) and fish co-occurring metalloid concentration.

In Loumbila reservoir, significant negative relationships between logBAFs (Hg) and fish relative weight (W_r) were reported for *O. niloticus* ($_{adj.}R^2 = 0.33$; $p < 0.001$) and *A. occidentalis* ($_{adj.}R^2 = 0.34$; $p < 0.01$) (Table 3.3). The logBAF for Se in these species did not present significant relationships with these three variables ($p > 0.05$).

Significant relationships were reported between the BAF (Hg) and fish W_r, fish total length and TSe concentration in fish for *O. niloticus* in Ziga ($_{adj.}R^2 = 0.37$; $p < 0.01$) and for *A. occidentalis* in Kompienga ($_{adj.}R^2 = 0.91$; $p < 0.001$). Relationships between W_r and log BAF are always negative.

Overall, fish species which displayed significant relationships between BAFs for Hg and Se and the three variables were at the base of the food webs. These variables did not significantly explain BAF for Hg and Se from the fish at the top of food webs (*S. intermedius* from Loumbila, and Ziga, *L. niloticus* from Kompienga), except for *B. bajad* from Ziga where both *B. bajad* total length and TSe significantly explained BAF (Hg) ($_{adj.}R^2 = 0.38$; $p < 0.001$) and W_r the BAF for Se ($_{adj.}R^2 = 0.23$; $p < 0.01$).

Table 3.3 Relationships between BAF of THg, TSe and fish size (Tl), fish body condition (W_r) for each fish species in the three study reservoirs: Loumbila, Ziga and Kompienga. Abbreviations: BAF refers to bioaccumulation factor, Tl is total length

Fish	Regression models	slope	$_{adj}R^2$	P-value
Loumbila				
O niloticus	log(BAF)(Hg) = 4.05 – 0.015* W_r	- 0.015	0.33	< 0.001
A occidentalis	log(BAF)(Hg) = 4.52 - 0.012*W_r	-0.012	0.34	<0.01
C. anguillaris	log(BAF)(Hg) = 3.08 + 0.0034*Tl + 1.50*(TSe)		0.74	< 0.001
	log(BAF)(Se) = 3.6 + 0.074* log (THg)	0.074	0.18	<0.05
Ziga				
O niloticus	log(BAF)(Hg) = 0.42 +0.89*log(Tl) -2.2*(TSe) - 0.012*(Wr)		0.37	<0.01
	log(BAF)(Se) = 2.85 +0.15*log (Tl)	0.15	0.19	<0.01
B bajad	log(BAF)(Hg) = -5.5 +5.6*log (Tl)+1.31*(TSe)		0.38	<0.001
	log(BAF)(Se) = 3.94 - 0.003*(Wr)		0.23	< 0.01
Kompienga				
O niloticus	log(BAF)(Hg) = -1.28 + 0.93* log (Tl)	0.93	0.46	< 0.001
	log(BAF)(Se) = 2.65 +0.08*Log(Tl) - 0.04*Log (THg) -0.0008*(Wr)		0.09	> 0.05
A. occidentalis	log(BAF)(Hg) = 4.94 +0.003*(Tl) +0.26*$\log_{10}$ (TSe) - 0.027* (Wr)		0.91	< 0.001

Complex relationships between THg concentration and fish size (total length) were reported (Table 3.3). For instance, THg concentrations from *O. niloticus* and *A. occidentalis* showed significant negative relationships with total length from Loumbila and positive relationships in Kompienga. Significant and positive relationship between THg and *C. anguillaris* total length was observed only from Loumbila reservoir ($_{adj.}R^2 = 0.70$, $p < 0.001$). *B. bajad,* the fish at the top of food webs in Ziga and Kompienga, showed a similarly complex pattern for the relationship between THg and total length, with a positive regression in Ziga ($_{adj.}R^2 = 0.29$; $p < 0.01$) and a non significant relationship for a Kompienga ($p > 0.05$).

3.4.2.3 Metal(loïd) biomagnification in food webs

Significant positive linear relationships were observed between log_{10}-Hg (THg and MeHg) concentrations and adjusted $\delta^{15}N$ for all biota from each reservoir (Table 3.4; $p < 0.001$) except in Loumbila for THg, whereas no such relationships were found for selenium ($p > 0.05$). When invertebrates (zooplankton, Unionidae and Gastropoda) were not included, THg and MeHg biomagnified through fish food webs in the three dams and TSe in the Kompienga reservoir (bold values in parentheses, Table 3.4). Based on fish food web, the greatest TMF for Hg was from Kompienga reservoir (6.54 ±1.4 for both THg and MeHg) and the lowest was reported from Loumbila (2.87 ± 1.4 and 3.10 ± 1.4 for THg and MeHg respectively).

Table 3.4. Relationships between log_{10}-Metal(loid) concentration versus adj.$\delta^{15}N$ for THg, MeHg and TSe from three freshwater systems (Burkina Faso) over rainy and dry seasons and their corresponding Trophic magnification factors (TMF). (TMF =10^m, m = slope × 3.4). Bold text (in parentheses) indicates regression values calculated after removing all invertebrates.

Reservoir	Regression Equation	Slope ± SD	adj.R^2	p- value	TMF
Loumbila	log_{10}THg vs adj.δ15N	0.06 ± 0.03 **(0.13 ± 0.04)**	0.08 **(0.26)**	0.07 **(0.008)**	1 **(2.87 ± 1.4)**
	log_{10} MeHg vs adj.δ15N	0.09 ± 0.03 **(0.14 ± 0.04)**	0.25 **(0.34)**	0.004 **(0.002)**	2.02 ± 1.2 **(3.10 ± 1.4)**
	log_{10}TSe vs adj.δ15N	-0.002 ± 0.01 **(0.02 ± 0.02)**	0.00 **(0.008)**	0.9 **(0.29)**	1 **(1)**
Ziga	log_{10}THg vs adj.δ15N	0.12 ± 0.04 **(0.15 ± 0.05)**	0.2 **(0.27)**	0.008 **(0.004)**	2.61 ± 1.4 **(3.29 ±1.5)**
	log_{10} MeHg adj.δ15N	0.13 ± 0.04 **(0.14 ± 0.04)**	0.24 **(0.25)**	0.004 **(0.006)**	2.91 ± 1.4 **(3.10 ± 1.1)**
	log_{10}TSe vs adj.δ15N	0.01 ± 0.01 **(0.015 ± 0.01)**	0.00 **(0.04)**	0.34 **(0.16)**	1 **(1)**
Kompienga	log_{10}THg vs adj.δ15N	0.24 ± 0.03 **(0.24 ± 0.04)**	0.46 **(0.44)**	< 0.001 **(< 0.001)**	6.54 ± 1.2 **(6.54 ±1.4)**
	log_{10} MeHg vs adj.δ15N	0.25 ± 0.03 **(0.24 ± 0.03)**	0.56 **(0.48)**	< 0.001 **(< 0.001)**	7.45 ± 1.2 **(6.54 ± 1.3)**
	log_{10}TSe vs adj.δ15N	0.026 ±0.02 **(0.038 ± 0.02)**	0.008 **(0.06)**	0.23 **(0.046)**	1 **(1.34 ± 1.2)**

Mercury biomagnification was 2 times more efficient in Kompienga compared to the Ziga and Loumbila reservoirs and similar differences among reservoirs were also observed for THg biomagnification rates (Table 3.4).

MeHg biomagnified at a similar rate as THg in the three reservoirs

The TMF values for THg and MeHg were greater than 1 and indicated that Hg biomagnification occurred in these reservoirs systems. A significant relationship between $\log_{10}$-TSe and $\delta^{15}N_{adj}$ of fish (TMF greater than 1) was found in the Kompienga reservoir, suggesting biomagnification of this element through food webs ($p > 0.05$, Table 3.4).

3.5 Discussion

3.5.1 Metal(loïd) levels in reservoir systems

We observed relatively low levels of mercury and selenium concentrations in all environmental compartments in the three reservoirs. Our previous study (Ouedraogo and Amyot, 2012) reported the same range of THg and TSe contamination in these systems. Mercury contamination levels were similar to those reported from other African freshwater systems (Campbell et al., 2003a; 2003b) and from other tropical areas (He et al., 2008). The similarly low ratio of MeHg/THg (2 - 6.6 %) reported in each reservoir suggested similar fate of mercury from these freshwater systems. Difference between MeHg concentrations in littoral and pelagic zones observed in Loumbila an Ziga may be due to greater photodregradation or

sediment demethylation of MeHg in shallower littoral zones of these sites. This hypothesis is consistent with the lack of significant variation of MeHg between littoral and pelagic zones Kompienga reservoir where littoral zone is deep.

Mercury concentrations in sediments (7 to 27 ng/g) were in the range of those reported from Tanzanian lakes (Campbell et al., 2003c). The profundal sediments had higher levels of THg and TSe, compared to littoral ones, whereas no such differences in aqueous THg concentrations were reported from these two zones. These results suggest a sink of Hg into profundal sediments which sequesters and reduces its bioavailability. Higher Hg in profundal sediments may be due to sediment focusing by which particulate matter entering the reservoir is redistributed by particule size as a result of water movements and differences in water velocity at different reservoir depths. Fine inorganic particles (fine silt, 2 – 32 µm, clays,< 2 µm diameter) and organic particles are transported from the littoral zone to the center of lake (Blais and Kalff, 1995; Wetzel, 2001). As 20 - 90% of THg were associated to particulate matter (Annexe 3, Table S3.1), profundal sediments had higher Hg than that of the littoral.

Concentrations of THg, MeHg and TSe in fish reported in the present study were low and did not pose health risk for humans considering the World Health Organization guideline of 0.5 µg THg/g to protect groups vulnerable to mercury toxicity (FAO/WHO, 2004) for THg and Se threshold of 3 µg/g (Lemly, 1993). Fish contamination patterns in freshwater and potential health hazard posed to wildlife and human from Burkina Faso has been further discussed in the previous study (Ouedraogo and Amyot 2012).

3.5.2 Food webs structure

The food webs of the three freshwater reservoirs from Burkina Faso had similar structures, with relatively short food chain lengths (3 – 4 levels). Fish species occupied the same trophic position across reservoirs and relied mainly upon littoral carbon sources. The, highest depletion in $\delta^{13}C$ signature of fish fauna from Loumbila reservoir (-24.8 ± 2 ‰) compared to those of the other ones (-21.5 ± 3 ‰) could be due to inputs of organic matter from agriculture practice in wetlands surrounding Loumbila dam. Significant differences in carbon fractionation ($\delta^{13}C$) were observed for *O. niloticus* between Loumbila (mean $\delta^{13}C$ = -27.4 ± 0.7), Ziga (-18.4 ± 2.80) and Kompienga (-18.18 ± 1.08). It is likely that *O. niloticus* relies on pelagic preys in Loumbila and littoral ones in the other reservoirs. This change on carbon source for *O. niloticus* did not affect Hg and MeHg bioaccumulation as indicated by similar BAFs (Hg) values across sites.

Changes in habitat use were also documented for *C. anguillaris* with $\delta^{13}C$ signature (‰) varying significantly within and across reservoirs ($\delta^{13}C$ = -22.98 ± 1.8 from Loumbila, -21.52 ± 1.6 in Ziga and -19.87 ± 1.08 from Kompienga). This may be due to opportunistic feeding habit of this species as indicated by $\delta^{13}C$ variation and stomach content analysis (Annexe 3, Table S3.5). Stomach contents indicated that fish was the dominant food items for *C. anguillaris* from Loumbila and Kompienga reservoirs whereas invertebrates dominated its diet in Ziga.

Within Ziga reservoir, Hg levels in top predator fish were related to habitat use. Species relying on pelagic carbon sources such as the planktivorous species *S. membranaceus* ($\delta^{13}C$ =

-25.67 ± 0.6 ‰, Table S3.5) had the highest Hg concentration; similarly, the piscivore fish *B. bajad* which was related to littoral carbon sources had relatively low Hg content suggesting a key role of pelagic dwellers (zooplankton and invertebrates) on Hg transfer to fish communities in this site. That is consistent with reports from previous studies (Chetelat et al., 2011; France, 1995b; Kidd et al., 2001; Post, 2002). Assuming an average increase of 3.4‰ $\delta^{15}N$ per trophic level (Kidd et al., 1995), the wide range of $\delta^{15}N$ values (6.23‰ to 10.27‰) of *S. membranaceus* from Ziga reservoir indicate the occurrence of two trophic levels within this species. One explanation of this $\delta^{15}N$ variation within the same reservoir should be an ontogenic diet shift between small and large fish, but the relationship between $\delta^{15}N$ and fish body size was not significant for this species ($p > 0.05$). Identification of taxonomic composition of zooplankton along ontogenic development could enhance our understanding on variation in $\delta^{15}N$ composition of this species.

Overall, many fish relied on littoral food webs (Fig. 3.4). This suggests inter-specific competition for resource use. We propose that opportunistic feeding habits, as shown by stomach contents and isotopic analyses (Table S3.5), is a common behavior of fish from these tropical ecosystems. We hypothesize that this omnivorous behavior may also affect Hg concentrations in fish from these systems. We propose that lower Hg levels could be observed during dry seasons than during rainy ones, since scarcity of food during dry seasons could promote omnivory and affect Hg bioaccumulation.

3.5.3 Metal(loïd) bioaccumulation and biomagnification in reservoir systems

The TMF values reported in this study suggested that biomagnification of mercury occurs in aquatic systems from Burkina Faso. However there is little evidence to support Se biomagnification through food webs.

Overall, the mean logBAFs for Hg from fish were greater than regulatory criterion of 3.7, indicating that mercury is a bioaccumulative substance for freshwater fish from Burkina Faso. A clear increase of BAFs (Hg) from lower trophic level fish to predatory fish indicates that Hg was not metabolized and is biomagnified in the food webs (Arnot and Gobas, 2006).

The mean logBAF for Se in fish was 3.4. According to the BAF criteria of 5000 (log BAF $\geq$ 3.7), it behaved as a nonbioaccumulative substance. In addition we did not find a clear increase of log BAF (Se) with fish trophic position. This result suggests that selenium does not biomagnify in food webs from the studied reservoirs in Burkina Faso.

Among the factors selected to assess biological and ecological influence on BAF, negative relationships between BAF (Hg) and W_r were reported for some species, suggesting that Hg bioaccumulation efficiency of these fish were inversely related to their body condition. This inverse relation between relative weight (W_r) and BAF (Hg) of some fish may be due to the effect of physiology on food intake. For instance, seasonal changes of lipid content depending on food availability can influence the BAF of contaminants (Arnot and Gobas, 2006). Our sampling period corresponds to rising waters and rising food availability (Ouéda et al., 2007; Wetzel, 2001; Winemiller and Jepsen, 1998). Evidence of feeding inhibition with increased blood levels of fatty acid has been documented (Peter, 1979). We hypothesized that

within each fish species, fish with W_r approaching optimum conditions (W_r = 100) may have relatively higher levels of fatty acid compared to those with lower W_r. Consequently, the former fish will have lower Hg burden due to a reduced feeding rate. Further investigations are needed to assess role of fish body condition on metal(loids) bioaccumulation processes.

Several studies have shown that mercury and selenium concentrations in fish generally increase with both size (Campbell et al., 2003a; Cossa et al., 2012; 1995; Kidd et al., 2003) and trophic position as the result of contaminant accumulation with the exposure time and magnification (Cabana et al., 1994; Chen et al., 2000). We report here non-significant, positive and negative relationships between Hg concentrations and fish size (total length). Our results are consistent with those reported from previous studies carried out in tropical freshwater systems (Campbell et al., 2006; Desta et al., 2007; Sampaio Da Silva et al., 2005; Tadiso et al., 2011). As Hg concentrations in fish usually increases with their trophic position (Cabana and Rasmussen, 1994), Sampaio Da Silva et al. (2005) have invoked the shift of some tropical fish species to lower trophic levels during their life cycle to explain the decrease in Hg levels with size. Understanding the BAF - W_r relationships may yield insight on wide variation of Hg accumulation with fish size in many tropical species.

The TMF values reported in this study were in agreement with data reported from tropical lakes and rivers. For instance TMF for THg from Lake Malawi was 4.8 with BMF value of 0.20 (Kidd et al., 2003). BMFs of 0.13, 0.15 and 0.24 for THg and 0.14, 0.14 and 0.24 for MeHg reported from respectively Loumbila, Ziga and Kompienga reservoirs were in the range of those reported in similar water systems from Africa which range from 0.12 to 0.30 (Campbell et al., 2008; Kidd et al., 2003; Tadiso et al., 2011). Similar BMFs were reported in lakes from temperate studies (0.17 - 0.29) (Kidd et al., 2012; Wyn et al., 2009). Among site

differences in Hg biomagnification rates are no yet well understood (Kidd et al., 2012). Our results indicated that Hg concentration in fish did not always increases with fish size. Fish W_r could play a certain role in Hg bioaccumulation and probably in TMF. Therefore assessing causes of among site difference in mercury biomagnification should considered fish bioenergetics that may influence dietary contaminants bioaccumulation and magnification (Trudel and Rasmussen, 2006).

3.6 Conclusion

This study describes the food web structures, Hg and selenium bioaccumulation and trophic transfer to fish from fluvial reservoirs in Burkina Faso, using stable isotopes analyses. We reported BAFs of Hg greater than the regulatory agencies bioaccumulation criterion set at 5000 (logBAF $\geq$ 3.7) indicating that Hg bioaccumulates in the freshwater fish in Burkina Faso reservoirs. However, log BAFs reported for selenium suggests no bioaccumulation for this contaminant. Among the factors potentially important to explain Hg bioaccumulation, we observed inverse relationships between BAF and some fish W_r suggesting that fish body conditions could play a role in mercury bioaccumulation. We reported also from all studied reservoirs a short food chain length (3 - 4 levels) with many fish relying on littoral sources for their carbon supply. Despite these observations mercury was biomagnified with a TMF of 2.87, 3.29 and 6.54 in Loumbila, Ziga and Kompienga reservoirs, respectively. These findings suggest that biomagnification occurs in these fluvial systems as observed in other aquatic

systems. There is little evidence for selenium biomagnification through food webs in these systems.

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Chapitre 4

Influence of season on mercury and selenium dynamics in sub-tropical freshwater food webs (Burkina Faso)

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Article en préparation pour soumission à journal of Environmental Monitoring

4.1 Abstract

We measured Hg and Se concentrations in water, sediments and aquatic organisms (zooplankton, invertebrates and fish) from three water reservoirs of Burkina Faso during the rainy season (in July and August of 2010) and the dry season (February and March 2011). $\delta^{15}N$ and $\delta^{13}C$ signatures in organisms were used to track diet shifts in order to assess how contaminant dynamics in food webs was affected by season. We report significant decrease during dry season of mercury (THg and MeHg) concentrations in water from all study sites and in some fish, particularly in the deepest reservoir Kompienga, compared to the rainy season. Total selenium (TSe) levels in water did not show significant changes between the two seasons but a general decrease was observed with some fish. Seasonal variation of fish Hg concentrations was consistent with lower trophic magnification factors for MeHg observed in two reservoirs during dry season compared to those of rainy season. However, this seasonal shift could not be attributed to variations in food web structure as inferred by stables isotopes of $\delta^{13}C$ and $\delta^{15}N$ despite a general decrease in $\delta^{15}N$ signature during dry season. With the exception of some fish, most of them exhibited the same trophic position and relied on the same carbon sources for their food supply over the two seasons. As for Hg, seasonal changes in TSe concentration in fish is not explained by the variation of food web structure. Gut content analyses yielded evidence of a diet shift in fish suggesting a central role of prey availability and prey taxonomy on mercury uptake by fish in these systems. These results indicate seasonal influence on metal(loïd) dynamics in freshwater food webs from Burkina Faso and suggest that stable isotope measurements in aquatic food web studies should be

combined with prey abundance and functional feeding dynamics data to further improve interpretations of contaminant ecosystem dynamics.

Keywords : Food webs, mercury, selenium, biomagnification, stable isotopes, gut contents.

4.2 Introduction

Several factors influence trace metal levels in aquatic environment such as lake physicochemistry (Schäfer et al., 2006), catchment area (Chasar et al., 2009), and ecological factors such as productivity (Chen and Folt, 2005; Pickhardt et al., 2002), trophic position (Kidd et al., 2012), growth rate (Lavigne et al., 2010; Simoneau et al., 2005), fish age (Tremblay et al., 1998) and food web structure (Cabana and Rasmussen, 1994). Sub-tropical freshwater environments are characterized by a rainy and a dry season. The rainy season is the annual rainfall period typically occurring between June and October, and is marked by high water flow intensity, primary production and food availability in aquatic systems (Winemiller and Jepsen, 1998). Dry season is characterized by low rainfall runoffs and water scarcity with some riverbeds becoming dry during several months (November-May). Soil erosion via rainfall runoffs is the main source of trace metal transportation and inputs into aquatic systems in tropical regions (Donkor et al., 2006; Roulet et al., 1998). Further, high plankton densities during high productivity periods may reduce trace metal uptake and biomagnification (Chen and Folt, 2005). Therefore, differences between these two periods may influence inputs, availability and uptake of trace elements in biota and food webs. Understanding the influence of season on food web structure and metals accumulation in these systems is important to implement risk assessment and conservation programs.

Stable isotope analyses of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) are routinely used in aquatic ecology to describe food web structures (Peterson and Fry, 1987; Vander Zanden et al., 1997). $\delta^{15}N$ is used to describe trophic position based on the well-established average enrichment of 3.4 (± 1 ‰) between a consumer and its preys (Cabana and Rasmussen, 1994;

Peterson and Fry, 1987), whereas, ^{13}C presents relative little fractionation with trophic level (< 1 ‰) (Vander Zanden and Rasmussen, 2001) and is used to differentiate between sources of energy having distinct signature (France, 1995a,1995b). Stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) have been successfully used to track diet shifts in food webs in relation to seasonal change in prey availability (Rimmer et al., 2010; Sampaio Da Silva et al., 2005). Stable isotopes analyses are often preferred to track food web relationship than traditional stomach content analyses because they integrate information on feeding patterns over long periods of time (weeks to years, depending on body size and growth rate). In the case of rapid seasonal changes, gut content analyses may be advantageous because it provides short-term snapshots of diet; it also gives taxonomic information on diet shifts that may prove crucial in understanding contaminant transfer.

In this study, we used stable isotopes complemented by gut content analysis to assess seasonality in contaminant accumulation and magnification in freshwater food webs from Burkina Faso. We hypothesized that mercury (Hg) and selenium (Se) concentrations will decrease in biota from rainy season to dry season. The main objectives of this study were: (1) to quantify changes in mercury and selenium concentrations in water, sediment and fish from the 2010 rainy season to the 2011 dry season, (2) to investigate ecological and biological factors that influence this seasonal changes in metal and metalloid concentrations in fish, and (3) to assess how metal(loïd)s biomagnification will be affected by seasonal variation in their concentrations in fish community.

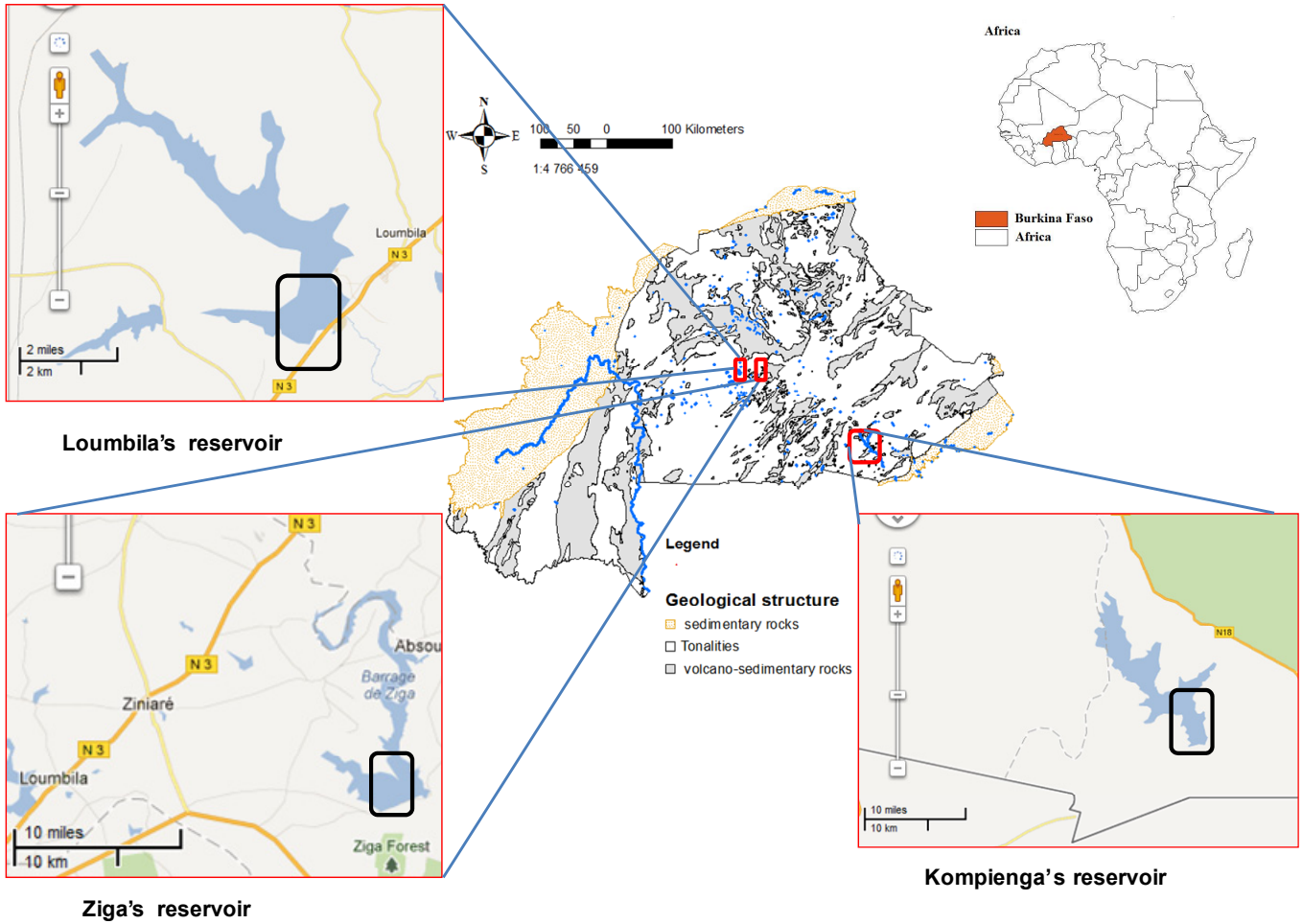

Figure 4.1. Map of Burkina Faso showing the locations of the three sampling sites.

4.3 Material and Methods

4.3.1 Study sites

Study sites were those described in Ouedraogo and Amyot (2012), namely Loumbila, Ziga and Kompienga reservoirs. Located in the central part of Burkina Faso in the Nakambe river catchment (Fig.4.1), the first one, Loumbila dam (12° 29'N , 1° 24' W) is a small man-made water reservoir built in 1947 and used to provide water for people, livestock and agriculture. It has an average area of 1500 ha, and a mean depth of 6.5 m. The second system, Ziga dam (12° 30' N, 1° 4' W) with a surface area of 7.000 ha was built ten years ago for drinking water supply. The third reservoir Kompienga (11° 5' N, 0° 41' W) is one of the two largest hydroelectric reservoirs in the country with surface areas ranging from 16 to 20.000 ha and built in 1988. These sites differ by the reservoir size (depth, areas), age and in watershed use, and are representative of the main types of water bodies found in this country. All the three sites have warm waters (20-30 °C), low levels of DOC (average 2 mg/L) and have a pH close to neutral (Annexe 4, Table S4.1). In 2010 and 2011 sampling, temperature and dissolved oxygen profiles indicated that sites were not stratified and were well oxygenated (Annexe 4, Fig. S4.1).

4.3.2 Field sampling

In February and March 2011 (dry season, decreasing water levels), water, sediment, zooplankton, shellfish and fish were collected in these freshwater reservoirs. We used the same sampling protocols detailed in Ouedraogo and Amyot (2012) at the same sites. The rainy season samples were taken from July to August of 2010 (rainy season, raising water).

4.32.1 Water sampling

Ultra clean protocols for trace metals (US-EPA, method 1669) were used to collect water from the same station at each site in both rainy season of 2010 and dry season of the 2011. Filtered and unfiltered water samples were taken at 0.5 m over the sediments for THg and MeHg analyses, stored in 125 mL amber glass bottles, and placed in double ziplock bags. Filtration was done onboard using a Whatman syringe filter of 0.45 µm pore size. Prior to water collection, bottles were rinsed three times with dam water. All aqueous mercury samples were preserved at pH 2 with ultra high purity hydrochloric acid (VWR) (0.5 %, v/v), kept in a field cooler and refrigerated (+ 4 °C) upon return to laboratory until analysis.

Two separate 30 mL Nalgene HDPE bottles were also filled with water at the same depth for ancillary chemical analyses. One bottle was acidified using hydrochloric acid (0.5 %, v/v) for major cations [potassium (K^+), calcium (Ca^{2+}), sodium (Na^+) and magnesium (Mg^{2+})], the second one was left unacidified for analysis of anions [chloride (Cl^-), nitrite-nitrate (NO_3^-), sulphate (SO_4^{2-})]. For dissolved organic carbon (DOC) analyses, water samples were filtered on 0.45 µm Whatman filters and collected in glass bottle (pre-heated at 550 °C during 1h).

Filtered water samples for selenium analysis were collected in 30 mL Nalgene HDPE bottles samples and preserved with 1% v/v EDTA (Bednar et al., 2002).

4.3.2.2 Sediment sampling

Sediments were sampled with a sediment trap. The surface sediment was transferred to a polyethylene bottle and stored at -20 °C, freeze dried, and shipped to Montreal, stored in double Ziploc bags at 4 °C until analysis

4.3.2.3 Fish sampling

Fish samples were bought from local fishermen. Twenty to thirty individuals from three size groups (small, median and large) (Table S4.2) were sampled for each main fish species. After sex identification and body measurements, sections of dorsal muscle tissue were taken for analyses of trace elements and isotope ratios. These samples were kept in polyethylene bag at -20 °C, then freeze-dried and shipped to Canada for laboratory analysis. More than 300 individual fish were collected each season including *Oreochromis niloticus*, (Cichlidae, detritivore), *Clarias anguillaris* (Clariidae, omnivore), *Bagrus bajad* (Bagridae, piscivore), *Auchenoglanis occidentalis* (Claroteidae, invertebrates feeders), *Schilbe intermedius* (Schilbeidae, invertebrates feeders -piscivore). Additional species such as the Nile perch, *Lates niloticus* (Centropomidae, piscivore), *Synodontis membranaceus* (Mochokidae, planktivore), *Hydrocynus forskalii* (Alestidae, piscivore) were also collected.

4.3.2.4 Zooplancton and benthos sampling

Zooplankton was sampled with a 0.25 m diameter net of 100 µm mesh size at the profundal zone of each reservoir during both rainy and dry seasons by vertical hauls. For density calculation, three vertical hauls were taken at each site, transferred into containers preserved with alcohol (10%) until analyses. For trace metal and isotopic ratio analyses a large volume of water was filtered to collect enough biomass. Sample were transferred into clean Teflon containers, placed in double Ziploc at -20 °C, freeze-dried and stored in double Ziploc bag at 4 °C until analysis.

Pelagic unionids were collected using Ekman grab, and gastropods were hand removed by fishermen from their nets. All invertebrates were prepared with the same protocol as for fish for trace metal and isotopic analyses.

4.3.3 Laboratory analysis

4.3.3.1 Total mercury analysis.

Water samples (36 samples, filtered and unfiltered) were analyzed for total mercury (THg) by cold vapor atomic fluorescence spectrometer (CVAFS) following U.S. Environmental Protection Agency (U.S. EPA) method 1631 with Tekran 2600 (Tekran Instruments Corporation, Knoxville, TN, USA). The detection limit for this analysis was 0.1 ng THg/L and the mean relative recoveries was 106 ± 5 % (n=6).

Solid tissues from aquatic organisms (fish, zooplankton, bivalves, gastropods) and sediments were analyzed for total mercury using a direct mercury analyzer (DMA 80,

Milestone inc., Pittsburgh, PA), in which samples were combusted at 750 °C and mercury vapors were retained on a gold trap for analysis by cold vapor atomic absorption spectrometry. DMA threshold analysis was between 0.12 and 600 ng and the detection limit was 0.05 ng THg/sample, with average analytical variance of 5 %. Certified reference materials (CRM), TORT-2 (lobster hepatopancreas, National Research Council, Canada) and DORM-2 (National Research Council, Canada) were used for quality control (Table S4.3).

4.3.3.2 Methylmercury analysis.

Water samples (50 mL) were analyzed for methylmercury (MeHg) by CVAFS based on the method of Bloom (1989). Briefly, water samples for analysis were acid-distilled to remove matrix interferences, then derivatized by aqueous-phase ethylation with $NaB(C_2H_5)_4$, purged on Tenax (Tenax Corporation, Baltimore, MD, USA), separated by gas chromatography and quantified by CVAFS with a Tekran 2500 (Tekran Instruments Corporation, Knoxville, TN, USA). Field and procedural blanks contained less than 1 ± 1 pg MeHg and revealed no contamination during sampling, filtration, distillation, and analysis. Analytical accuracy was checked by analysis of TORT-2 (Table S4.3).

For MeHg analysis in solid tissues, 10 to 50 mg of dried tissues (sub-samples) were digested in 5 mL of 4M HNO_3 at 55 °C for 16 h. Digested samples then underwent aqueous-phase ethylation followed by gas chromatography separation with CVAFS detection (Tekran 2500). Analytical accuracy was checked by analysis of TORT-2 at each 10 samples (Table S4.3). Method detection limits (MDL) based on three times the standard deviation of 10 blanks were 0.06 ng/L

4.3.3.3 Selenium determination

The protocol for selenium determination is the same as in Ouedraogo and Amyot (2012). For total selenium (TSe) analysis in water, 4 ml of water samples were digested in an acid mixture of HCl (4 mL) and HNO_3 (0.48 mL)) to allow the reduction of Se (VI) to Se (IV). $NaBH_4$ (1.1 % m/v in 0.1M NaOH v/v) was added to the digested samples to produce hydrides and the amount of TSe was detected by Hydride Generation Atomic Fluorescence Spectrometry (HG-AFS; PSA 10.055, Millenium Excalibur; PS Analytical, Orpington, Kent, UK). For TSe analysis in solid samples (fish, zooplankton, gastropod and bivalve), 20 to 50 mg of solid tissues were submitted to microwave digestion with a mixture of HNO_3 and H_2O_2 based on a method developed by Corns et al. (1993) in order to extract elements from solid matrices. An aliquot was then taken and underwent the same steps as for aqueous samples. Se analysis was performed on the same fish used for MeHg analysis. Efficacy of Se (VI) conversion to Se (IV) was checked by using a solution of Se (VI) which was analyzed together with the samples. Procedural blanks were 21 ± 8 $ng.L^{-1}$ (n=8) for analyses performed in 2010 and 19 ng/L L ± 8 (n=8) in 2011. The method detection limit (MDL) was 22 ng L^{-1}(aqueous Se) and 0.022 µg/g dry weight (d.w.) for solids samples at both seasons. Se (VI) (200 $ng.L^{-1}$) conversion to Se (IV) averaged 109 ± 9% in 2010 analysis and 114 ± 3% for 2011 analysis.

4.3.3.4 Zooplankton density calculation

Quantitative analyses were done on sub-samples diluted by a factor of 10. Counting was done on 2 to 5 aliquots of 1 mL according to the density of sample. Density was calculated based on the following formula:

$$\text{Density (Ind. / L)} = n.V_e / V_a. V_f$$

Where n is the individual number of zooplankton taxa in 1 mL, V_e is the standardized volume of the sample, V_a, is the volume of sub-sample analysed and V_f is the total volume filtered.

4.3.3.5 Physico-chemical data set analysis

Anions (Cl^-, SO_4^{2-}, NO_3^-) were analysed by ion chromatography using a DIONEX-DX500 (MDLs: 1 µmol L^{-1} for all anions). Cations (Ca^{2+}, Mg^{2+}, K^+ and Na+) were analysed by atomic absorption spectrometry with MDLs of 0.5, 0.1, 0.5 and 0.5 µmol/L, respectively.

Vertical profiles of water temperature (°C), dissolved oxygen concentration (%), pH, and conductivity (µS/cm) were measured at 0.5 m intervals from each site using a YSI-650 DMS multiprobe. Oxygen and pH calibrations were completed every sampling day.

4.3.3.6 Stable isotope analyses

Stable isotope analyses were made in the stable isotope laboratory of the GEOTOP research centre at the Université du Québec à Montréal (UQÀM). Prior to analyses, freeze-dried fish and invertebrate tissue samples were homogenized into a powder. Stable isotope

analysis was performed on the same fish used in MeHg and selenium analyses. Zooplankton samples were analyzed in bulk due to their small size. Small sub-samples of ground tissues were weighed in tin cups and analyzed on a Micromass Isoprime^{TM} isotope ratio mass spectrometer in continuous flow mode coupled to an Elemantar Vario Micro Cube^{TM} elemental analyzer. $\delta^{15}N$ and $\delta^{13}C$ values were normalized using 3 internal reference materials calibrated against nitrogen gas (N_2) in ambient air, and a PeeDee belemnite (CO_2) respectively as follows:

$$\delta X = [(R_{sample}/R_{standard})-1]\times 1000$$

where: X = ^{13}C or ^{15}N, R_{sample} and $R_{standard}$ are the molar ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample and in an international standard, respectively.

Analytical precisions for $\delta^{13}C$ and $\delta^{15}N$ measurements were 0.1 ‰ and 0.2 ‰, respectively

4.3.3.6 Fish stomach content analysis

In addition to isotope analysis, fish stomach contents were analyzed at the Laboratoire de Biologie et Ecologie Animales (LBEA) of Université de Ouagadougou (Burkina Faso) in order to examine diet composition. The gut contents of each fish were identified under a dissecting microscope or stereomicroscope. Identified food items were grouped into four main classes: detritus (sediment, particulate matters), plants (phytoplankton, aquatic macrophytes and terrestrial plants), invertebrates (insects, zooplankton, shrimps) and fish items (fish, scales). Main food item (MFI) (Rosecchi and Nouaze, 1987) was used as index to assess dominant prey species in the fish diet and was calculated with the following equation:

$$MFIi = \sqrt{P_i((N_i + F_i)/2)}$$

where P_i, F_i and N_i were the ratios of amount of the prey i on total preys on weight, occurrence and numerical bases, respectively.

The percentage of total MFI for a prey i consumed by a species (MFI_i %) was calculated according to Rosecchi and Nouaze (1987)

$$MFI_i\ \% = MFI_i/\Sigma MFI_i \times 100.$$

Principal preys with cumulated MFI_i % greater than 50 % of the total MFI were considered as main food items.

4.3.3.7 Fish body condition calculation

Fish relative weight (W_r), used as index of fish body condition was determined to allow better interpretation of food web dynamics. Calculation was done as in Dittman and Driscoll, (2009), using the following equation:

$$W_r = W/W_s \times 100$$

where W is the actual weight of fish and W_s is the length-specific standard weight of fish. W_s can be predicted by the relation $W_s = a \times L^b$, where L is the total length of fish**.** The $\log_{10}$ – transformation allowed calculation of W_s by the linear weight-length regression: $\log_{10}(W_s) = \log_{10}(a) + b \times \log_{10}(L)$. Fish W_r values are given in Table S4.4

4.3.3.8 Data statistical analysis

We used Wilcoxon signed rank-test to compare metal(loid) concentrations in water from the two seasons. Mean concentrations of THg, MeHg and TSe and isotopic ($\delta^{13}C$ and $\delta^{15}N$) signatures of fish belonging to the same size class were also compared among rainy

season of 2010 and dry season of 2011 by paired t-test to evaluate general trend of fish contamination over seasons. Within site, seasonal comparisons were done for each fish class by Wilcoxon rank-test, when sample size was three or more. Non parametric Kruskal-Wallis test was used to assess among sites differences in fish metal(loid)s concentrations and isotopic ($\delta^{13}C$ and $\delta^{15}N$) signatures. Simple linear regression between $\log_{10}$-tranformed metal(loïd) concentrations in biota (fish and invertebrates) and their adjusted $\delta^{15}N$ value was run to assess biomagnification rates in food web of the study sites at the two seasons. Within each site, trophic magnification factors (TMF_s) of the two seasons were compared by t-test. Difference was statistically significant when $p < 0.05$.

Analyses were performed with the R software (version R-2.13.1) (http://www.r-project.org/)

4.4 Results

4.4.1 Seasonal dynamics of mercury and selenium concentrations in water

For all sites, total mercury (THg) levels in water were 2 (Ziga and Kompienga) to 4 (Loumbila) times higher in samples of 2010 rainy season than in those of the 2011 dry season (Fig. 4.2A). These differences were statistically significant (Wilcoxon signed rank test, $p < 0.001$).

In Loumbila and Kompienga reservoirs, dissolved mercury (DHg) levels were 3 and 1.6 times higher during the rainy season compared to the dry one, respectively (Fig.4.2B; Wilcoxon signed rank test, $p < 0.001$).

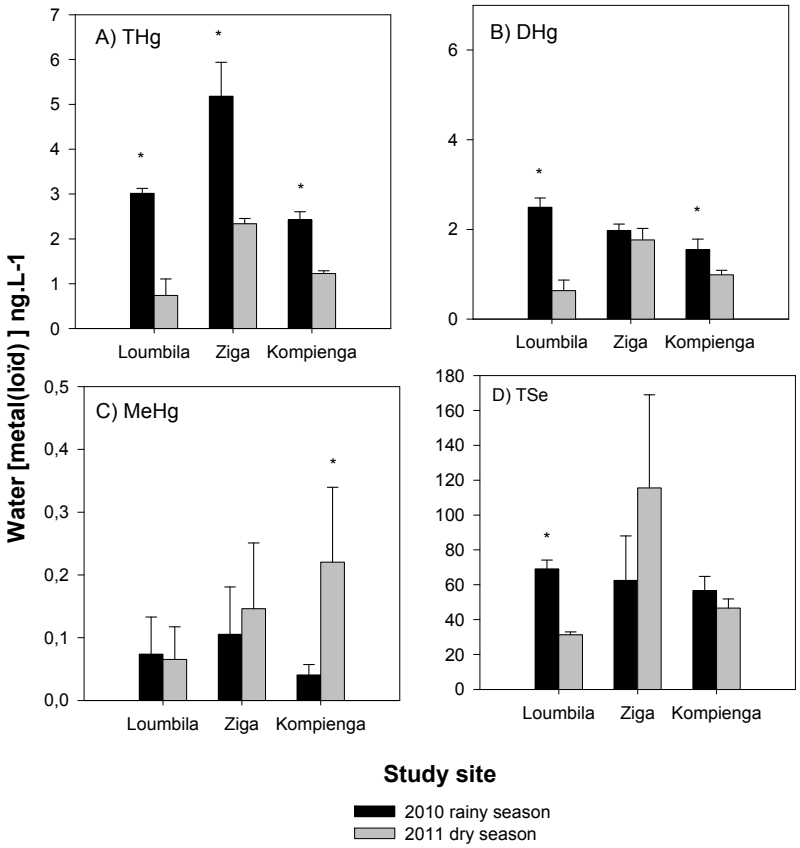

Figure 4.2 Variation of aqueous mercury (Fig.4.2A, B and C) and selenium (Fig.4.2D) levels in freshwater from Burkina Faso across 2010 rainy and 2011 dry seasons. Bars represent mean + standard deviation of 6 samples and asterisk (*) indicated the significant highest value ($p < 0.05$; Wilcoxon signed rank test).

DHg levels did not show significant variation between the two seasons in Ziga reservoir. Particulate Hg (PHg) (difference between unfiltered THg and DHg) represents 23 ± 7 %, 35 ± 6 % and 91 ± 15 % of the THg concentrations in Loumbila, Kompienga and Ziga respectively during 2010 rainy season. In the dry season, these ratios represent 14 ± 27 %, 20 ± 8 % and 24 ± 10 % in these sites respectively (Table S4.1).

No significant difference between MeHg levels in samples from rainy season and those of dry were observed, except from Kompienga reservoir (Fig.4.2C), where higher MeHg concentrations were reported in the dry season (Wilcoxon signed rank test , $p = 0.015$).

Selenium concentration in water (Fig.4.2D) across the 2 seasons at the three reservoirs did not show clear trends. Lower TSe concentration in samples from dry season was reported in Loumbila reservoir (Wilcoxon signed rank test, $p < 0.05$). However, no significant difference was reported in TSe levels from samples from Ziga and Kompienga reservoirs ($p > 0.05$).

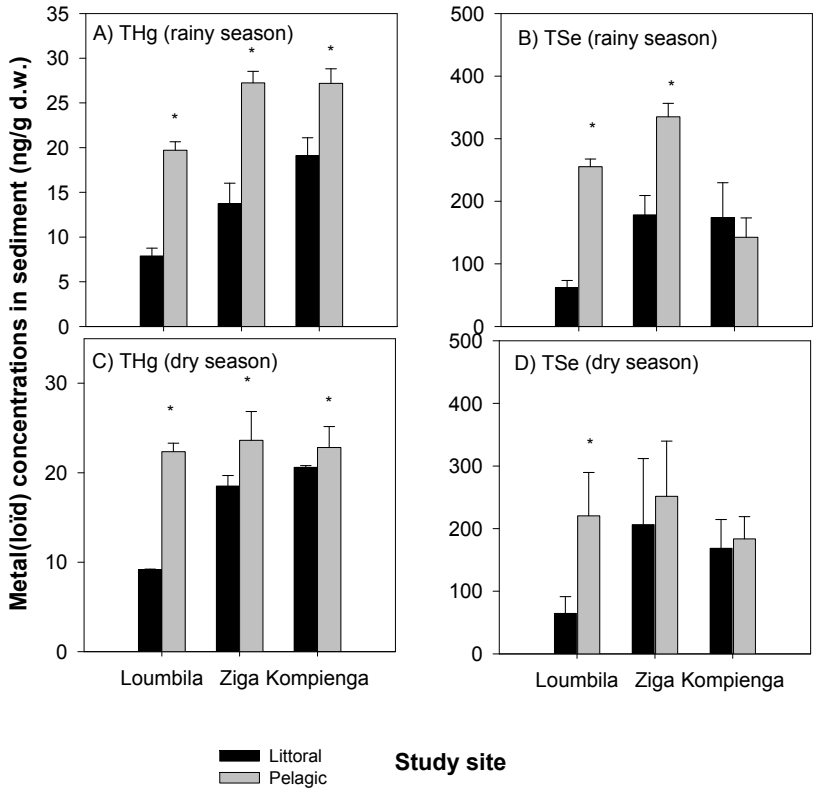

Figure 4.3 Mercury (THg) and selenium (TSe) concentrations in littoral and pelagic surficial sediments of three freshwater reservoirs (Burkina Faso) during rainy and dry seasons. Bars represent mean + standard deviation of 3 samples and asterisk (*) indicated the significant highest value ($p < 0.05$; Wilcoxon signed rank test).

In the three reservoirs, metalloid levels in sediment (Fig.4.3) followed the same trends in both 2010 rainy season (Fig.4.3A, B) and 2011 dry season (Fig.4.3C, D) with higher THg and TSe concentrations in pelagic than littoral sediments. However, differences of THg concentration in sediment from each reservoir between the two seasons were not significant (Wilcoxon signed rank test, $p > 0.05$).

4.4.2 Mercury and selenium levels in fish sample across the 2 seasons

In general, THg, MeHg levels in fish collected during dry season of 2011 were lower than those from fish collected in the rainy season of 2010 (Fig.4.4, paired t-test, $p < 0.05$).

In Loumbila reservoir, during the two seasons, piscivorous fish *S. intermedius* showed the greatest levels of total mercury contamination with mean (± SD) of 0.221 ± 0.091 µg/g w.w. and 0.143 ± 0.133 µg/g for 2010 rainy and 2011 dry seasons respectively (Table S4.5). *O. niloticus* had the lowest mercury content in both seasons, with mean concentration (µg/g) of 0.005 ± 0.003 for medium size class from 2010 rainy season, and 0.013 ± 0.006 for small size class from 2011 dry season. Seasonal comparisons based on the same size class of each fish species from Loumbila (Fig.4.4A) showed significant increase of THg concentrations in the dry season for *O. niloticus* (small size) and *A. occidentalis* (medium size) and significant decrease for *S. intermedius* (small size) (Wicoxon-test, $p < 0.05$).

The range of THg concentration (µg/g) in fish from Ziga reservoir were from 0.010 ± 0.001 (*O. niloticus,* large size) (Table S4.5) to 0.178 ± 0.104 (*S. membranaceus*, medium size) and from 0.013 ± 0.006 (*O. niloticus* small size) to 0.141 ± 0.05 (*B. Bajad,* medium size)

during the 2010 rainy season and the 2011 dry season, respectively (Table S4.5). Differences in THg burden between rainy and dry seasons for specimens of the same class size were not significant (Fig.4.4B, Wilcoxon-test, $p < 0.05$) for any species.

Seasonal variations of THg levels in fish from Kompienga dam (Fig.4.4C) indicated significant decrease of THg from rainy to dry season for, *A. occidentalis, C. anguillaris* (Wilcoxon-test, $p < 0.05$). A significant increase was observed with *O. niloticus* medium size ($p < 0.05$). For the other species, *B. bajad*, *S. intermedius* and *L. niloticus,* differences were not significant (Wilcoxon-test, $p > 0.05$).

MeHg levels in fish between the two seasons did not show any significant difference in Loumbila reservoir (Fig.4.4D). Only MeHg level in *S. intermedius* from Ziga decreased significantly from rainy to dry season (Fig.4.4E). For Kompienga, MeHg levels decrease from rainy to dry season in *C. anguillaris* (large size) (Fig.4.4F).

Significant decrease of TSe levels in fish occurred from rainy to dry season (paired t-test, $t = 2.2805$, $df = 21$, $p = 0.01656$). Within sites, *C. anguillaris* (small size) and *S. intermedius* (small and medium size) had lower TSe concentrations during dry season in Loumbila dam (Wilcoxon-test, $p < 0.05$). *O. niloticus* (small size) from Ziga and *S. intermedius* in Kompienga dam also presented lower selenium levels in the dry season ($p < 0.05$).

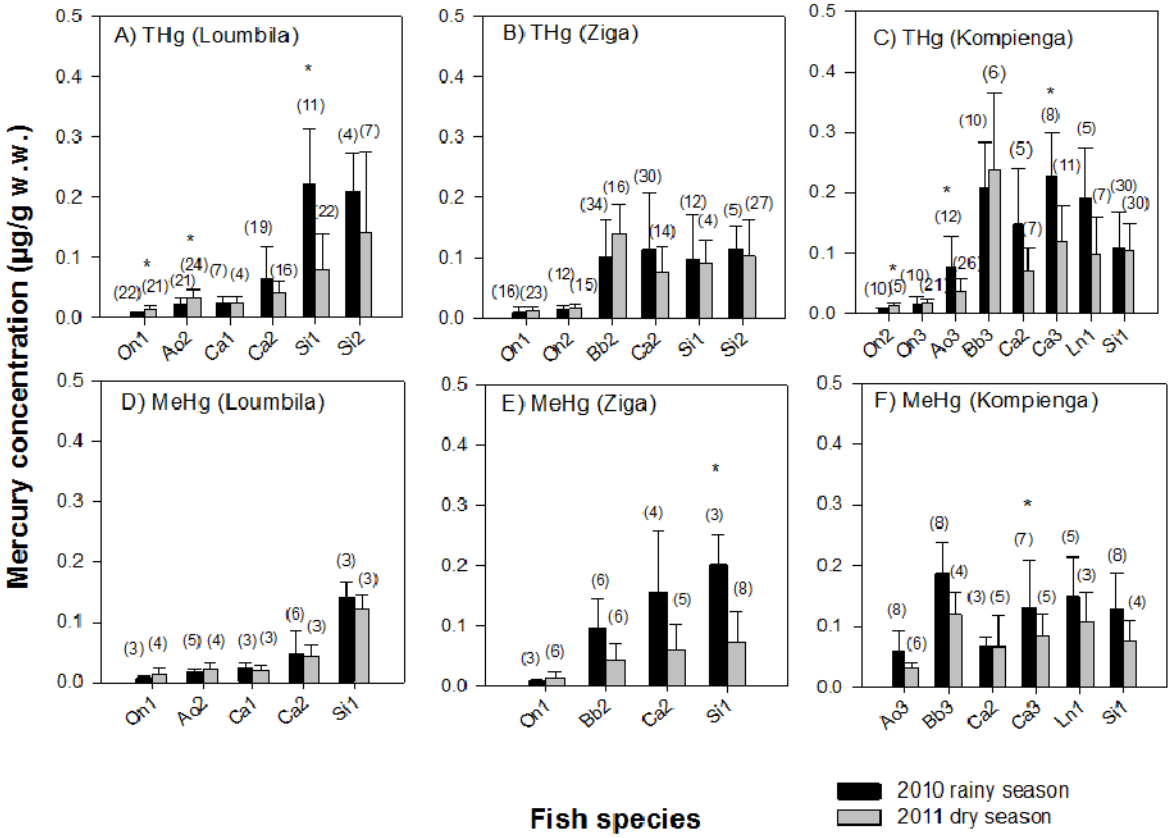

Figure 4.4 Influence of season on mercury levels in fish from freshwater of Burkina Faso. The top figures 4.4A, 4B, and 4C represent the variations of fish THg from Loumbila , Ziga and Kompienga reservoirs between the 2 seasons. Bottom figures 4.4D, 4E and 4F were the variation of fish MeHg in the same reservoir during the 2 seasons. Sample sizes are in parentheses and significant difference between the 2 seasons was shown by asterisk (*) after Wilcoxon rank test ($p < 0.05$). Abbreviations: fish name is designated by the 2 first letters of scientific name followed by the class size. On1 = *O. niloticus small size*; Ao = *A. occidentalis; Ca = C. anguillaris; Si = S. intermedius; Bb = B. bajad; Ln = L. niloticus.*

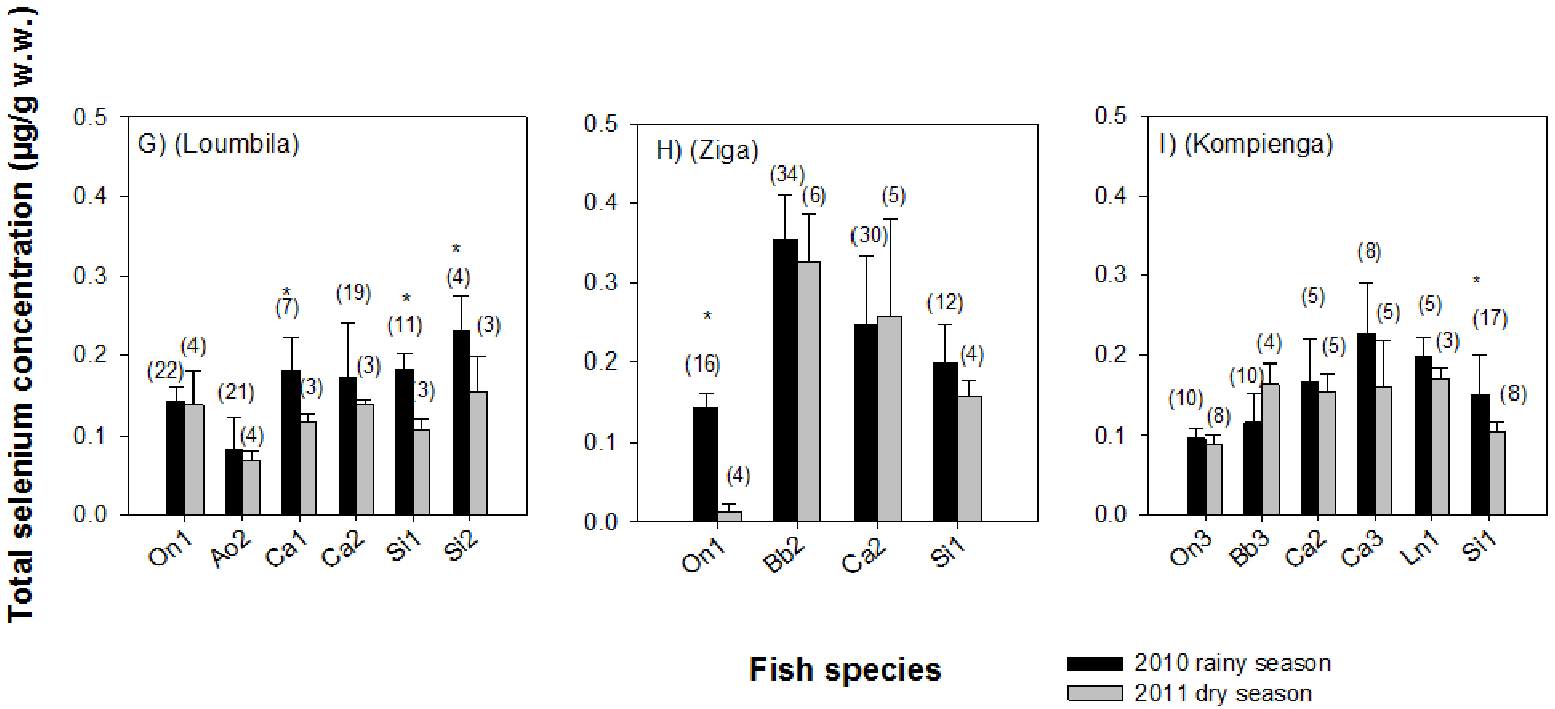

Figure 4.4. (continued)

4.4.3 Zooplankton dynamic

During the dry season, zooplankton density was maximal in all reservoirs (total density of zooplankton was 7, 1.6 and 2 times higher in 2011 samples than those of 2010 rainy season from Loumbila, Ziga and Kompienga respectively) (Table 4.1). However, this trend was mainly driven by high densities of rotifers. Cladocerans were 2.3 to 4.4 times more abundant in the rainy season than in the dry season, whereas copepods did not display a consistent trend.

Table 4.1. Zooplankton density from three freshwater reservoirs of Burkina Faso during rainy season of 2010 and dry season of 2011

Zooplancton group	**Loumbila**		**Ziga**		**Kompienga**	
	Rainy season (2010)	Dry season (2011)	Rainy season (2010)	Dry season (2011)	Rainy season (2010)	Dry season (2011)
Cladoceran (ind./L)	96	38	22	5	88	38
Copepods (ind./L)	172	166	76	59	57	113
Rotifers (ind./L)	148	2687	18	125	12	155
Total density of Zooplankton(ind./L)	417	2890	116	189	157	306

4.4.4 Seasonal variation of fish feeding habits

Diet analysis indicated that many freshwater fish from Burkina Faso exhibited flexible dietary habits. Main food items (MFI) of *O. niloticus* were dominated by detritus during both

the rainy and the dry season in all sites (Table 4.2). Detritus and invertebrates were the main food items for *A. occidentalis* in all study sites at both seasons. Ratios of these two components in the diet of this species varied with season and study site. For instance, invertebrates represented 42 % of the diet of this species in Loumbila reservoir during rainy 2010 rainy season, and 56 % during 2011 dry season. In Kompienga, invertebrates represented 62 % and 45 % of the diet in the rainy and dry seasons, respectively. During 2010 rainy season, food items of *C. anguillaris* were dominated by fish (49 %) and invertebrates (19 %) in Loumbila, invertebrates in Ziga (63 %) and fish in Kompienga reservoir (71 %). From 2010 rainy season to dry 2011 season, plants became one of the main prey items of this species in Loumbila (32%), and detritus (64 %) was the main food item of *C. anguillaris,* from Kompienga. From Ziga reservoir, invertebrates (98 %) remain the dominant food item for this species across the two seasons. Similar variations of feeding habits among sites and between seasons were seen with other species such as *B. bajad* and *S. intermedius. S. intermedius* was a piscivore in Loumbila (66 % fish in rainy season and 99 % in dry season) and clearly more invertebrates feeders in Kompienga (up to 100 % invertebrates during dry season). From rainy season to dry season, *B. bajad* feeding shifted from invertebrates feeders (56 %) to piscivore (80 %) in Ziga reservoir. In Kompienga it became more piscivorous during dry season.

Table.4.2 Main food items (MFI) for fish species in the three reservoirs of Burkina Faso at both rainy season and dry season. Bold values represent the main food items

Fish	**2010 rainy season MFI (%)**				**2011 dry season MFI (%)**			
	Detritus	**Plants**	**Invertebrates**	**Fish**	**Detritus**	**Plants**	**Invertebrates**	**Fish**
Loumbila								
O. niloticus	**74.4**	23.8	0.4	1.4	**90.4**	7,1		2.5
A. occidentalis	**56.1**	1.1	42.6	_	24.2	0.9	**53.3**	21.5
S. intermedius	10.9	0.4	22.8	**66.2**	_	_	1	**99**
C. anguillaris	12.7	18.8	**19.2**	**49.3**	_	**32.1**	**39.5**	28.4
Ziga								
O. niloticus	**56.6**	19.6	3.7	20.2	**96.3**	0.4		3.2
A. occidentalis					**55.9**	4.3	35.7	4.0
S. membranaceus	21.4	4.6	**70.3**					
B. bajad		0.2	**56.0**	41.6	_	_	19.1	**80.9**
C. anguillaris	2.04	3.0	**62.9**	32.1	_	0.2	**96.5**	3.3
S. intermedius					_	_	16.6	**83.4**
Kompienga								
O. niloticus	**92.1**	6.9		1.0	**94**.0	2.6	1.1	2.2
A. occidentalis	36.2	1.2	**62.6**	_	**54.5**	0	45.3	_
B. bajad	_	8.6	30.4	**60.8**	_	_	31.2	**68.8**
C. anguillaris	2.9	4.0	21.7	**71.4**	**64.2**	2.3	25.4	3.4
S. intermedius	_	_	**85.7**	14.35	_	_	**100**	**_**

4.4.5 Food web structures and metal(loid) biomagnification.

4.4.5.1 Seasonal dynamics and stable isotopes ratios of $\delta^{15}N$ and $\delta^{13}C$ in fish

During the rainy season, mean $\delta^{13}C$ signatures of fish sampled from Ziga and Kompienga were more enriched ($\delta^{13}C$ = –21.46 ‰) than those collected from Loumbila ($\delta^{13}C$ = –24.8 ‰). The same trend was observed during the dry season (Table 4.3). The variations of $\delta^{13}C$ signature between the two seasons of most fish were not statistically significant (paired t-test, $p > 0.05$). However, $\delta^{13}C$ ratios from piscivorous fish *S. intermedius* at Loumbila showed significant depletion of 2.6 ‰ during the dry season compared to ratios from rainy season (Table 4.3, Wilcoxon-test, $p < 0.05$). From Kompienga reservoir, the same piscivorous fish displayed a $\delta^{13}C$ signature lower by 2.3 ‰ during the dry season than that of the rainy season.

During 2010 rainy season, an average enrichment of 4 ‰ in $\delta^{15}N$ signature was reported from piscivorous fish at the top of food web from Loumbila (*S. intermedius* and *C. anguillaris)* compared with those at the base (*O. niloticus*) (Table 4.4). The gradient in $\delta^{15}N$ increase between fish at the base of food web and those at the top was 3.8 ‰ and 3 ‰ in Ziga and Kompienga reservoirs respectively (Table 4.4).

Table 4.3. Means and standard deviations of the $\delta^{13}C$ ratios for fish species of three freshwater reservoirs from Burkina Faso in 2010 rainy season and 2011 dry season. Sample sizes are in parentheses, significant difference between the 2 seasons was indicated by bold p- value (Wilcoxon rank test, $p < 0.05$).

Site	Organism	$\delta^{13}C$ (‰)		p value
		2010 rainy season	2011 dry season	
Loumbila	*O. niloticus*	- 27.4 ± 0.7 (5)	- 25.92 ± 2.4 (6)	0.329
	A. occidentalis	- 25.86 ± 1.4 (5)	- 26.79 ± 2 (6)	0.246
	B. bajad	_	- 27.26 ± 0.02 (2)	
	C. anguillaris	- 22.98 ± 1.81 (9)	- 24.47 ± 1.4 (9)	0.077
	S. intermedius	- 24.16 ± 0.9 (3)	- 26.76 ± 1.14 (6)	0.023
	Zooplankton	-25.43 ± 0.23	_	
	Unionid	- 28.3 ± 0.13 (4)	-30.88 ± 0.64 (3)	
	Gastropod	- 25.69 ± 2.4 (3)	- 21.52 ± 1.89 (3)	
	Sediment	- 22.25 ± 0.9	-21.52 ± 1.19	
Ziga	*O. niloticus*	- 18.7 ± 2.86 (6)	- 16.67 ± 1.7 (7)	0.0513
	A. occidentalis		- 21.20 ± 0.88	
	S. membranaceus	- 25.67 ± 0.58		
	B. bajad	- 20.01 ± 0.75 (6)	- 20.70 ± 0.81 (9)	0.181
	C. anguillaris	- 21.52 ± 1.6 (5)	- 20.04 ± 1.42 (7)	0.202
	S. intermedius	- 21.62 ± 0.77 (2)	- 21.80 ± 1.6 (8)	0.711
	Zooplankton	- 25.26	_	
	Unionid	- 29.55 ± 0.8 (2)	- 29.55 ± 0.8 (2)	
	Gastropod	- 24.93 ± 0.09 (3)	- 24.93 ± 0.09(3)	
	Sediment	- 19.42 ± 0.9	-19.27 ± 0.8	
Kompienga	*O. niloticus*	- 18.18 ± 1.05 (6)	- 19.90 ± 4.53 (9)	0.528
	A. occidentalis	- 23.87 ± 3.45 (8)	- 22.67 ± 2.97 (7)	0.054
	S. membranaceus	- 24.43 ± 1	_	
	B. bajad	- 21.04 ± 1.04 (10)	- 21.19 ± 2.52 (3)	0.05
	C. anguillaris	- 19.87 ± 1.08 (16)	- 21.70 ± 2.63 (8)	0.582
	L. niloticus	- 20.11 ± 0.74(5)	- 19.62 ± 2.37 (3)	0.785
	S. intermedius	- 21.78 ± 1.55 (16)	- 24.30 ± 2.17 (8)	< 0.05
	H. forskalii		- 17.89 ± 1.34	
	Zooplankton	- 25.60	- 24.62 ± 0.01	
	Gastropod	- 21.19 ± 0.3 (3)	- 21.2 ± 1.12 (3)	
	Sediment	- 21.02 ± 2.32	-19.94 ± 0.19	

Table 4.4. Means and standard deviations of the adj. $\delta^{15}N$ ratios for fish species of three freshwater reservoirs from Burkina Faso in 2010 rainy season and 2011 dry season. Sample sizes are in parentheses, significant difference between the 2 seasons was indicated by bold p value (Wilcoxon rank test, $p < 0.05$).

Site	Organism	Adj. $\delta^{15}N$ (‰)		p value
		Rainy season	Dry season	
Loumbila	*O. niloticus*	3.3 ± 0.5 (5)	3.35 ± 1.44 (6)	0.93
	A. occidentalis	5.44 ± 1 (5)	4.01 ± 1.14 (6)	0.030
	B. bajad	_	7.97 ± 0.03	
	C. anguillaris	6.7 ± 2.2 (9)	5.71 ± 1.02 (9)	0.545
	S. intermedius	7.3 ± 0.4 (3)	4.78 ± 1.53 (6)	0.023
	Zooplankton	2	_	
	Unionid	4.5 ± 0.25	3.56 ± 0.36	
	Gastropod	0	0	
Ziga	*O. niloticus*	3.33 ± 2.36 (6)	3.62 ± 0.35 (7)	0.0513
	A. occidentalis	_	4.12 ± 1.01	
	S. membranaceus	7.13 ± 1.52	_	
	B. bajad	7.05 ± 0.86 (6)	7.32 ± 0.52 (9)	0.954
	C. anguillaris	4.92 ± 0.4 (5)	5.98 ± 0.32 (7)	0.005
	S. intermedius	4.93 ± 0.25 (2)	4.73 ± 0.75 (8)	0.888
	Zooplankton	3.73	_	
	Unionid	4.6 ± 0.5	4.63± 0.52	
	Gastropod	0	0	
Kompienga	*O. niloticus*	4.55 ± 0.92 (6)	2.72 ± 2.13 (7)	0.0011
	A. occidentalis	4.26 ± 0.4 (8)	4.86 ± 1.1 (7)	> 0.05
	S. membranaceus	7.00 ± 0.7		
	B. bajad	5.35 ± 0.83 (10)	7.46 ± 1.1 (3)	0.46
	C. anguillaris	7.37 ± 0.7 (12)	5.75 ± 1.3 (10)	0.069
	L. niloticus	5.76 ± 0.95 (5)	6.16 ± 1.05 (3)	0.142
	S. intermedius	4.5 (16)	5.58 ± 1.4(8)	0.528
	H. forskalii		6.04 ± 0.89	
	Zooplankton	4.48	3.84 ± 0.00	
	Gastropod	0	0	

Table 4.5. Means and standard deviations of the adj. $\delta^{15}N$ derived - trophic positions for biota of three freshwater reservoirs from Burkina Faso in 2010 rainy season and 2011 dry season. Sample sizes are in parentheses, significant difference between the 2 seasons was indicated by bold p value (Wilcoxon rank test , $p < 0.05$).

Site	Organisms	Trophic position		p.value
		2010 rainy season	2011 dry season	
Loumbila	*O. niloticus*	3 ± 0.16 (5)	2.98 ± 0.42 (6)	0.93
	A. occidentalis	3.6 ± 0.3 (5)	3.18 ± 0.33 (6)	0.030
	B. bajad		4.34 ± 0.008	
	C. anguillaris	3.96 ± 0.7 (9)	3.68 ± 0.3 (9)	0.545
	S. intermedius	4.14 ± 0.11 (3)	3.40 ± 0.45 (6)	0.03
	Zooplankton	2.6	_	
	Unionid	3.3 ± 0.07	2	
	Gastropod	2	2.0 ± 0.08	
Ziga	*O. niloticus*	2.98 ± 0.7 (6)	3.06 ± 0.1 (7)	0.0513
	A. occidentalis	_	3.21 ± 0.3	
	S. membranaceus	4.09 ± 0.45	_	
	B. bajad	4.07 ± 0.25 (6)	4.15 ± 0.15 (9)	0.954
	C. anguillaris	3.44 ± 0.12 (5)	3.76 ± 0.09 (7)	0.005
	S. intermedius	3.45 ± 0.07 (2)	3.39 ± 0.22 (8)	0.888
	Zooplankton	3.09	_	
	Unionid	3.4 ± 0.15	3.36 ± 0.15	
	Gastropod	2	2	
Kompienga	*O. niloticus*	2.86 ± 0.12 (6)	2.8 ± 0.6 (7)	0.0011
	A. occidentalis	3.34 ± 0.3 (8)	3.43 ± 0.32 (7)	0.054
	S. membranaceus	3.25 ± 0.11	_	
	B. bajad	4.05 ± 0.2 (10)	4.2 ± 0.34 (3)	0.46
	C. anguillaris	3.57 ± 0.24 (12)	3.69 ± 0.4 (10)	0.069
	L. niloticus	4.17 ± 0.2 (5)	3.81 ± 0.31 (3)	0.142
	S. intermedius	3.7 ± 0.3 (16)	3.64 ± 0.4 (8)	0.528
	H. forskalii	_	3.77 ± 0.26	
	Zooplankton	3.3	3.13 ± 0.00	
	Gastropod	2	2.02 ± 0.03	

During the 2011 dry season, enrichment in $\delta^{15}N$ ratios from fish at the base of food webs to those at the top reported from the three study sites average 4.6 ‰, 3.7 ‰ and 4.7 ‰ from Loumbila, Ziga and Kompienga, respectively. A paired t-test applied to data from all fish of the two seasons indicated a significant decrease of $\delta^{15}N$ signatures during the dry season compared to those of rainy ($p < 0.05$). When considering each fish species, *S. intermedius* from Loumbila showed significant decrease of 2.5 ‰ in $\delta^{15}N$ signature (Wilcoxon rank test, $p < 0.05$) (Table 4.4). A similar decrease of 1.8 ‰ in $\delta^{15}N$ was observed for *O. niloticus* from Kompienga ($p < 0.05$), whereas, *C. anguillaris* from Ziga exhibited an increase of $\delta^{15}N$ from rainy to dry season of 1 ‰ (Wilcoxon-test, $p < 0.05$).

In agreement with the seasonal decrease in adj. $\delta^{15}N$ enrichment ratios of fish, the $\delta^{15}N$ - derived trophic position of fish belonging to the same size class showed significant decrease (Table 4.5, paired t-test, $p < 0.05$). However, at the species level, only *S. intermedius* from Loumbila and *C. anguillaris* from Ziga displayed significant shifts in trophic position during these two periods ($p < 0.05$).

4.4.5.2 Mercury and selenium biomagnification in food webs

Biomagnification of mercury (THg and MeHg) through entire food webs (expressed as Trophic Magnification Factors,TMF) in the three dams during both rainy season and dry season were significantly greater than 1 except for THg in Loumbila during the 2010 rainy season (Table 4.6).

Table 4.6. Relationships between log_{10}-Metal(loid) concentration versus adj. $\delta^{15}N$ for THg, MeHg and TSe from three freshwater systems (Burkina Faso) over rainy and dry seasons and their corresponding Trophic magnification factors (TMF).Bold text (in parentheses) indicate regression values calculated after removing all invertebrates.

Reservoir	Regression Equation	Slope ± SD	Adj.R^2	p- value	TMF
Rainy season (2010)					
Loumbila	log_{10}THg vs adj.δ15N	0.06 ± 0.03 **(0.13 ± 0.04)**	0.08 **(0.26**)	0.07 **(0.008**)	1 **(2.87 ± 1.4)**
	log_{10} MeHg vs adj.δ15N	0.09 ± 0.03 **(0.14 ± 0.04)**	0.25 **(0.34**)	0.004 **(0.002)**	2.02 ± 1.2 **(3.10 ± 1.4**)
	log_{10}TSe vs adj.δ15N	-0.002 ± 0.01 **(0.02 ± 0.02)**	0.00 **(0.008**)	0.9 **(0.29**)	1 **(1)**
Ziga	log_{10}THg vs adj.δ15N	0.12 ± 0.04 **(0.15 ± 0.05)**	0.2 **(0.27)**	0.008 **(0.004)**	2.61 ± 1.4 **(3.29 ±1.5)**
	log_{10} MeHg adj.δ15N	0.13 ± 0.04 **(0.14 ± 0.04)**	0.24 **(0.25)**	0.004 **(0.006)**	2.91 ± 1.4 **(3.10 ± 1.1)**
	log_{10}TSe vs adj.δ15N	0.01 ± 0.01 **(0.015 ± 0.01)**	0.00 **(0.04)**	0.34 **(0.16)**	1 **(1)**
Kompienga	log_{10}THg vs adj.δ15N	0.24 ± 0.03 **(0.24 ± 0.04)**	0.46 **(0.44)**	< 0.001 **(< 0.001)**	6.54 ± 1.2 **(6.54 ±1.4)**
	log_{10} MeHg vs adj.δ15N	0.25 ± 0.03 **(0.24 ± 0.03)**	0.56 **(0.48)**	< 0.001 **(< 0.001)**	7.45 ± 1.2 **(6.54 ± 1.3)**
	log_{10}TSe vs adj.δ15N	0.026 ±0.02 **(0.038 ± 0.02)**	0.008 **(0.06)**	0.23 **(0.046)**	1 **(1.34 ± 1.2)**

Table 4.6. (continued**).**

Reservoir	Regression Equation	Slope ± SD	adj.R^2	p- value	TMF
Dry season (2011)					
Loumbila	log_{10}THg vs adj.δ15N	0.07 ± 0.03 **(0.13 ± 0.04)**	0.10 **(0.27)**	0.04 **(0.002)**	1.67 ± 1.2 **(2.87 ± 1.4)**
	log_{10} MeHg vs adj.δ15N	0.13 ± 0.03 **(0.11 ± 0.04)**	0.35 **(0.18)**	0.0002 **(0.01)**	2.86 ± 1.2 **(2.39 ± 1.4)**
	log_{10}TSe vs adj.δ15N	-0.016 ± 0.01 **(0.02± 0.02)**	0.008 **(0.02)**	0.27 **(0.22)**	1 **(1)**
Ziga	log_{10}THg vs adj.δ15N	0.11 ± 0.03 **(0.20 ± 0.03)**	0.21 **(0.48)**	0.001 **(0.001)**	2.40 ± 1.2 **(4.73 ± 1.3)**
	log_{10} MeHg adj.δ15N	0.11 ± 0.03 **(0.16 ± 0.03)**	0.27 **(0.42)**	0.0002 **(0.001)**	2.40 ± 1.2 **(3.57 ± 1.3)**
	log_{10}TSe vs adj.δ15N	0.05 ± 0.02 **(0.015 ± 0.015)**	0.15 **(0.002)**	0.018 **(0.33)**	1 **(1.46 ±1.2)**
Kompienga	log_{10}THg vs adj.δ15N	0.16 ± 0.02 **(0.17 ± 0.02)**	0.65 **(0.64)**	< 0.001 **(< 0.001)**	3.59 ± 1.2 **(3.78 ± 1.2)**
	log_{10} MeHg vs adj.δ15N	0.14 ± 0.02 **(0.13 ± 0.02)**	0.53 **(0.53)**	< 0.001 **(< 0.001**)	3.10 ± 1.2 **(2.76 ± 1.2)**
	log_{10}TSe vs adj.δ15N	0.022 ± 0.01 **(0.045 ± 0.01)**	0.033 **(0.21)**	0.12 **(0.0015)**	1 **(1.42 ± 1.1)**

When removing all invertebrates (zooplankton, Unionidae and Gastropoda) (bold values in parentheses, Table 4.6) THg and MeHg biomagnified through fish food webs in the three dams at both 2010 rainy season and 2011 dry season (Fig 4.5).

Slopes of the regression between $\log_{10}$-TSe and $\delta^{15}N_{adj.}$ in entire food webs of each site during both 2010 rainy season and 2011 dry season were similar and did not significantly differ to zero (Table 4.5, $p > 0.05$). They corresponded to TMF not different from 1, indicating that no biomagnification of this element occurred in these ecosystems. However, when considering only fish food webs, TSe biomagnified in Kompienga reservoir during both rainy and dry seasons with slope of regression $\log_{10}$-TSe – $\delta^{15}N_{adj.}$ significantly greater than 0 (Fig 4.5, $p < 0.05$). Biomagnification of TSe was also observed in Ziga reservoir during 2011 dry season ($p < 0.05$).

Seasonal comparison of metal(loïd) biomagnification was done based on the fish food webs (Fig. 4.5). From rainy to dry season, the trophic magnification factor of THg through food webs remain unchanged in Loumbila reservoir ($p > 0.05$), increased 1.43 times in Ziga and decreased 1.73 times in Kompienga reservoir. These latter two variations were statistically significant (t-test, $p < 0.05$, Table S4.6). TMF values of MeHg decreased 1.3 and 2.4 times in Loumbila and Kompienga respectively from rainy season of 2010 to dry season of 2011. In Ziga reservoir, TMF of MeHg did not change significantly among the two seasons ($p > 0.05$). TSe biomagnified through fish food web in Kompienga reservoir during both 2010 rainy season and 2011 dry season with the same efficiency. (t-test, $p > 0.05$).

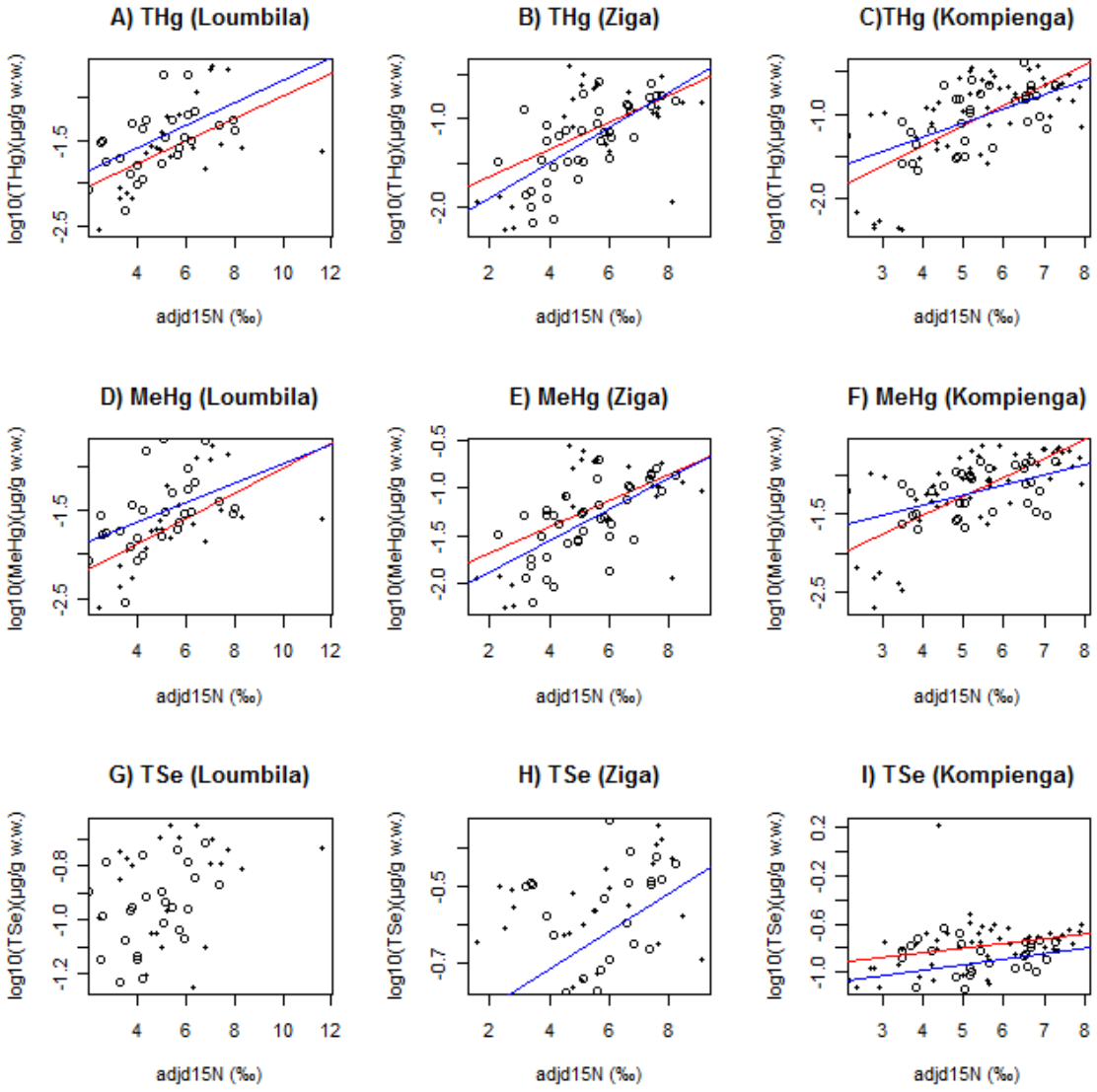

Figure 4.5. Relationships between $\log_{10}$(metalloid) concentration and adjuste d-δ^{15}N of fish in the three food webs from Burkina Faso. Individual organisms (biota) are represented by circles. Closed circles represent an individual from 2010 rainy season and the open circle from 2011 dry season. Red and blue lines are the linear regression lines from rainy and dry seasons, respectively

4.5 Discussion

4.5.1 Influence of season on metal(loids) concentrations in aquatic systems

We found significant decreases in mercury (THg and MeHg) and total selenium (TSe) concentrations in water, and sediment of the three freshwater reservoirs during dry season of 2011 compared with those of rainy season of 2010. The highest ratios of particulate Hg reported in all sites in the rainy season suggest that soil erosion via rainfall runoff is the main source of trace metal transportation and inputs into aquatic systems. Therefore, lack of rainfall during dry season may reduce trace metals inputs and their availability into aquatic systems.

Significant variation of THg, MeHg and TSe concentrations in some fish between the two seasons was also observed. A significant decrease of THg concentrations in fish during the dry season was most clearly observed in the Kompienga reservoir (Fig. 4.4C). The overall dry season decrease of THg levels in water and some fish suggested an influence of season on mercury dynamics in these systems. Among several hypotheses invoked to explain the seasonal variation of biotic Hg concentrations, variation of feeding habits over time depending on availability of food resources has been documented in a number of studies (Rimmer et al., 2010; Sampaio Da Silva et al., 2005). Since the trophic level of an organism is a good indicator of its Hg content (Cabana and Rasmussen, 1994), variations of Hg levels may reflect diet shifts. Habitat use is also known to reflect Hg concentration in organisms in lakes with pelagic dwellers having higher concentrations than littoral organisms (Chetelat et al., 2011; France, 1995; Post, 2002).

In this study, seasonal variation of Hg and TSe concentrations of individual fish could not be explained by variation of stables isotopes of $\delta^{13}C$ and $\delta^{15}N$ signatures. With the exception of some fish, most of them exhibited the same trophic position and relied on the same carbon sources for their food supply over the two seasons. However, traditional approach by gut contents analysis (Table 4.2) and zooplankton density (Table 4.1) yielded insights to understand variation of fish mercury level between rainy and dry seasons. For instance, the seasonal change of trophic status of *C. anguillaris* which shift from piscivorous fish at the rainy season to detritivorous fish at the dry season in Kompienga could explain its significant decrease of THg concentrations (Fig. 4.4C). Significant increase of THg levels of *A. occidentalis* from Loumbila and Kompienga reservoirs was observed during dry season compared with those of the rainy season. When we consider that Hg uptake by *A. occidentalis* was mainly from aquatic invertebrates, seasonal variation of THg content in this species in both Loumbila and Kompienga was consistent with the observed change in invertebrates ratio in the diet across seasons. The diet shift of *C. anguillaris* from fish as diet to detritus in Kompienga was expected to affect $\delta^{15}N$ signature. $\delta^{15}N$ signatures (‰) of *C. anguillaris* decreased from 7.37 ± 0.7 to 5.75 ± 1.3 during the dry season but this variation is not significant. Perhaps, the period of six month between the two sampling date is not long enough to integrate the turn-over time of ^{15}N in this species.

Further studies are needed to confirm our findings on the overall decrease of fish THg concentrations during dry season compared to rainy season from the Kompienga reservoir.

This study did not differentiate the forms of selenium in water but, it is known that selenium in the oxidized conditions is maily in the form of selenate (Simmons and Wallschläger, 2005). Therefore, low availability in our study sites of selenite, which is the

more bioaccumulative form of selenium could explain the observed low level of TSe in fish. Lower TSe level in fish in the dry season compared to that of rainy may be explained by seasonal variation of selenium speciation. Since microbial activities and primary production is important in selenium biotransformation (Simmons and Wallschläger, 2005), rainy season marked by higher primary production and high microbial activities in aquatic environment (Winemiller and Jepsen, 1998) is expected to favor a greater production of selenite forms compared to dry season, potentially leading to higher levels in fish in the rainy season. As chemical forms of Se influence its uptake by algae and microorganisms and its subsequent transfer to upper biota (Orr et al., 2005; Simmons and Wallschläger, 2005) seasonal difference in Se speciation may be factor explaining variation of Se levels in upper organisms such as fish. Seasonality of selenium uptake by biota has been previously reported (Mason et al., 2000) In this study, Mason et al. (2000) assessed the factors controlling bioaccumulation of metal(loïd)s in biota. For trout, selenium bioaccumulation increased of a factor of two with age in july, and less of the trend in April, suggesting a seasonal Se net depuration in this species.

4.5.2 Food webs structure and metal(loid)s biomagnification

No significant seasonal change was observed for $\delta^{13}C$ signatures in fish; however, a general decrease of $\delta^{15}N$ signatures was observed in the dry season. Most fish relied mainly on littoral carbon sources. Food webs were consistently short, with 2 to 3 levels, for all reservoirs and for both seasons.

Changes in $\delta^{13}C$ content in biota may reflect variation on endogenous or allochthonous carbon use for food supply, whereas changes in $\delta^{15}N$ values may reflect effects of prey availability or food chain length. It is well known that biota relying on pelagic carbon sources have higher mercury concentrations than those using littoral sources (France, 1995b). Furthermore, top predators from lakes with longer food chains have higher mercury concentration than those from lakes with short food chains (Cabana and Rasmussen, 1994). In this study, diet shifts that affect THg concentrations in fish from rainy to dry season was not necessarily linked to change in $\delta^{15}N$ signatures among the two seasons. For instance, variation in Hg concentrations from *A. occidentalis*, *C. anguillaris* from Kompienga and MeHg concentration of *S. intermedius* in Ziga was not linked to changes of the trophic position of these fish. Only change of trophic position of *S. intermedius* from Loumbila corresponded to change in Hg concentration. The reasons by which most fish Hg concentrations did not follow the temporal change in $\delta^{15}N$ signatures are not clear. Tissue turnover rate in isotopic shifts, high levels of omnivory and flexibility in feeding habits as well as fish migration in these fluvial systems are factors that could affects $\delta^{15}N$ enrichment over time (Jardine et al., 2006). Gut content analysis provide a shorter-term picture of trophic status (compared to isotopic signatures) that could

better explain this seasonal decrease of Hg in some fish without variation of $\delta^{15}N$ signature. This analysis suggests that within the same trophic level of prey, types of invertebrates used by fish over time may be important to their Hg uptake.

With respect to food web biomagnification, TMF values of MeHg were 1.3 and 2.4 times higher in rainy season food webs than that of dry season for Loumbila and Kompienga, respectively. This suggests a higher efficiency of mercury transfer from primary producer/consumer to fish at the top of food webs during rainy season compared to dry season in these food webs. This may explain lower Hg in fish from these site during dry season than that of rainy season. According to Trudel and Rasmussen (2006) fish bioenergetics vary seasonally and may play a key role on Hg bioaccumulation and biomagnification.

Change in prey availability was another hypothesis to explain variation in TMF (MeHg) among seasons. We think that changes in prey selection by fish over seasons may be an important factor on mercury uptake as Hg concentration in potential preys such as invertebrates can vary among and within species (Chetelat et al., 2011). Seasonal changes in prey taxonomic functional groups seem important in Hg uptake and its transfer through food webs. Logistic constraints did not allow us to follow up the dynamics of zooplankton over the two seasons for better interpretation of the systems dynamics. TSe did not show biomagnification in entire food webs and TSe concentrations in fish remained low. Since study sites were well oxygenated, selenate was the main form of selenium produced in these systems (Plant et al., 2004). However, when eliminating invertebrates in the food webs, we observed TMF (TSe) greater than 1 suggesting biomagnification of Se through fish food webs of Kompienga at both seasons and Ziga at the dry season. Evidence of TSe biomagnification was not found in Loumbila. This result could be related to the difference in site depth. In the

deepest reservoir, relative reducing conditions may be involved in the production of selenite (Simmons and Wallschläger, 2005) leading to observed biomagnification of TSe in Kompienga and Ziga.

Our results indicated seasonal influence on metal(loïd) dynamics in freshwater food webs from Burkina Faso. They suggested that stable isotope measurements in aquatic food web studies should be combined with prey abundance and functional feeding dynamics data to further improve interpretations of studied ecosystem dynamics.

4.6 Conclusion

This study was the first to measure the influence of season on metal(loïd) dynamics in freshwater reservoirs from sub-tropical areas of Africa using stable isotope analysis. We report significant decrease of aqueous mercury (THg and MeHg) concentrations from all study sites and from some fish particularly from the deepest reservoir Kompienga during dry season compared to what is observed in the rainy season. TSe levels in water did not show significant changes between the two seasons but, lower TSe concentrations in fish was reported in dry season compared with those of rainy season. The changes of THg levels in fish could not be related to changes in food web structures. However gut content analysis gave evidence of flexibility of feeding habits of many freshwater fish from Burkina Faso and indicate diet shifts in some fish species that may influence metal(loïd)s uptake. TMFs comparison indicated a decrease of MeHg biomagnification rates in Loumbila and Kompienga during the dry period likely leading to low Hg content in fish compared to that of rainy season. TSe dynamics in

food webs of our study sites (oxygenated and unstratified) may be related to its biogeochemical speciation and seasonal variation of uptake rates of aquatic primary producer and fish.

Stable isotope of ^{13}C and ^{15}N analyses provide a time-integrated measure of trophic position and have the potential to track energy or mass flows through food webs. However, they failed to track seasonal shifts of contaminants in food webs in this study, and should be combined with prey abundance and functional feeding dynamics data to further improve interpretations of studied ecosystem dynamics and fish ecology. Further investigations are needed to assess biogeochemical transformations that affect Hg and Se in these aquatic systems to enhance our understanding on their seasonal dynamics.

4.7 Acknowledgement

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Chapitre 5

Effects of various cooking methods and food components on bioaccessibility of mercury from fish

Ousséni OUÉDRAOGO and Marc AMYOT

5.1 Abstract

Fish consumption is the main source of human exposure to mercury. Studies from specific human populations have reported Hg levels lower than those modeled from consumption data. These discrepancies between expected and measured Hg levels may be explained by differences in dietary habits such as cooking methods and food components on fish Hg bioavailability. We assessed the effects of three cooking methods (no cooking, frying and boiling) and of the co-ingestion of selected food items (tea, coffee and corn starch) on Hg bioaccessibility in three fish species (tuna, shark and mackerel) containing between 1 and 4 µg/g dry weight of Hg. We used *in vitro* techniques simulating human digestion and each experiment was repeated three times with at least three different individuals for each fish species. For all fish species, Hg concentrations (dry weight) in boiled fish were slightly but not significantly higher than those in fried or raw fish. Boiling and frying reduced Hg bioaccessibility by 40 % and 60 % respectively, compared to raw fish Hg bioaccessibility. Black coffee as well as green and black tea significantly reduced raw fish Hg bioaccessibility by 50- 60 %, whereas, corn starch did not. The combined effect of cooking and addition of tea or coffee led to very low levels of Hg bioaccessibility. This study suggests that Hg bioaccessibilty from fish can be modified by cooking and by the co-ingestion of tea and coffee. These results should be further validated *in vitro* with different fish species before proceeding with *in vivo* approaches using animal models.

Keywords: Mercury, bioaccessibility, cooking methods, in vitro digestion.

5.2 Introduction

Mercury (Hg) exposure poses a risk to human health due to its adverse neurological effects (Clarkson and Magos, 2006; W.H.O., 1991; W.H.O., 2007). Mercury is classified by the International Agency for Research on Cancer (IARC) as a possible carcinogen (2B group) to humans (IARC, 1993). Teratogenic effects of mercury have also been observed (Grandjean et al., 2005) and its cardiovascular effects have been suggested(Choi and Grandjean, 2008).

In aquatic environments, inorganic mercury can readily be transformed into methylmercury (MeHg) which bioaccumulates and biomagnifies through the food chain and lead to high concentrations at the top of aquatic food webs (Morel et al., 1998). Predatory fish such as shark and tuna have the highest concentrations of MeHg (Boudou et al., 2006; Roulet et al., 1998). Consequently, fish consumption is the main source of human exposure to mercury (Clarkson et al., 2003; US-EPA, 2009).

Epidemiologic studies use mercury concentrations in hair, urine or blood as biomarkers to estimate human exposure to MeHg through fish consumption (Dolbec et al., 2001; Lebel et al., 1997). In current risk assessment, oral dose from a specific product is considered to be equal to bioavailability found in systemic circulation. A dose-response relationship between MeHg oral dose and body burden is an important tool used to establish guidelines on safe levels of MeHg exposure. Canuel et al. (2006a) conducted a study using data sets on fish consumption habits, Hg levels in edible fish, and corresponding Hg accumulation in hair, for different communities. Using a model based on pharmacokinetic parameters obtained from

NRC (2000), they calculated daily exposure using fish consumption patterns, and modeled Hg levels in hair. They then compared modeled and measured levels of Hg in hair. Expected hair Hg levels were 369 to 586 % higher than the measured concentrations, for inhabitants from Inuit, Cree and Objiwa communities in Canada, and from three Japanese communities. In the case of a Canadian Innu community, hair Hg levels were 14 fold lower than the expected average concentration in hair based on the model (Canuel et al., 2006a). These observations demonstrate that Hg levels in hair do not always reflect the reported level of MeHg intake via fish consumption.

Several hypotheses have been proposed to explain these discrepancies between measured and expected values in hair. One of them is that oral bioavailability of Hg from fish may be reduced due to complexation of mercury by chelating agents such as fibers and phytates found in other co-ingested food items during digestion (Shim et al., 2009). Only a fraction in a consumed item may thus be bioavailable after oral exposure. Indeed, mercury toxicity was found to be reduced by several compounds such as selenium (Cabanero et al., 2004; Cabanero et al., 2007), omega-3 fatty acid, vitamin E, or by alcohol (Dunn et al., 1981; PNUE, 2007). Field studies have also shown that the strong relationship between fish consumption and Hg exposure was significantly modified by fruit consumption in Brazilian region (Passos et al., 2003; Passos et al., 2007). Canuel et al. (2006b) have further suggested that the consumption of large amounts of tea by Canadian aboriginal populations may partly explain the discrepancies between measured and expected mercury levels in hair. Tea contains significant amounts of flavonoids which may chelate mercury and inhibit it bioavailability.

For that reason, it has been suggested that, for each community, risk assessment should consider the impact of dietary habits on mercury bioavailability.

Dietary habits may include foods components and cooking processes. Fish is usually eaten after various cooking treatments such as boiling, grilling, baking or frying, but sometimes it is eaten raw (e.g. in sushi meal). It is known that cooking may affect the amount and speciation of chemical pollutants in foods (Burger et al., 2003; Ersoy and Özeren, 2009; Ersoy et al., 2006; Gokoglu et al., 2004; He et al., 2010; Perello et al., 2008). Some studies have been published either on Hg bioaccessibility from fish (Shim et al., 2009; Torres-Escribano et al., 2010) or on the effect of cooking on Hg content in fish (Ersoy et al., 2006; Perello et al., 2008). However, no study has yet evaluated Hg bioaccessibility in cooked fish. Bioaccessibility is defined here as the fraction of ingested Hg that is solubilized into the gastrointestinal tract (Oomen et al., 2003; Ruby et al., 1999). If Hg bioaccessibility differs in cooked and raw fish following digestion, this can have significant implications for risk assessment.

In vitro digestion experiments are useful tools to assess mercury bioaccessibility. These experiments make use of simulated gastric and intestinal juices, which are applied to samples in order to predict the availability of metals for human absorption. They mimic processes that occur in typically two or three distinct, but linked, areas of the human digestive system (mouth, stomach and small intestine). Several approaches have been developed to assess metal bioaccessibility from soils (Brandon et al., 2006; Garrett et al., 1999; Intawongse and Dean, 2006; Pouschat and Zagury, 2006; Rodriguez et al., 1999; Ruby et al., 1996; Zagury et al., 2009), and from fish samples (Cabanero et al., 2004; Laird et al., 2009; Laird et al., 2007; Leaner and Mason, 2002; Shim et al., 2009).

The investigation detailed below assesses the effect of cooking methods and food components on the bioaccessibility of Hg in three fish species using an in vitro digestion model. The first objective was to verify if different cooking methods altered Hg concentrations (on a dry weight basis) in fish tissues, prior to digestion. We predicted that cooking would alter water content and Hg levels on a wet weight basis, but not on a dry weight basis. The second objective was to assess Hg bioaccessibility after simulated digestion of raw, boiled and fried fish muscles. We predicted that heat from boiling and frying would alter mineral content and protein structure (Gokoglu et al., 2004) leading to a change in Hg bioaccessibility. We further hypothesized that cooking and digestion would not alter the ratio of MeHg over total Hg. The third objective was to determine the impact of the co-ingestion of food items on Hg bioaccessibility of raw, boiled and fried fish samples. We anticipated that food items with known chelating properties toward metals such as tea, coffee and corn starch would reduce fish mercury bioaccessibility.

5.3 Materials and methods

5.3.1 Food items and reagents

Food samples were purchased in 2010 from markets and grocery stores in Montreal, Canada. They included three marine fish species: spanish mackerel (*Scomberomorus maculatus*), Mediterranean shark (*Scyliorhinus canicula*) and red tuna (*Thunnus thunnus*),

green and black tea (Golden Sail, China), instant coffee (Nescafé, Switzerland), corn starch (Ideal, China) and corn oil (Mazola, U.S.A.).

Fish samples were prepared by removing the skin and bones. The edible (muscle) portion was divided into three samples that were boiled, fried or kept raw. Green and black teas were infused in warm ultrapure water (250 ml for one pocket) and the solution was lyophilized to obtain a powder. Instant coffee, tea powder and corn starch were solubilized in 5 mL of ultrapure water [Milli-Q (Millipore Corporation, Billerica, MA, USA) $\geq$ 18 MΩcm^{-1}] before being used in test meals. For tea and coffee, the amounts of powder used (40, 80, 120 and 160 mg) to prepare test meals correspond to 1, 2, 3 or 4 cups of 250 mL reduced to 5 ml.

Enzymes and bile salts were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA) and included porcine pepsin (enzymatic activity 800. 2,500 units/mg protein), porcine pancreatin (activity equivalent to 4X U.S.P) and porcine bile (glycine and taurine conjugates of hyodeoxycholic acid and other bile salts).

The following reagents were of analytical or reagent grade: monobasic anhydrous sodium citrate (Sigma Aldrich), DL-malic acid disodium salt (Sigma Aldrich), lactic acid (Fisher Scientific, Fair Lawn, NJ, U.S.A.), hydrochloric acid (Fisher Scientific), nitric acid (Fisher Scientific), glacial acetic acid (Fisher Scientific), sodium hydroxide (Fisher Scientific), tin(II) chloride dehydrate (Fisher Scientific) and sodium hydrogen carbonate (BDH , Toronto, Canada).

5.3.2 Test meals

Test meal preparation was done according to Shim et al. (2009). A portion of fish soft tissues (boiled, fried or raw) and the same amount (weight/weight) of a saline solution (0.9 % NaCl) were homogenized in a food processor. Stainless tools were used to cut and homogenize fish tissues.

5.3.3 Digestive juices

For the batch extraction method, we selected one of the most commonly used gastric solutions, developed by Ruby et al. (1996) and Garett et al. (1999). Briefly, we adjusted 1L of ultrapure water to pH 2 with 2 ml of 12 N HCl and adding 1.25 g of porcine pepsin, 0.50 g of sodium citrate, 0.50 g of malic acid, 420 µL of lactic acid, and 500 µL of acetic acid. Intestinal juices were prepared in sample tubes by homogenizing 2.4 mg mL^{-1} of salt bile extract and 0.6 mg mL^{-1} of pancreatin in 9 ml of $NaHCO_3$ (Shim et al., 2009).

5.3.4 Experiments

5.3.4.1 Effect of cooking methods on mercury content in fish

A portion of each fish (n = at least 3 samples) was boiled, fried or left uncooked and mercury content were measured before and after treatment. Each experiment was repeated at least three times with three different individuals for each fish species. Frying was done in a teflon skillet using corn oil for 10 min. The temperature of oil during the frying process was 160˚C. Boiling was performed in a teflon saucepot filled with ultrapure water. Fish tissues were dipped into boiling water for 15 min at 80˚C. The weight/weight ratio of oil: fish and water: fish were 1 and 2, respectively.

5.3.4.2 In vitro digestion protocol

We performed the in vitro digestion according to the static digestion model SBET (Simple Bioaccessibility Extraction Test) described by Ruby et al. (1996). Briefly, 30 mL of gastric solution were added to 0.5, 1, 2, and 5 g of homogenized fish meal in a 50 mL polyethylene vial to determine a suitable amount of fish to use in the digestion process. The results of these preliminary tests are reported on Figure S5.1 (Annexe 5, supplementary data) and indicated that a fish meal of 1 g was appropriate for our experimental conditions. Therefore, this amount was used for the rest of the experiments. 1 g of each homogenized fish (raw or cooked), correspond to 0.42 ± 0.1 g dry weight. This amount of tissue was added to 42

± 2 ml of test liquid, leading to a dry weight to volume ratio of 1:100. This ratio has been shown to be appropriate by Van de Wiele et al. (2007).

Each test meal was done in triplicate and all aliquots were incubated at 37° C for 1h in a shaking incubator at 100 rpm. To mimic digestion in the small intestine, 9 mL of intestinal juice were added to gastric solution after rising the pH to 5.3 with saturated $NaHCO_3$. Final intestinal juice pH was adjusted with NaOH 1M to 7.0 ± 0.5 and aliquots were placed in the shaking incubator for 2 h at 37° C, 100 rpm.

For experiments on the effect of food items, the following diet components were added to homogenates: green tea powder (40, 80, 120 and 160 mg), black tea powder (40, 80, 120 and 160 mg), corn starch (50, 100, 500 and 1000 mg), or black coffee (40, 80 120 and 160 mg). The volume of the controls (no food added) were adjusted with water in order to be equal to the volume of the treatments with food addition.

After digestion, solutions were centrifuged at 3000 g for 15 min to separate the aqueous phase from residual materials. The supernatant of each aliquot was sampled in triplicate in 1.5 mL Eppendorf tube and stored at -20° C prior to mercury (THg and MeHg) analysis. The pellet was lyophilized prior to Hg analysis. THg in the aqueous phase was defined as bioaccessible, and percent THg bioaccessibility (% BA) can be calculated by dividing the amount in the digestive juice by the total amount in the test food before the digestion experiment using the following equation:

$$\% \text{ BA} = ([\text{THg}]_{(aq)} \times V) / ([\text{THg}]_{(meal)} \times M) \times 100 \quad (1)$$

where $[THg]_{(aq)}$ is the total Hg concentration (μg mL^{-1}) in the aqueous phase, V is the total water volume of the gastrointestinal fluid (mL), $[THg]_{(meal)}$ is the THg concentration in the test food (μg g^{-1}), and M is the total mass (g) of the food digested through the in vitro process. THg in the pellet after gastrointestinal digestion was measured and percent of THg recovery was assessed according to following equation (all values in μg):

THg recovery (%) = (THg in aqueous phase + THg in pellet) / (THg in pre-digested fish) × 100 (2)

To assess the impact of phytochemical-rich food on mercury bioaccessibility, 1 g of each fish meal was used and the same in vitro digestion process was applied. The ratio of liquid/meal was set at 100:1. Recovery rates for all digestions under the different experimental conditions averaged 98 ± 21 % (n = 97).

5.3.5 Mercury analysis

Fish samples were subjected to gastrointestinal digestion and total mercury (THg) and MeHg were determined in aqueous phase and in the residual pellet. THg content of each fish used in the experiments was assessed in raw, boiled and fried samples to determine effect of cooking type on fish mercury level and their impact on mercury bioaccessibility.

THg of aqueous phase of each aliquot was performed by cold vapor atomic fluorescence spectrometer (CVAFS, Tekran 2600, Toronto, Canada) following U.S. Environmental Protection Agency (U.S. EPA) method 1631. Briefly, 50 mL of diluted sample was digested with 200 mL of BrCl, and excess of BrCl was neutralized with 50 μL of hydroxylamine. Samples were then reduced with stannous chloride ($SnCl_2$,3 % w/v) prior to analysis. The detection limit for this analysis was 0.1 ng THg/L and the mean relative recoveries was 99.3 ± 5.1 % (n=34).

Fish tissues and centrifugation pellets were analyzed for total mercury using a direct mercury analyzer (DMA 80, milestone inc., Pittsburgh, PA), in which samples were combusted at 750°C and mercury vapors were retained on a gold trap for analysis by cold vapor atomic absorption spectrometry. DMA threshold analysis was between 0.12 and 600 ng and detection limit was 0.05 ng THg/sample, with average analytical variance of 5 %. Certified references materials (Tort-2 and Dorm-2, Institute for Environmental Chemistry, National Research Council of Canada, Ottawa, Canada) were used to standardize the instrument. TORT-2, and Dorm-2 were analyzed every 10 samples. Tort-2 recovery averaged 307. 26 ± 15.80 ng g-1 (n = 19), corresponding to 102 ± 5 % of the certified value. Dorm-2 recovery averaged 4628.84 ± 233.29 ng g-1 (n = 49) corresponding to 99.75 ± 5 % of certified value. The relative standard deviation of analytical triplicates averaged 1.51 % (n=19).

MeHg concentrations were determined in pellets and in the supernatant of some aliquots. Prior to analysis, 10 to 50 mg of dried pellets were digested in 5mL of 4M HNO_3 at 55 ˚C for 16 h. For the supernatant, 1 mL of aqueous phase was digested in 5mL of 4M HNO_3 at 55 ˚C for 16 h. Digested samples then underwent aqueous-phase ethylation with $NaB(C_2H_5)_4$, followed by gas chromatography separation with CVAFS detection (Tekran

2500), based on the method of Bloom (1989). Procedural blanks contained 1 ± 1 pg MeHg. Analytical accuracy was checked by analysis of TORT-2 (lobster hepatopancreas certified reference material of the National Research Council, Canada). Recoveries for TORT-2 averaged 148 ± 9 ng g^{-1} (n= 6), corresponding to 97 ± 6 % of the certified value (TORT-2 = 152 ± 13 ng g^{-1} MeHg).

Mercury contents of chemical reactants (pepsin, pancreatin, bile extract) and food components (coffee, tea, corn starch) were close to DMA-80 detection limit and were therefore not considered a significant source of contamination in our experiment.

5.3.6 Data analysis

Data analysis was done using the statistical software R-2.11.1. Results are expressed as means ± standard deviation of mean. Each experiment was repeated at least three times to insure results reliability. Differences among treatments were assessed, using a Kruskal-Wallis signed rank test (Scherrer, 2007). Wilcoxon Signed Rank test for paired samples was used in case of group heterogeneity to check difference among the k groups. To evaluate if cooking methods affected fish THg bioaccessibility, we compared THg bioaccessibility following in vitro digestion of raw and cooked (boiled and fried) fish. Food items effect were assessed by comparison between THg bioaccessibility from raw fish tissues in the presence of other food items to that from raw fish tissues alone following in vitro digestion process. A significance level of $P < 0.05$ was adopted for all comparisons.

5.4 Results

5.4.1 Effect of various cooking methods on fish Hg concentration

As part of our cycle of experiments on Hg bioaccessibility, we first tested the effect of cooking methods on Hg levels in fish tissues used in subsequent experiments. For all fish species, THg concentrations (dry weight) in boiled fish were slightly but not significantly higher than those in fried or raw fish (Table 5.1; Kruskal-Wallis Signed Rank test; $p > 0.05$). On average, raw fish was 13 % less contaminated than boiled fish. Mean THg levels in raw and fried fish were very similar, and differed by 3 to 6 % depending of the species. During cooking, water content of fish tissues decreased on average by 10 – 20 % for boiled samples and by 35 – 48 % for fried ones. Only moisture loss during frying was statistically significant in each case (Wilcoxon Signed Rank test; $p < 0.05$). The relatively high variance in Hg levels in fish in Table 5.1 mainly reflects natural variability in Hg content in different individuals, rather than analytical variability.

Table 5.1 THg concentration and average moisture of fish samples (tuna,shark and mackerel) following cooking processes.

Fish species	Cooking methods	[THg] (µg/g dry weight)	Moisture (%)
Tuna (n=3)	raw	1.37 ± 0.62	72.3 ± 2.6
	boiled	1.57± 0.76	62.1 ± 7.6
	fried	1.30 ± 0.53	35.9 ± 11.9
Shark (n=3)	raw tissues	3.50 ± 2.57	80.3 ± 6.3
	boiled	4.00 ± 3.00	71.5 ± 3.4
	fried	3.38 ± 2.64	60.4 ± 1.9
Mackerel (n=4)	raw tissues	1.05 ± 0.45	72.4 ± 4.7
	boiled	1.22 ± 0.40	66.8 ± 2.9
	fried	1.06 ± 0.30	58.9 ± 7.9

5.4.2 THg bioaccessibility in digested raw and cooked fish.

Hg bioaccessibility was assessed in digested raw, boiled and fried muscles of three fish species (tuna, shark and mackerel) (Fig. 5.1). Total Hg in all fish species was more bioaccessible in digested raw tissues (up to 80 % in aqueous phase), compared to boiled (40 %) and fried fish (< 20 %). Differences between treatments were shown to be statistically significant (using a Kruskal-Wallis test (Annexe 1, Supplementary data, Table S5.1)), except between boiled vs. fried shark (Wilcoxon signed rank test, $p < 0.05$ (n=3); Fig. 5.1).

5.4.3 Cooking effect on MeHg partitioning after in vitro digestion.

MeHg partitioning between dissolved and particulate (pellet) phases after digestion was assessed on a subset of raw, boiled and fried tuna samples. The total mass of MeHg and THg found before and after digestion were similar, with recoveries ranging from 93-144 % for MeHg and from 95-111 % for THg (Table 5.2). This indicates that digestion did not result in a production or degradation of MeHg. MeHg in digested raw fish was mostly found in the liquid phase, with masses 11 times higher than in pellets. In sharp contrast, MeHg burdens in pellets from digested boiled and fried tuna were 2 and 12 times higher than in the liquid phase, respectively.

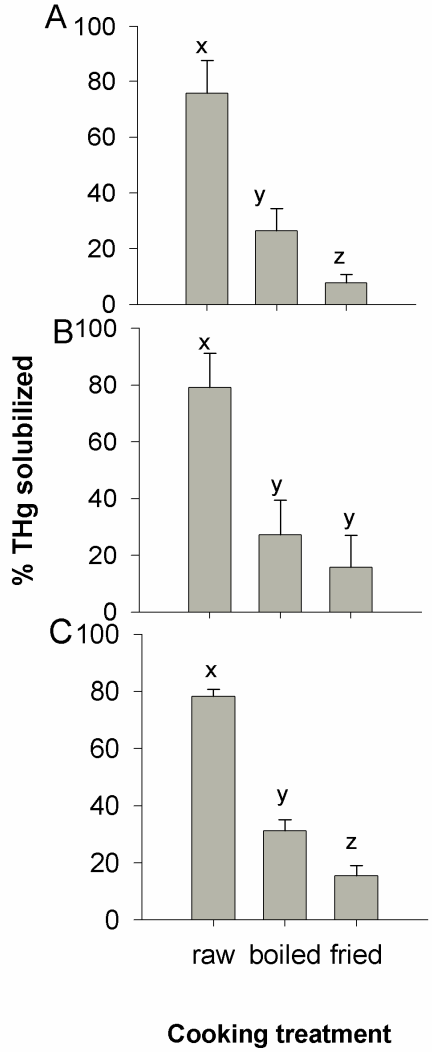

Figure 5.1 Effect of cooking treatments (boiling, frying or keeping raw) on THg bioaccessibility for three fish species: **A**) tuna (*Thunnus tunnus),* B) mediteranean squale *(Scyliorhinus canicula) and* C*)* spanish mackerel *(Scomberomorus maculatus).* Values represent means (% THg solubilized) ± standard deviation for 3 replicates. Letters x, y and z indicate significance at the $\alpha = 0.05$ level, from a Wicoxon signed rank test.

Table 5.2 MeHg contents in tuna digestion produces (pellet and liquid phase).

Sample	THg (ng)	MeHg (ng)	(%) MeHg
Raw (n =3)			
Pre-digestion	500 ± 3	390 ± 2	78 ±4
Pellet	56 ± 13	47 ± 11	84 ± 1
Liquid phase	474± 11	516 ± 30	109 ± 7
Total post-digestion	531± 4	563 ± 32	106 ± 6
(%) Recovery	106 ± 1	144 ± 8	
Fried (n =3)			
Pre-digestion	1289 ± 10	1095 ± 9	85 ± 0.6
Pellet	1271 ± 84	996 ± 19	79 ± 5
Liquid phase	166 ± 50	82 ± 9	53 ± 17
Total post-digestion	1437 ± 83	1078 ± 28	75 ± 2,5
(%) Recovery	112 ± 6	98 ± 3	
Boiled (n =3)			
Pre-digestion	943 ± 5	802 ± 4	85± 4
culot	607 ± 24	506 ± 23	83 ± 8
Liquid phase	291 ± 35	237 ± 36	81 ± 3,5
Post- digestion	899 ± 50	743± 60	82 ±5
(%) Recovery	95 ± 5	93 ± 8	

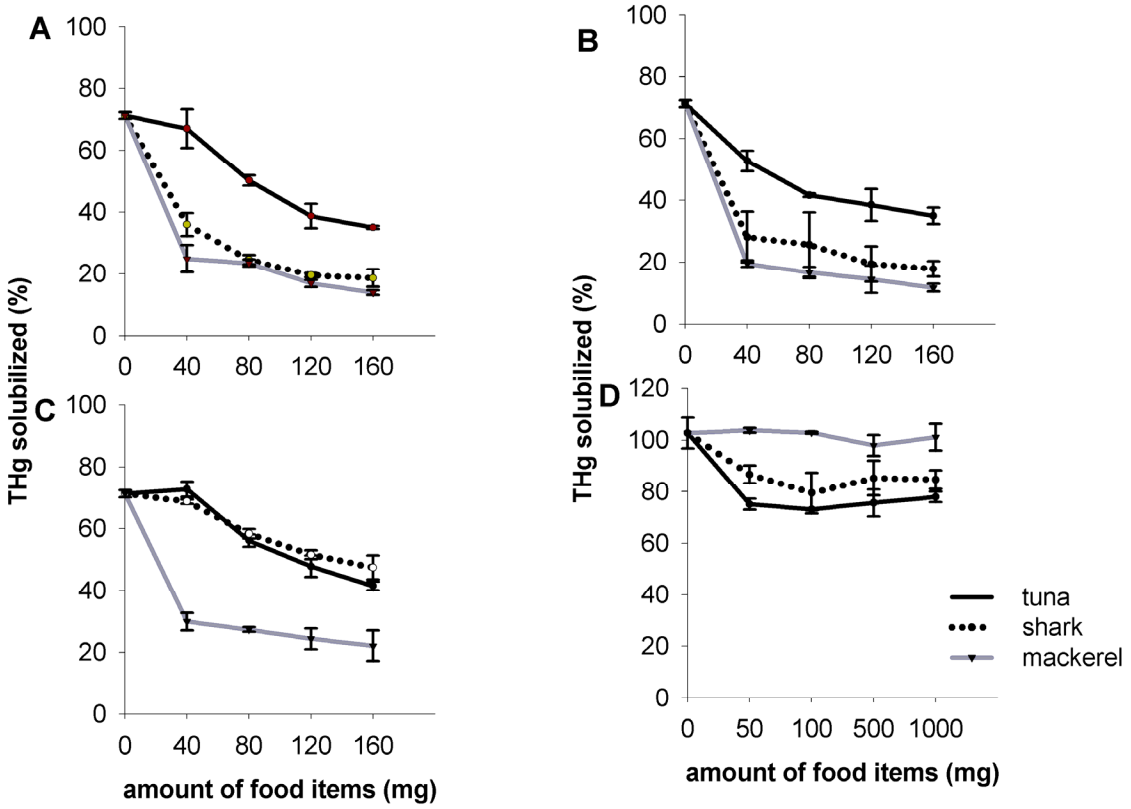

Figure 5.2 Effects of THg bioaccessibility from three fish species (red tuna, shark and green spanish mackerel) following in vitro digestion processes of **1** g fish tissue in the presence of increasing amounts of food items. **A**) green tea , **B**) black tea, **C**) black coffee and **D**) corn starch. Values represent means (% THg solubilized) ± standard deviation for 3 replications.

5.4.4 Effect of food items on fish mercury bioaccessibility

Effects of food components (black coffee, green and black tea, corn starch) added to each fish meal (raw fish) on THg bioaccessibility were investigated. Green tea, black tea and coffee significantly reduced Hg bioaccessibility in all fish species, whereas corn starch addition had a weaker effect (Fig. 5.2; Kruskal-Wallis rank test (Table S5.2). Hg

bioaccessibility usually decreased as a function of the amount of tea or coffee added, eventually reaching a plateau in most instances.

For green tea and black tea (Fig. 5.2A , B), this plateau was reached after the addition of 40 mg of tea to shark and mackerel tissues, and the maximal loss of bioaccessibility ranged from 50 to 60 %. For tuna, the decrease was weaker, with a maximum loss of bioaccessibility of 35 % after the addition of 160 mg of tea.

For coffee, the maximum loss in Hg bioaccessibility of 50 % was observed for mackerel, and a plateau was reached for this species after the addition of 40 mg. Hg bioaccessibility decreased more slowly for tuna and shark, with a maximum loss ranging from 10 to 30 % (Fig. 5.2C).

For corn starch, no significant decrease in bioaccessibility was observed for mackerel and shark (Table S5.2). For tuna a decrease of up to 20 % was reported after the addition of 50 mg of corn starch, but no further decrease occurred after the addition of more corn starch (Fig. 5.2D, Table S5.2).

Additional loss of Hg bioaccessibility in tuna occurred when we combined the effect of cooking and of ingestion of tea or coffee (Fig. 5.3). Hg bioaccessibility in cooked tuna was nearly eliminated when more than 40 mg of green or black tea was added (Fig. 5.3A and 3B). Co-ingestion of coffee had a lesser effect on Hg bioavailability, leaving about 5- 25 % of Hg bioaccessible, after the addition of 160 mg (Fig. 5.3C). Corn starch addition did not alter Hg biaccessibility of cooked tuna, regardless of the amount added (Fig. 5.3D). These results indicate that there is an additive effect of cooking and of co-ingestion of food items on Hg bioaccessibility in fish.

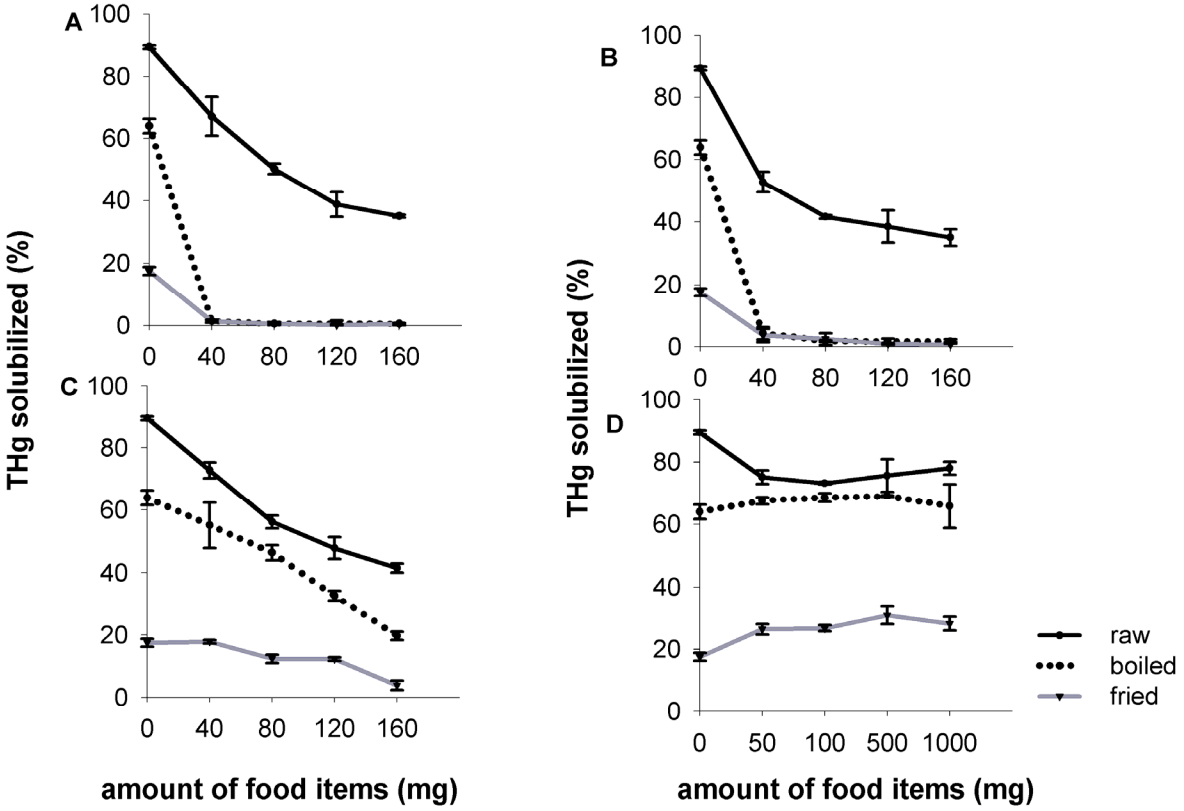

Figure 5.3. Effects of THg bioaccessibility from tuna fish meal following in vitro digestion processes of **1** g fish tissue (raw, boiled and fried) in the presence of increasing amounts of food items. **A**) green tea , **B**) black tea, **C**) black coffee and **D**) corn starch.

5.5 Discussion

Our results indicate that cooking did not significantly modify Hg concentrations in fish tissues, when expressed on a dry weight basis. Previous studies on different fish species have similarly observed no clear change in Hg levels after cooking, on a dry weight basis

(Armbruster et al., 1988; Morgan et al., 1997). However, some authors are reporting their results on a wet weight basis, in which cases cooking usually tends to increase Hg levels due to variable losses of water and fat (Burger et al., 2003; Morgan et al., 1997; Perello et al., 2008). Therefore, it seems that mercury in fish muscles is not significantly driven off during cooking and likely remains bound to proteins (Burger et al., 2003). Studies on effect of cooking methods on composition and mineral contents of fish have shown an increase of protein content in cooked samples compared to raw fish due to water loss occurring during cooking process (Ersoy and Özeren, 2009; Gokoglu et al., 2004).

Even though cooking did not significantly modify Hg concentrations in fish compared to uncooked fish, it had a profound effect on Hg bioaccessibility during *in vitro* digestion. It is likely that boiling and frying are modifying protein structure by heating, rendering Hg-protein complexes less available for solubilization during digestion. Mercury is indeed recognized for its strong affinity for proteins (George et al., 2008; Harris et al., 2003) and its relatively weak lipophilic nature (Mason et al., 1996). In some studies, protein denaturation by heat has been shown to alter the reactivity of protein-bound Hg. For instance, Dunn et al. (1981) observed that treating mouse liver homogenates with heat (74^{o}C water bath for 10 to 15 minutes) hampered the conversion of Hg^{2+} to volatile elemental Hg in vitro. They argued that heat denaturation may create additional binding sites and thus effectively sequester the metal from active sites. In the present study, the change of protein structure with cooking conditions may similarly explain the observed change in solubilization in boiled and fried samples. In addition to protein content, higher levels of fat content in fried fish samples compared to raw or other cooked sample (Ersoy and Özeren, 2009) may also lower THg bioaccessibility.

Cooking and digestion did not alter the total mass of MeHg in the samples, and therefore there was no conversion between inorganic and organic forms of Hg. This is in agreement with the study of George *et al*. (2008) in which no Hg conversion was observed in fish digested in simulated gastric fluid. However, cooking had a strong effect on the post-digestion partitioning of MeHg, with most MeHg found in the liquid phase for digested raw fish, and most MeHg found in less bioaccessible pellets for boiled and fried fish. This effect of cooking on MeHg partitioning is similar to what was observed in this study for THg (see section 3.2).

The co-ingestion of tea and coffee decreased Hg bioaccessiblity during digestion of cooked and uncooked fish, whereas corn starch had a weaker effect. Shim *et al*. (2009) in their study on the impact of phytochemical-rich foods on bioaccessibility of mercury from uncooked fish similarly obtained reduction rate of 82–92 % and 88–91 % respectively for green and black tea. The chelating role of these components which are rich in phytates was hypothesized to explain the decrease in Hg bioaccessibility. Phytate has been reported to form complexes with proteins at both low and high pH values. This complexation alter protein structure, which may result in decreased protein solubility (Kumar et al., 2009; Shim et al., 2009). According to Kumar et al. (2009), phytate forms complexes with dietary minerals, especially iron and zinc, and causes mineral-related deficiency in humans. These authors noticed that diet supplementation of phytase (an enzyme degrading phytates) results in increase in mineral absorption. These observations support the “complexation hypothesis” but we are not aware of studies on the effect of phytates on mercury bioaccessibility. Indeed, corn starch is a cereal which may content fibres and phytates and it did not reduce mercury

bioaccessibility. Further investigation is necessary for better understanding impact of food components on mercury bioaccessibility.

5.6 Conclusion

Frying and boiling fish decreased Hg bioaccessibility when compared to raw fish. This decrease was higher in fried fish than in boiled fish. Moreover, foodstuffs like green tea, black tea, and coffee simultaneously ingested with fish meal decreased fish mercury bioaccessibility. Corn starch did not show significant impact on mercury bioaccessibility. These results stress the importance of dietary habits on fish THg bioaccessibility. The exact mechanisms by which cooking affects Hg bioavailability should be further investigated.

We are not aware of the existence of previous data comparing various cooking effects on THg bioaccessibility from fish consumption. Since Hg in raw fish is highly bioaccessible, people who have fish as a major dietary source might reduce raw fish consumption to limit their mercury exposure. However, an important step to validate the results and conclusions from this work would be to conduct *in vivo* experiments with animal models. If our results were confirmed by *in vivo* studies, toxicological risk assessments associated with fish consumption would have to be modified to take into account cooking methods and dietary habits in a given community.

5.7 Acknowledgement

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Chapitre 6.
Discussion générale

Cette étude a été conduite pour évaluer les niveaux de contamination des systèmes aquatiques au Burkina Faso par le mercure (Hg), l'arsenic (As) et le sélénium (Se) dans une perspective d'évaluation de risque. Elle se veut aussi une des premières études à décrire la bioaccumulation et le transfert trophique de ces contaminants dans des milieux aquatiques d'eau douce en Afrique au sud du Sahara. Dans cette section, nous discuterons les principales observations de cette étude tant en ce qui concerne le niveau de la contamination des écosystèmes, le transfert au sein des réseaux trophiques que sur l'aspect risque d'exposition avec notre essai expérimental sur la bioaccessibilité du mercure de poisson en lien avec les habitudes alimentaires des communautés.

6.1 Contamination métallique des systèmes aquatiques au Burkina Faso et risque potentiel pour les organismes aquatiques et les humains.

Nous avons rapporté des concentrations en Hg, As et Se relativement faibles dans l'eau, en deçà des limites recommandées par les agences de réglementation internationales pour la préservation de la santé environnementale (FAO/WHO, 2004; USEPA, 2001). Ces concentrations sont comparables aux résultats observés sur des sites non contaminés en Afrique (Taylor et al., 2005) et ailleurs dans le monde (Burger et al., 2001; May et al., 2008).

Pour investiguer le rôle de la physico-chimie de l'eau sur les niveaux de concentration des métal(loïd)s nous avons choisi une approche d'évaluation qui prend en compte la présence simultanée de ces éléments dans le même milieu en considérant les interactions bien

documentées entre d'une part le Hg et le Se (Gailer, 2007; Ganther et al., 1972; Parizek et Ostadalova, 1967) et d'autre part l'As et le Se (Gailer, 2007). Les analyses multivariées mettant en relation les variables physico-chimiques et les teneurs des 3 métal(loïde)s ont permis d'identifier 4 variables (température de l'eau, la conductivité, les nitrates et la couleur de l'eau) comme variables majeures modulant la co-occurence de ces 3 métal(loïde)s dans les eaux des reservoirs étudiés au Burkina Faso.

Nous avons observé de très faibles teneurs en méthylmercure (MeHg) (2% du mercure total (HgT)) dans l'eau de ces réservoirs. Nos résultats qui sont comparables à ceux observés dans l'étude conduite en Afrique du Sud (Walters et al., 2011) contrastent avec ceux rapportés d'autres régions dans des environnements similaires où le ratio MeHg/HgT peut compter jusqu'à 50 % (Balogh et al., 2006; He et al., 2007; Roulet et al., 2001; Zhang et al., 2010). Cette observation permet d'expliquer en partie les faibles teneurs en Hg chez les poissons unanimement observées dans les études menées dans les lacs et réservoirs d'eau en Afrique (Agorku et al., 2009; Black et al., 2011; Campbell et al., 2006; 2005; Desta et al., 2007; Donkor et al., 2006; Ikingura et al., 2006; Kidd et al., 2004; Kwaansa-Ansah et al., 2011; Machiwa, 2005; Tadiso et al., 2011).

En l'absence de données sur la géochimie du Hg notamment sur la métylation du Hg dans ces environnements aquatiques nous avons spéculé sur les raisons potentielles de ces faibles teneurs en MeHg. Nous avons invoqué:

1) Le rôle des températures élevées sur les transformations biotiques qui peuvent affecter le MeHg en milieu aquatique. La température influencerait le ratio méthylation/déméthylation et in fine, les teneurs nets en MeHg (Barkay et al., 2003).

2) Le rôle des radiations UV solaires dans la photodéméthylation du Hg (Amyot et al., 1997a)
3) Le rôle des sols latéritiques (riches en Fe et Al) qui pourraient séquestrer le Hg dans le fond des lacs et limiter sa méthylation (Ikingura et Akagi, 2003).

Cette dernière hypothèse ne serait pas justifiée au regard des faibles teneurs de Hg dans les sédiments que nous avons rapportées aux chapitres 3. et 4. L'hypothèse de la photodégradation du mercure reste parmi les plus plausibles pour expliquer les faibles teneurs nettes en MeHg dans ces milieux. En effet, il a été demontré que la photoréduction du Hg était plus élevé dans des lacs à faible teneur en DOC (Amyot et al., 1997b). L'insolation qui a cours toute l'année dans notre zone d'étude, conjuguée aux faibles teneurs en COD observées dans ce milieu soutiennent l'hypothèse de la photodégradation du Hg.

Nos résultats indiquent aussi de faibles concentrations de ces trois éléments traces dans les poissons avec seulement 20% des poissons qui seraient potentiellement en danger quant à leur teneur en Hg. La prise en compte des effets antagonistes Hg/Se entraine quasiment l'annulation des effets potentiels du Hg avec plus de 99% des poissons ayant suffisamment du Se pour amoindrir leur propre risque d'exposition ainsi que celui encouru par leurs consommateurs (potentiels) à la toxicité du Hg. Ces résultats tendent à montrer que les poissons d'eau douce du Burkina Faso pourraient être moins exposés contre les effets du Hg par la présence simultanée du sélénium dans le milieu. D'autres études avaient rapporté des résultats similaires sur les poissons d'eau douce (Peterson et al., 2009; Yang et al., 2010). Cependant la prise en compte des effets antagonistes As/Se conduit à nuancer ces résultats puisque la proportion des poissons moins exposés aux effets du Hg est ramenée à seulement

83%. Notre étude est probablement l'une des premières à utiliser le ratio As/Se comme facteur correctif de l'impact de l'antagonisme Hg/Se sur les effets potentiels du Hg. Les résultats de cette approche exploratoire démontrent l'importance d'une meilleure connaissance des interactions multiples en toxicologie environnementale. Bien que de nombreux travaux de laboratoire (Dang et Wang, 2011; Yang et al., 2010) et de terrain (Belzile et al., 2006; Chen et al., 2001) aient mis en évidence l'antagonisme Se/Hg, de vives controverses entourent encore certaines questions telles l'utilisation du ratio molaire Se:Hg $\geq$ 1 comme valeur de protection du Se contre la toxicité du Hg (Burger et Gochfeld, 2012; Gochfeld et al., 2012) ou encore l'effet antagoniste Hg/Se chez les humains (Choi et al., 2008; Mergler et al., 2007; Saint-Amour et al., 2006). Par principe de précaution, nous avons suggéré des recommandations de consommation des différentes espèces de poissons sur une base conservatrice, c'est-à-dire en considérant la teneur du contaminant mesurée dans le muscle des poissons. Cette considération est vraiment très conservatrice car elle considère que toute la quantité du contaminant chez le poisson passe chez le consommateur. Ce à quoi notre étude expérimentale appelle à nuancer.

6.2 Bioaccumulation et bioamplification du Hg et du Se dans les systèmes aquatiques au Burkina Faso

Les modèles sur la dynamique des contaminants ont suggéré qu'une substance s'accumule dans un organisme avec le temps lorsque son absorption dépasse son élimination et la croissance de l'organisme. Ainsi les concentrations en Hg de poissons d'une même population varient en fonction de l'âge et la taille des individus.

Notre étude sur les réseaux trophiques de 3 réservoirs d'eau au Burkina Faso a montré que le Hg est une substance capable de bioaccumulation (avec un facteur de bioaccumulation (BAF) $\geq$ 5000), mais que cette bioaccumulation n'était pas toujours en rapport avec la taille des organismes au sein d'une même population. D'autres auteurs avaient déjà rapporté cette observation notamment les études portant sur la contamination au Hg de poissons en zones tropicales et subtropicales (Campbell et al., 2003a; Sampaio Da Silva et al., 2005; Tadiso et al., 2011).

Malgré une abondante littérature produite depuis quelques décennies sur les mécanismes de bioaccumulation de contaminants, les facteurs précis qui contrôlent la bioaccumulation du MeHg demeurent insuffisamment compris (Kidd, 2005; Trudel et Rasmussen, 2006). La teneur d'un métal dans l'eau n'est pas toujours indicatrice du niveau de contamination chez les organismes aquatiques, principalement des poissons piscivores vivant en haut des chaines trophiques (Chen et Folt, 2000). Il a été rapporté la présence de poissons avec des teneurs en Hg au-dessus des seuils admissibles pour la consommation humaine dans des lacs dont les teneurs en mercure de l'eau étaient en dessous de la limite de détection (Chen et al., 2000).

Inversement, des teneurs élevées en Hg ont été mesurées dans des lacs où les poissons présentaient relativement de faibles teneurs en Hg. C'est l'exemple de ce qui est convenu d'appeler le paradoxe africain où plusieurs études ont rapporté de faibles teneurs en Hg chez les poissons que ce que l'on était en droit d'espérer étant donné les teneurs élevées dans l'eau (Black et al., 2011; Campbell et al., 2003; Desta et al., 2007).

Cette apparente contradiction soulevée par ces précédentes études pose la nécessité de mieux comprendre comment se fait le transfert des métaux au sein des réseaux trophiques des lacs (c'est - à - dire la bioaccumulation et la bioamplification) afin de déterminer les paramètres qui permettront de mieux prédire les niveaux de contamination des organismes.

Dans notre étude, nous avons observé chez certains poissons tels *O. niloticus* et *A. occidentalis*, que le poids relatif (W_r) utilisé comme indice de condition pour l'appréciation de la santé des poissons affectait la bioaccumulation du Hg. Plus les conditions de vie de ces espèces de poissons s'amélioraient plus le degré d'accumulation du Hg diminuait. Cette diminution de la bioaccumulation du Hg se comprend lorsqu'on considère que l'amélioration du W_r des poissons entraîne une baisse des coûts métaboliques associés à leur activité. Dans ces conditions, comme l'ont montré Trudel et Rasmussen (2006) avec leur modèle de bioaccumulation, pour un taux d'ingestion et pour un niveau de contamination des proies donné, une baisse des coûts métaboliques conduit à la réduction de la concentration de Hg chez les poissons. La baisse du taux de consommation due à l'accumulation de lipides dans le sang, facteur limitant l'alimentation (Arnot et Gobas, 2006) justifierait aussi la baisse du BAF (Hg) étant donné que le Hg se transfert principalement par la voie alimentaire (Trudel et Rasmussen, 2006). L'importance de la bioénergétique donc de la physiologie ainsi que d'autres facteurs tels l'alimentation, la croissance, la teneur du Hg des proies et le taux

métabolique dans la dynamique d'accumulation du Hg a été largement discuté dans des études précédentes (Trudel et Rasmussen, 2001; Trudel et al., 2000). Trudel et Rasmussen (2006) attribuent un rôle central à la bioénergétique chez les poissons dans l'accumulation du Hg. Nous avons suggéré d'approfondir les recherches sur le rôle du W_r dans l'accumulation du Hg chez les poissons pour aider à comprendre la grande variabilité de la relation Hg –taille des poissons rapportée dans plusieurs études. Puisque les coûts métaboliques associés à l'activité des poissons jouent un rôle dans le potentiel de bioaccumulation, nous avons émis l'hypothèse d'une baisse des teneurs en Hg et Se des poissons en saison sèche par rapport à celles de la saison pluvieuse étant donné la variation de la disponibilité en proies.

Nous avons noté une baisse des teneurs du Hg et du Se dans l'eau et une tendance générale à la baisse des teneurs en Hg des poissons au cours de la saison sèche. L'utilisation des isotopes stables d'azote ($\delta^{15}N$) et de carbone ($\delta^{13}C$) ne nous a pas permis d'expliquer la baisse significative du Hg observée chez certains poissons dont la position trophique et la source d'alimentation n'ont pas changé entre les deux saisons. Tout comme le Hg, la baisse des concentrations en Se chez les poissons au cours de la saison sèche n'était pas toujours en lien avec une diminution de leur niveau trophique ou avec un changement dans la source d'approvisionnement en énergie. Cependant, l'analyse des contenus stomacaux, bien que livrant une image instantanée des aliments consommés par les poissons, suggère que la nature des proies notamment la composition taxonomique des invertébrés serait importante pour expliquer l'accumulation et le transfert du Hg aux poissons. La baisse significative de la bioamplification du MeHg dans deux des trois réservoirs d'eau en saison sèche rapportée au chapitre 4 renforce l'hypothèse d'une accumulation saisonnière du Hg et du Se dans les systèmes aquatiques au Burkina Faso.

Si cette tendance se confirme avec d'autres investigations notamment dans le plus grand réservoir, à savoir le barrage hydroélectrique de la Kompienga, nous suggérons la mise en place de mesures limitatives de la consommation des poissons les plus contaminés au Hg en accord avec cette variation saisonnière afin de minimiser le risque d'exposition des populations locales utilisant ces ressources.

La plupart des avis de consommation du poisson sont calculés sur la base des teneurs dans les tissus comestibles comme le muscle. Cependant, plusieurs facteurs peuvent influencer sa biodisponibilité et sa toxicité. Cela nous a conduit à évaluer l'exposition au Hg en relation avec les modes de cuisson du poisson et les habitudes alimentaires au chapitre 5.

6.3 Effets des modes de cuisson et des habitudes alimentaires sur la bioaccessibilité du Hg de poisson chez les humains

Notre étude est l'une des premières à avoir simulé la digestion de poissons préalablement préparés afin d'en évaluer la bioaccessibilité du Hg, les précédentes études s'étant limitées à évaluer l'effet des cuissons sur les teneurs des métaux dans les poissons (Ersoy et Özeren, 2009; Gokoglu et al., 2004) ou à évaluer la bioaccessibilité sur la base du tissus frais (Shim et al., 2009). L'une des conclusions majeures de cette section est de montrer que si les modes de cuisson affectent peu les teneurs en Hg des poissons, la bioaccessibilité (fraction soluble dans le tractus digestif après digestion in vitro) s'en trouve considérablement modifiée. Nous avons observé pour la première fois que les différents traitements appliqués aux poissons avant consommation par les communautés humaines peuvent avoir une incidence

sur la bioaccessibilité du Hg. En effet, nous avons observé une baisse de 80% et 60% de la bioaccessibilité du Hg respectivement pour la portion frite et bouillie par rapport à celle de la portion du poisson consommée crue.

Nous avons en plus montré que l'ingestion au cours du repas de certains composants alimentaires tels le café et le thé diminuait considérablement la bioaccessibilité du Hg. Cette diminution est d'autant plus importante que le poisson a préalablement subi des traitements de cuisson. Compte tenu de la forte affinité du Hg aux protéines (George et al., 2008; Harris et al., 2003) nous avons émis l'hypothèse de la dénaturation protéique par la chaleur pour expliquer la baisse de la solubilisation du Hg par sa séquestration au cours de la digestion (Dunn et al., 1981). De même, les phytates contenus dans le café et le thé pourraient, comme le suggèrent Kumar et al. (2009), avoir interagi avec les protéines et modifié leur solubilité et diminué la bioaccessibilité du Hg Hg lié à ces protéines. Une attention doit être portée sur les formes du Hg biodisponibles afin de mieux cerner les interactions avec d'autres éléments susceptibles de modifier sa toxicité tel le sélénium.

Ces résultats sont d'une grande portée en matière d'évaluation de risque d'exposition. Ils suggèrent, avec cependant la réserve de leur validation par des modèles animaux, la prise en compte des habitudes alimentaires de populations cibles dans l'approche d'évaluation de risques toxicologiques.

6.4 Conclusion générale et perspectives

Cette recherche a permis d'avoir une première évaluation du niveau de concentration du Hg, de l'As et du Se dans les écosystèmes aquatiques au Burkina Faso. Nous avons observé que les milieux aquatiques étudiés étaient peu affectés par ces éléments malgré l'essor de l'exploitation minière notamment sa composante artisanale (orpaillage) qui utilise et rejette du Hg dans les processus de récupération d'or. Cependant, notre recherche n'avait pas pour objectif d'évaluer le rôle de l'activité minière sur les teneurs en Hg, As et Se dans les réservoirs. Nous ne sommes donc pas à mesure de conclure sur l'impact direct ou non des activités minières sur les niveaux de concentrations de ces éléments observés dans les réservoirs. Dans l'ensemble les concentrations de ces trois métal(loïdes) sont faibles dans la chair des poissons bien que, quelques espèces notamment piscivores indiquent des teneurs de Hg susceptibles de présenter des risques toxicologiques pour eux-mêmes et pour leurs consommateurs y compris l'homme.

Nos résultats sur l'évaluation du potentiel de risque toxicologique que pourrait présenter les poissons étudiés, basés sur les interactions bien documentées entre Hg/Se et As/Se soulignent l'importance de la prise en compte des interactions multiples en écotoxicologie environnementale.

Une des observations les plus marquantes de cette étude est la mise en évidence de relatives faibles teneurs en MeHg en comparaison aux teneurs de THg dans les réservoirs d'eau. Peu d'études en Afrique avaient mesuré les concentrations en MeHg dans l'eau. Ces faibles teneurs en Hg expliqueraient en partie le paradoxe du « lower than expected Hg concentration

in fish from Africa » rapporté par plusieurs études. Cependant, Il convient de considérer que seule une étude sur la géochimie du Hg dans ces environnements aquatiques permettra d'élucider ces observations.

Au cours de ce travail, nous avons aussi décrit la structure des réseaux trophiques de trois réservoirs d'eau caractéristiques des types de plans d'eau du Burkina Faso au moyen d'isotopes stables du carbone (^{13}C) et d'azote (^{15}N). Tous présentaient des réseaux trophiques aux chaînes courtes et la majorité des poissons était reliée au benthos pour l'approvisionnement en ressources alimentaires. L'étude indique aussi que le Hg était capable de bioaccumulation et de bioamplification au sein de ces réseaux trophiques. En plus de la taille, nous avons identifié l'indice de condition du poisson (W_r) comme étant un facteur modulant la bioaccumulation chez certaines espèces de poissons laissant suggérer une dynamique saisonnière de la bioaccumulation du Hg chez ces espèces de poissons. En effet une baisse significative des concentrations en Hg a été observée au cours de la saison sèche en comparaison aux niveaux obtenus en saison pluvieuse avec certaines espèces dans l'un des réservoirs. L'utilisation des outils isotopiques n'a pas permis de noter ces changements. Par contre l'analyse complémentaire des contenus stomacaux laisse entendre que la dynamique saisonnière des proies pourrait expliquer cette variation des niveaux de contamination.

Il est clair qu'une étude sur la dynamique saisonnière des taxa d'invertébrés et de leur imprégnation en Hg couplée avec celles des poissons devrait améliorer notre compréhension du transfert trophique du Hg dans ces plans d'eau.

Enfin le volet expérimental de cette recherche soutient l'idée d'une prise en compte des habitudes alimentaires des communautés dans l'élaboration des avis de consommation de poissons sous réserve que nos résultats soient validés par des modèles animaux.

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Annexes

Annexe 1. Données supplémentaires du chapitre 1

Table S1.1. Propriétés physiques du mercure, de l'arsenic et du sélénium

Nom	**Mercure**	**Arsenic**	**Sélénium**
Symbole	Hg	As	Se
Numéro atomique	80	33	34
Group dans la table périodique des éléments	12	15	16
Masse atomique	200,59	74,9216	78,96
Classification	Métaux	Métalloïde	Nonmétal
Éléctronégativité	1,9	2,18	2,55
Densité ($kg.m^{-3}$)	13,5	5,727	4,808
Point de fusion (º C)	39,5	817(à haute pression)	220
Point d'ébullition (º C)	357	614 (sublimation)	685
Isotope naturel et abondance	^{196}Hg (0,15%) ^{198}Hg (9,97%) ^{199}Hg (16,87%) ^{200}Hg (23,10%) ^{201}Hg (13,18%) ^{202}Hg (29,86%) ^{204}Hg (6,87%)	^{75}As (100%)	^{74}Se (0,87%) ^{76}Se (9,02 %) ^{77}Se (7,58 %) ^{78}Se (23,52 %) ^{80}Se (49,82%) ^{82}Se (9,19 %)

Annexe 2. Données supplémentaires du chapitre 2

2.1. Supporting information (SI): Selenium and arsenic determination

2.1.1. Water sample preparation

Prior to analysis, sample was digested to allow the reduction of Se (VI) to Se (IV) and As (V) to As (III), the forms which will form a hydride with $NaBH_4$.

To determine Selenium, a polyethylene aliquot of the sample (4 mL) was added to 4 mL of concentrated hydrochloric acid (HCl) and 0.48 mL HNO_3. The acidified samples are heated at 120 ^{o}C for 1 hour to pre-reduce selenate Se (VI) to selenite Se (IV) which will form a hydride (selenium hydride) with $NaBH_4$. Once cooled, sample was dilute to 10 mL with ultra-pure water. To obtain As (V) reduction to As (III), sample (0.5 mL) was added to 1 mL of potassium persulfate ($K_2S_2O_8$) in NaOH (10 % m/v and 2 % m/v respectively)) and heating at 120 ^{o}C for further hour then, 5 mL of concentrated hydrochloric acid (HCl) were added. The mixture was heated again at 120 ^{o}C for 1 hour. Once cooled, 200 µL of potassium iodide/Ascorbic acid reagent (50 % m/v/10 % m/v) was added, and the solutions left for 30 minutes before being dilute to 10 mL with ultra-pure water.

2.1.2. Fish sample preparation: microwave digestion

For the determination of selenium and arsenic in fish tissues, a microwave digestion was performed in a mixture of HNO_3 and H_2O_2 in order to extract elements from solid matrix. Microwave digestion was performed with MARS Xpress microwave digestion system (CEM Corporation, Mathews, NC) based on the method developed by Corns et al. (1993). 20 to 50 mg of fish tissues was weighed into an acid washed Teflon microwave digestion vessel along with 3 mL HNO_3 then, heated under pressure in MARS Xpress microwave digestion system (CEM Corporation, Mathews, NC). Samples were subjected to 15 min at 800W and 70% power, before allowed to cool. Once cooled, 0.5 mL of perchloric acid H_2O_2 was added (in order to break down the sample matrix) and microwave step (15 mim at 800W and 70% power) was carried out. This step was repeated once more. Once completed the solutions were diluted to 25 mL with ultra-pure water. An aliquot was taken and proceed as the same with water sample.

For Arsenic determination. at the end of this heating step, Potassium persulfate $K_2S_2O_8$ oxidation stage was added to allow convertion of a non reducible form of arsenic (such as arsenobetaine which will not form a hydrid with $NaBH_4$) to As (V) which is then reduced to As (III) in the presence of KI.

2.2. Figures supplémentaires du chapitre 2

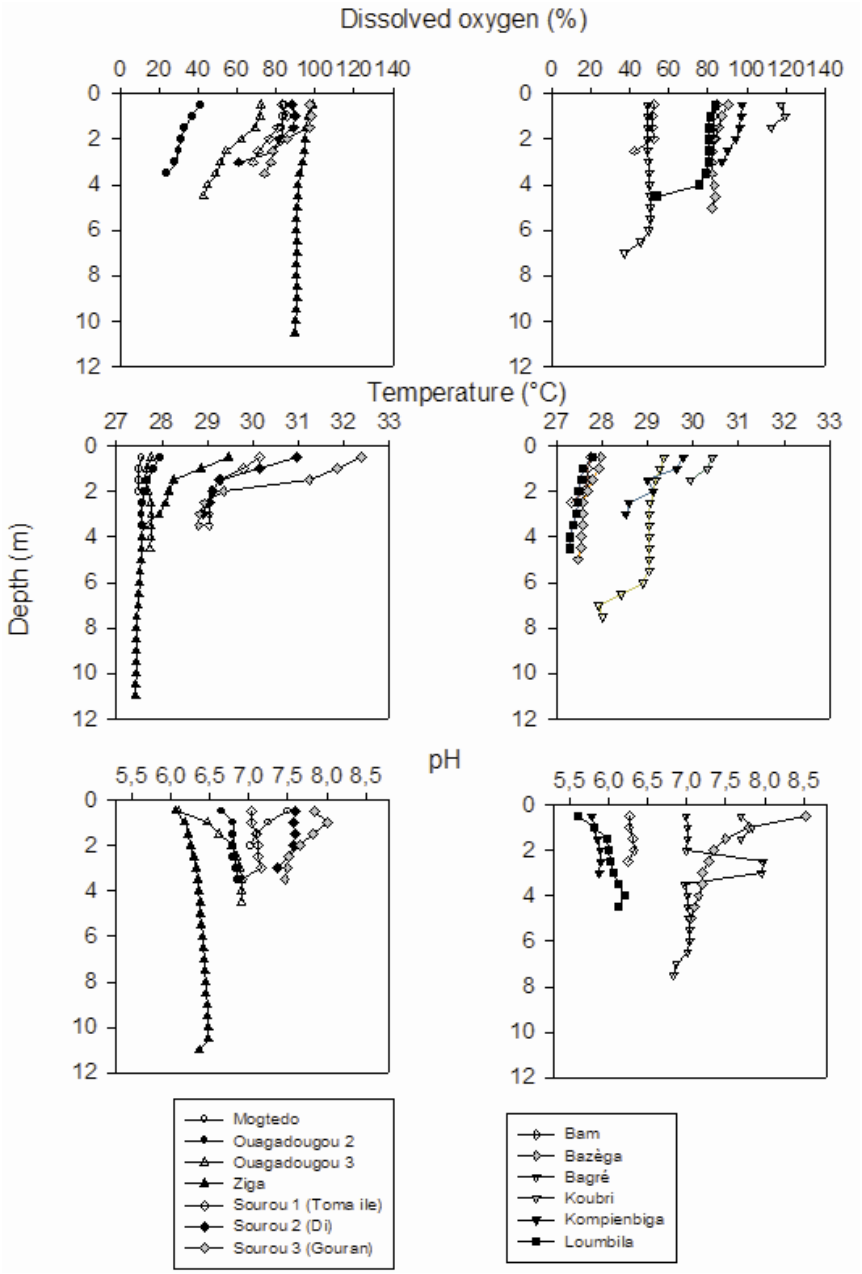

Figure S2.1. Physicochemical profiles of the study sites. Water temperature (T), Dissolved oxygen (DO) and pH. Bottom waters were well oxygenated (range 20 -100 %). None of the sites were stratified.

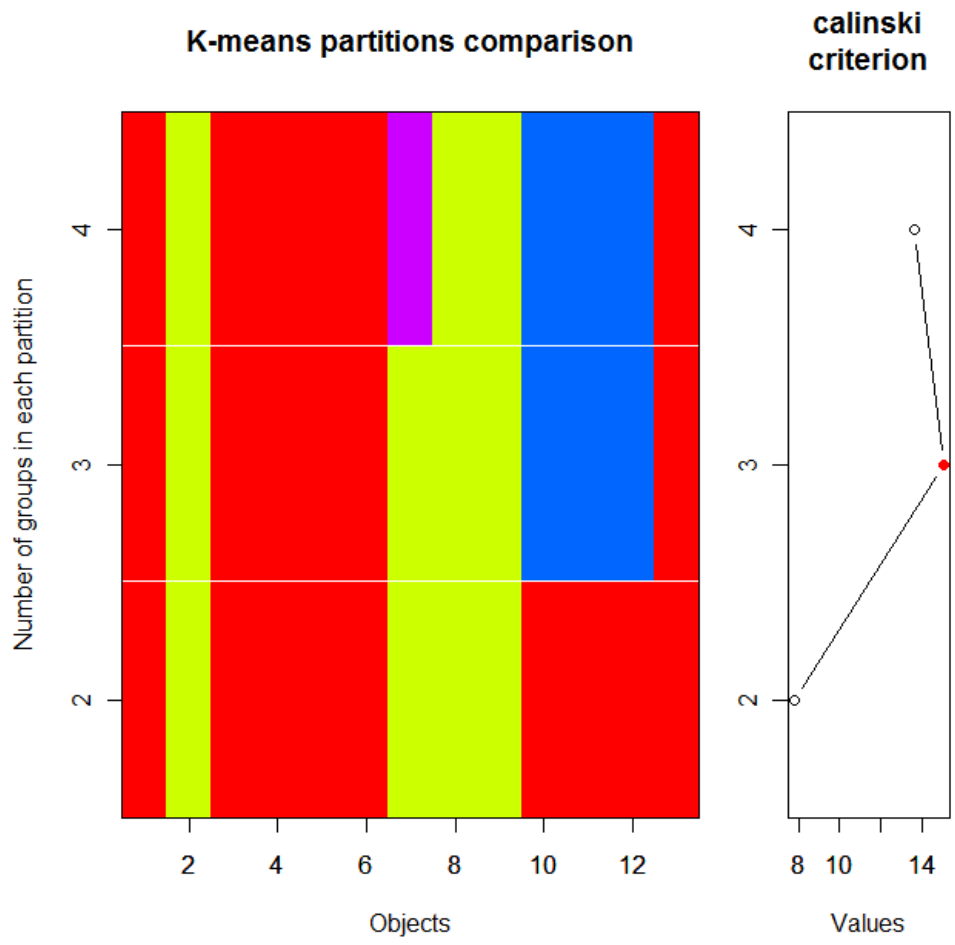

Figure S2.2. K-means cascade plot showing the group attributed to each study site (object) for each partition according to their trace metal concentrations. K =3 (maximum value of Calinski criterion) correspond to minimum total error sum of squares (E^2k) and then was the best number of group to classifying the sites.

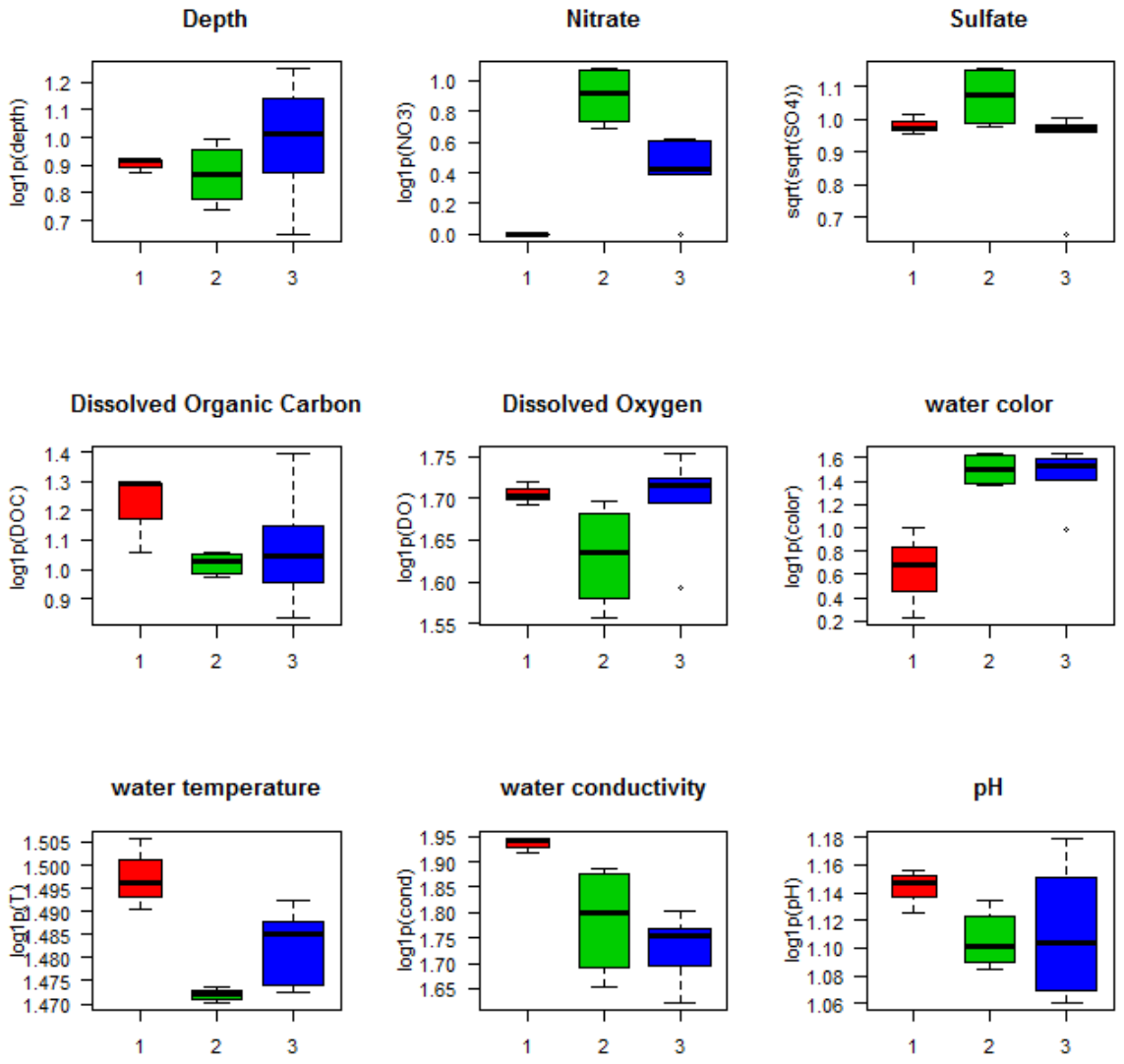

Figure S2.3. Boxplots of 9 physicochemical variables in three cluster groups (after some simple transformations to improve normality). Group. 1 = group with relative high MeHg, group 2 = enriched group of PHg, TAs and TSe and group 3 = highest arsenic enriched sites, log1p = log(x+1) transformation was applied. Temperature, conductivity, nitrate and water color were significantly different among clustering groups.

2.3. Tables supplémentaires chapitre 2

Table S2.1. Quality of analytical results of metal(loïd) in water and fish tissues. DORM-2, DORM-3, TORT-2 are certified reference materials (CRM) from the National Research Council of Canada.

Element	**TORT-2 (ng/g)**				**DORM-2 (Hg), DORM-3 (As,Se) (ng/g)**			
	Certified value	Our results	Sample (n)	% recovery	Certified value	Our results	Sample (n)	% recovery
THg	300 ± 15	313 ± 28	51	104 ± 9	4640 ± 260	4680 ± 370	16	101 ± 8
MeHg	152 ± 13	144 ± 20	7	95 ± 13	_	_	_	_
TSe	5630 ± 600	5060 ± 630	18	90 ± 11	3300 ±	3015 ± 230	18	91 ± 7
TAs	21600 ± 600	18800 ±1600	18	87 ± 7.4	6880 ± 300	6073 ± 740	18	88 ± 11

Table S2.2. Patterns of trace metals distribution among the three principal sites groups. (DHg = dissolved Hg; PHg = particulate Hg; MeHg = methylmercury; TSe = total selenium; and TAs = total arsenic).

Group	DHg (ng/L)	PHg (ng/L)	MeHg (ng/L)	TSe (ng/L)	TAs (ng/L)
Group 1 (6 sites) (Bagre, Bazega, Kompienga, Koubri, Loumbila, Ziga**)**	1.348± 0.35	0.973± 0.76	0.049± 0.02	67 ± 10	375 ± 59
Group 2 (4 sites) (Bam, Mogtedo, Ouaga 2, Ouaga 3**)**	2.478 ± 1.3	10.882 ± 4.93	0.020 ± 0.02	119 ± 39	606 ± 94
Goup 3 (3 sites) (Sourou1, Sourou2 , Sourou3**)**	0.08 ± 0.02	0.437 ± 0.06	<0.020	40 ± 4	662 ± 134

Table S2.3. Mass concentrations of total mercury, methylmercury and percent of mercury as MeHg from various fish groups. (n) = sample number of fish species at each location. TL = Total length (mm) (min = minimum, max = maximun). Fish species *O.* = *Oreochromis* , *S.* = *Synodontis*, *C.* = *Clarias*, *H. niloticus* = *Heterotis niloticus*, *H. forskalii* = *Hydrocynus. forskalii* , *G.* = *Gymnarchus, A.* = *Auchenoglanis*, *L.* = *Lates*, *B.*= *Bagrus.*

Fish species	**Lake (n)**	**TL (mm)**	**[THg] (µg/g w.w.)**			**[MeHg] (µg/g w.w.)**			**MeHg/THg (%)**		
		mean (min,max)	min	mean	max	min	mean	max	min	mean	max
			Nonpiscivores								
O. niloticus	Bagré (21)	197 (120,245)	0.008	0.018	0.046	0.008	0.015	0.03	63	86	104
	Bam (10)	141 (100,180)	0.013	0.024	0.044	0.008	0.015	0.035	42	62	79
	Bazega (25)	135 (110,280)	0.01	0.022	0.045	0.009	0.018	0.037	52	82	91
	Kompga (7)	255 (220,295)	0.012	0.023	0.04	0.011	0.02	0.032	75	88	95
	Koubri (14)	146 (125,195)	0.006	0.01	0.02	0.004	0.009	0.018	72	89	112
	Loumbila(22)	141 (99,180)	0.014	0.019	0.026	0.011	0.018	0.029	55	91	132
	Ouaga (14)	108 (55, 135)	0.01	0.021	0.04	0.01	0.018	0.04	24	90	124
	Sourou (10)	153 (55, 218)	0.003	0.006	0.013	0.002	0.006	0.012	72	93	123
	Ziga (15)	147 (125, 180)	0.01	0.016	0.038	0.005	0.013	0.034	41	84	98
	all sites (138)	**159 (55, 295)**	**0.003**	**0.018**	**0.046**	**0.002**	**0.015**	**0.04**	**41**	**85**	**132**
A. occidentalis	Bagré (4)	170 (160,180)	0.034	0.046	0.067	0.024	0.04	0.065	70	83	97

Table S2.3. (continued)

Fish species	Lake (n)	TL (mm)	[THg] (µg/g w.w.)			[MeHg] (µg/g w,w.)			MeHg/THg (%)		
		mean (min,max)	min	mean	max	min	mean	max	min	mean	max
			Nonpiscivores								
A. occidentalis	Bazega (15)	175 (155,210)	0.019	0.051	0.1	0.018	0.045	0.069	58	92	120
	Kompga (5)	254 (220,290)	0.017	0.025	0.037	0.015	0.023	0.032	57	78	114
	Lumbila (23)	186 (140,223)	0.024	0.11	0.268	0.035	0.082	0.177	46	80	112
	Sourou (1)	310		0.008			0.008			94	
	Ziga (6)	258 (190,330)	0.035	0.084	0.152	0.024	0.063	0.118	57	74	88
	all sites (54)	**222 (140, 330)**	**0.017**	**0.054**	**0.268**	**0.015**	**0.043**	**0.177**	**46**	**83.5**	**120**
S. schall	Mogtedo	160		0.13			0.047			36	
	Bam (2)	105 (100,110)	0.013	0.024	0.044	0.008	0.015	0.035	42	62	79
	Bazega (3)	150 (135,165)	0.047	0.047	0.048	0.036	0.042	0.05	75	88	105
	Kompga (9)	168 (105,215)	0.093	0.26	0.514	0.051	0.177	0.36	51	70	87
	Koubri (5)	217 (205,230)	0.097	0.112	0.129	0.077	0.101	0.135	78	90	105
	all sites (20)	**162(100, 230)**	**0.013**	**0.115**	**0.514**	**0.008**	**0.076**	**0.36**	**42**	**69**	105
H. niloticus	Sourou (6)	418 (290, 660)	0.007	0.018	0.033	0.007	0.017	0.032	93	97	105
S. membranaceus	Bagré (21)	245 (200,330)	0.027	0.06	0.101	0.019	0.054	0.094	62	90	114

Table S2.3. (continued)

Fish species	Lake (n)	TL (mm)	[THg] (µg/g w.w.)			[MeHg] (µg/g w,w.)			MeHg/THg (%)		
		mean (min,max)	min	mean	max	min	mean	max	min	mean	max
			Nonpiscivores								
S. membranaceus	Ziga (4)	286 (240,315)	0.097	0.125	0.152	0.04	0.084	0.117	33	67	90
	all sites (25)	**265 (200,330)**	**0.027**	**0.092**	**0.152**	**0.019**	**0.069**	**0.117**	**33**	**78.5**	**114**
All nonpiscivores (243)			**0.003**	**0.06**	**0.514**	**0.002**	**0.044**	**0.36**	**33**	**82.6**	**132**
			Omnivores								
C. anguillaris	Bagré (1)	335		0.108			0.076			70	
	Bam (1)	380		0.102			0.08			78	
	Kompga (3)	500 (474,545)	0.125	0.167	0.235	0.1	0.12	0.16	67	72	79
	Koubri (8)	311 (180,590)	0.054	0.152	0.388	0.049	0.08	0.236	47	70	90
	Lumbila (4)	233 (180,300)	0.148	0.176	0.235	0.086	0.116	0.128	54	68	86
	Mogtedo (2)	271 (262,280)	0.119	0.12	0.123	0.095	0.095	0.096	78	79	80
	Ouaga (4)	324 (250,410)	0.042	0.101	0.18	0.04	0.082	0.15	74	83	93
	Sourou (14)	272 (200,530)	0.006	0.02	0.05	0.004	0.019	0.052	59	95	110
	Ziga (2)	272 (270-275)	0.175	0.206	0.237	0.127	0.151	0.176	73	73.5	74
all sites (39)		**321 (180, 590)**	**0.006**	**0.128**	**0.388**	**0.004**	**0.091**	**0.236**	**47**	**76.5**	**110**
All Omnivores (39)			**0.006**	**0.128**	**0.388**	**0.004**	**0.091**	**0.236**	**47**	**76.5**	**110**

Table S2.3. (end).

Fish species	Lake (n)	TL (mm)	[THg] (µg/g w.w.)			[MeHg] (µg/g w.w.)			MeHg/THg (%)		
		mean (min,max)	min	mean	max	min	mean	max	min	mean	max
			Piscivores								
B. bajad	Bagré (12)	336 (270,500)	0.04	0.278	0.56	0.034	0.221	0.471	67	82	126
	Kompga (5)	434 (310,520)	0.168	0.35	0.607	0.128	0.256	0.405	67	75	81
	Ziga (16)	298 (220,470)	0.033	0.14	0.322	0.041	0.112	0.228	67	89	136
	all sites (33)	**356 (220, 520)**	**0.033**	**0.256**	**0.607**	**0.034**	**0.197**	**0.471**	**66**	**82**	136
L. niloticus	Kompga (5)	309 (145,410)	0.071	0.130	0.197	0.056	0.091	0.144	63	72	79
	Koubri (4)	234 (95,425)	0.047	0.153	0.304	0.042	0.111	0.221	68	75	89
	Lumbila(1)	380		0.140			0.094			67	
	all sites (10)	**319 (145, 425)**	**0.033**	**0.141**	**0.304**	**0.042**	**0.098**	**0.22**	**63**	**69**	89
H. forskalii	Kompga (2)	435 (250,630)	0.109	0.138	0.167	0.075	0.114	0.153	69	80	91
Hemichromis sp	Bazega (5)	154 (150,160)	0.183	0.263	0.357	0.178	0.235	0.306	86	90	97
G. niloticus	Sourou (2)	617 (435,800)	0.023	0.033	0.042	0.02	0.034	0.048	86	100	114
All piscivores (52)			**0.023**	**0.166**	**0.607**	**0.02**	**0.135**	**0.471**	**63**	**84.2**	**136**

Table S2.4. Mass concentration of total selenium and total arsenic from various fish across study sites.

Fish species	Lake (n)	TL (mm)	[TSe] (µg/g w.w.)			[TAs](µg/g w.w.)		
		mean (min,max)	min	mean	max	min	mean	max
			Nonpiscivores					
O. niloticus	Bagré (21)	197 (120,245)	0.143	0.0202	0.246	0.045	0.07	0.166
	Bam (10)	141 (100,180)	0.312	0.36	0.397	0.045	0.092	0.289
	Bazega (25)	135 (110,280)	0.168	0.222	0.342	0.045	0.085	0.194
	Kompga (7)	255 (220,295)	0.114	0.128	0.154	0.045	0.045	0.045
	Koubri (14)	146 (125,195)	0.152	0.22	0.292	0.045	0.094	0.281
	Loumbila (22)	141 (99,180)	0.155	0.188	0.23	0.045	0.057	0.174
	Ouaga (14)	108 (55, 135)	0.123	0.212	0.317	0.045	0.090	0.323
	Sourou (10)	153 (55, 218)	0.097	0.137	0.19	0.045	0.107	0.228
	Ziga (15)	147 (125, 180)	0.25	0.347	0.473	0.045	0.08	0.217
All O. niloticus **(138)**		**159 (55, 295)**	**0.097**	**0.224**	**0.473**	**0.045**	**0.08**	**0.323**
A. occidentalis	Bagré (4)	170 (160,180)	0.093	0.114	0.125	0.039	0.039	0.039
	Bazega (15)	175 (155,210)	0.06	0.077	0.096	0.039	0.68	0.154
	Kompga (5)	254 (220,290)	0.102	0.128	0.154	0.039	0.039	0.039
	Loumbila (23)	186 (140,223)	0.057	0.098	0.162	0.039	0.055	0.185
	Sourou (1)	310		0.03			0.039	
	Ziga (6)	258 (190,330)	0.159	0.313	0.286	0.039	0.039	0.039
All *A. occidentalis* (54)		**222 (140, 330)**	**0.057**	**0.11**	**0.286**	**0.039**	**0.054**	**0.185**

Table S2.5. Mercury levels in fish from previous studies in Africa. (Feeding habit O = Omnivore, P = Piscivore, I = invertivore, pk = planktivore).

Locations	Site	Fish species	Feeding habit	Hg µg/g (range/mean)	MeHg µg/g (mean/range)	Reference
North Africa						
Egypt	River Nile	*Bagrus sp*	P	0.026 – 0.391	_	Campbell et al. (2003c)
West Africa						
Ghana	Lake	*Tilapia sp*	O	< 0.001 − 0.070	_	Agorku et al. (2009)
	Bosomtwi	*S. membranaceus*	I	0.011 - 0.076		Kwaansa-Ansah et al. (2011)
	Volta	*Bagrus docmac*	O	0.040 - 0.090		
		Chrys. nigrodigitatus	O	0.010 - 0.062		
	Reservoirs	*Tilapia zilli*	Pk	< 0.001 − 0.076		Agorku et al. (2009)
	Kpong	*S. granulosis*	O	0.020 − 0.042		
	Akosombo	*P. guntheri*	C	0.011 − 0.275		
		Chrys. Auratus	O	0.026 − 0.079		
	River	*Tilapia sp*	D	0.013 - 0.163		Oppong et al. (2010)
	Pra	*Syn. sp (Catfish)*	D	0.69 ± 0.57	0.0035 ± 0.001	Donkor et al. (2006)
		Hepsetus odoe	P	4.473 ± 0.42	0.0072 ± 0.0014	
		Clarias sp (Mud fish)	O	1.04 ± 0.05	0.0019 ± 0.0001	
		Chrysichthys sp	P	0.069 - 0.370		

Table S2.5. (continued)

Locations	Site	Fish species	Feeding habit	Hg µg/g (range/mean)	MeHg µg/g (mean/range)	Reference
East Africa	Lake					
Ethiopia	Awassa	*O. niloticus,*	O	0.0076 - 0.0160		Desta et al. (2007; 2006)
		Barbus intermedius	P	0.1086 - 0.6280		Desta et al. (2008)
		Clarias gariepinus	P	0.002–0.154		Tadiso et al. (2011)
Ouganda	Victoria	*Clarias gariepinus*	O	0.005 - 0.630		Campbell et al. (2003a)
Kenya		*O. niloticus*	D	0.0017 - 0.1		Van Straaten (2000)
Tanzania		*P. aethiopicus*	P	0.0018 -0.240		
		Lates niloticus	P	0.007 - 0.323		Campbell et al. (2003d)
	Nabugabo	*Lates niloticus*	P	10.6 -42.2		
		Tilapia zilli	O	1.9 -10.6		
Kenya	Turkana	*C. gariepinus*	O	0.0435 - 0.1113		Campbell et al. (2003d)
	Naivasha	*Lates niloticus*	P	0.0461 - 0.132		
	Baringo	*O. niloticus*	D	0.0033 - 0.0247		
		H. forskahlii	P	0.0312 - 0.636		
Tanzania -Burundi	Tanganyika	*O. tanganyikae*	D	0.015		Campbell et al. (2008)
Zambia- RD congo		*C. theodorae*	P	0.11– 0.33		

Table S2.5. (continued)

Locations	Site	Fish species	Feeding habit	Hg µg/g (range/mean)	MeHg µg/g (mean/range)	Reference
East Africa	Lake					
Zambia- RD congo		*Polypterus congicus*	P	3.23		
	Mtera- Ktu	*C. mossambicus*	O	0.005 - 0.062		Ikingura and Akagi (2003)
	Nyumba -P	*Bagrus orientalis*	P	0.0134 – 0.058		
		H. vittatus	P	0.021 – 0.143		
		Tilapia urolepis	D	0.0015 – 0.017		
	Rwamagasa mining centre	*O. niloticus*	D	0.002–0.031		Taylor et al. (2005)
		C. gariepinus	O	0.048 - 1.97		
		Barbus spp.	P	0.285–0.318		
		Haplochromis spp.	O	0.145 -2.65		
Malawi	Malawi	*O. lidole*	D	0.007 ± 0.0014		Kidd et al. (2003)
		B.nyasensis	O	0.055 ± 0.042		
		B. meridionalis	P	0.043 ± 0.034		
		R. ferox		0.200 ± 0.160		

Table S2.5. (end).

Locations	Site	Fish species	Feeding habit	Hg µg/g (range/mean)	MeHg µg/g (mean/range)	Reference
Central Africa						
Tchad	Lake Tchad	*O. niloticus*	D	0.007 ± 0.004		Kidd et al. (2004)
		C. anguillaris	O	0.033 ± 0.005		
		Bagrus sp	P	0.058		
		Lates niloticus	P	0.060 ± 0.023		
Southern Africa						
South Africa	Rivers	*Amphilius spp.*	I	_	0.020 - 0.085	Williams et al. (2010)
	Olifan-Upper Vaal	*G. Affinis*	I	_	0.010 -0.034	Walters et al. (2011)
	Inkomati WMAS	*Labeo barbus sp.*	D	_	0.014 - 0.218	
Botwana	Okavango	*O. macrochir*	D	0.0027 - 0.0232		Black et al. (2011)
		C. gariepinus	O	0.0057 - 0.216.6		
		H. vittatus	P	0.0251 - 0.168.8		
		S. intermedius	P	0.0082 - 0.2062		
Other tropical regions	Guizhou reservoirs	*Carnivores fish*		0.012 – 0.445		Yan et al. (2010)
		Oreochromis sp		0.006 – 0.315		

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Annexe 3. Données supplémentaires du chapitre 3

3.1 Figures supplementaires du chapitre 3

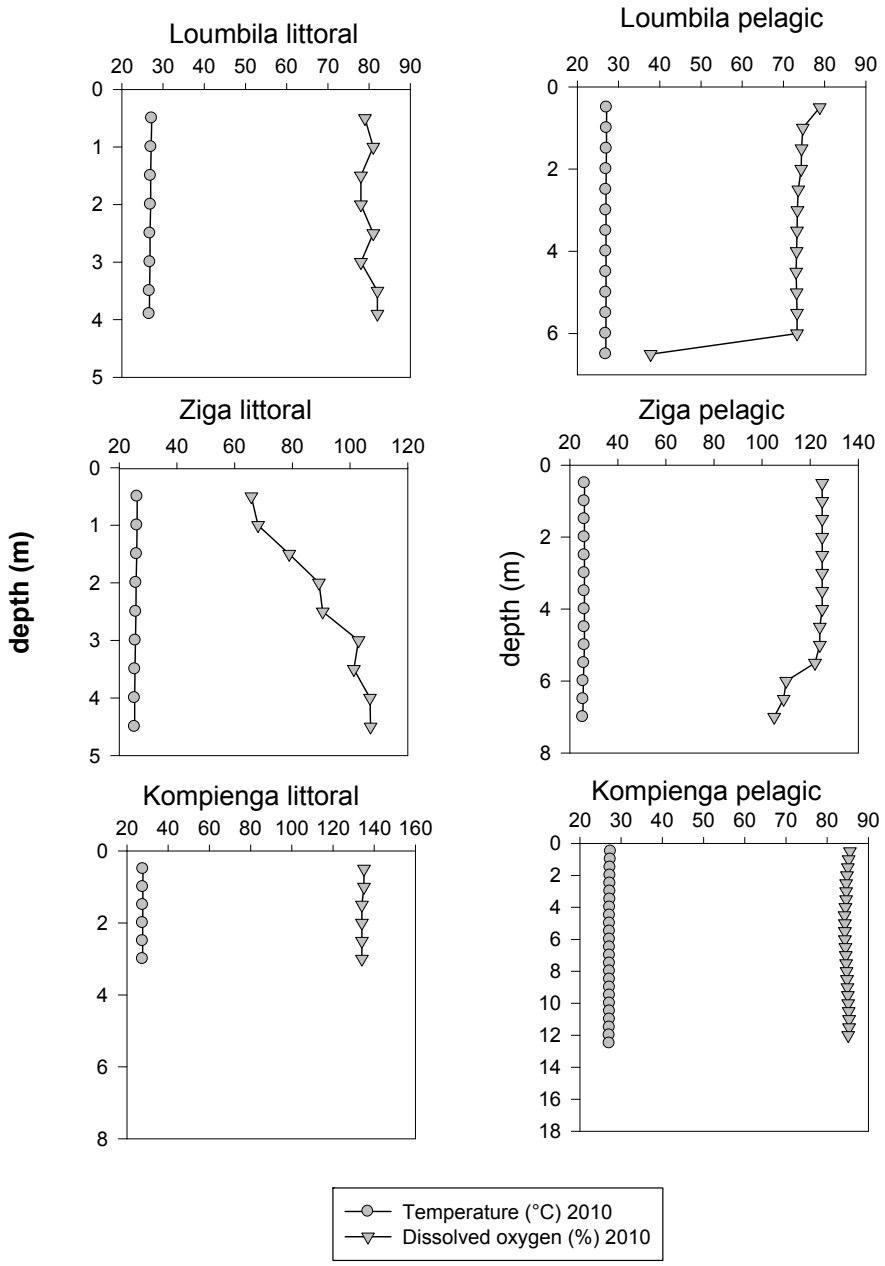

Figure S3.1. Aqueous temperature and dissolved oxygen profile across study site at the littoral and pelagic station.

3.2 Tables supplémentaires du chapitre 3

Table S3.1 Some environmental characteristics of sampled site

Site		**Loumbila**		**Ziga**		**Kompienga**	
Compartiment	variable	Littoral	Pelagic	Littoral	Pelagic	Littoral	Pelagic
Water							
	Depth (m)	3.5	6	4	6.5	2.5	12
	NO_3^- ($mg.L^{-1}$)	1.036	1.057	0.508	0.338	0.447	0.341
	SO_4^{2-}(mg.L-1)	0.593	0.558	0.773	0.736	0.725	0.7
	DOC (mg.L-1)	2.53	1.59	2.39	1.49	2.08	1.62
	Conductivity (µS.cm-1)	82	81	103	109	134	134
	T (° C)	26.7	26.93	25.36	25.57	27.6	27.17
	DO (%)	73	73.3	106.9	96	88.2	85.1
	pH	7.25	7.25	7.58	7.79	7.07	7.32
	THg ($ng.L^{-1}$)	3	3.04	4.93	5.42	2.38	2.48
	DHg (ng.L-1)	2.5	2.5	1.92	2.03	1.52	1.57
	PHg (ng.L-1)	0.5	0.9	3.01	3.39	0.86	0.91
	MeHg ($ng.L^{-1}$)	0.037	0.11	0.065	0.196	0.039	0.042
	Tse ($ng.L^{-1}$)	72.7	65.4	_	62.5	57.5	55.8
Sediment							
	THg (ng.g1)	7.87	19.7	13.74	27.24	19.11	27.19
	Tse ($ng.g^1$)	62	255	178	335	173	142

Table S3.2 Fish class size (total length)

Fish	Fish size class (total length) (mm)		
	Small (1)	Medium (2)	Large (3)
O. niloticus	≤ 150	151 - 200	> 200
A. occidentalis	≤ 150	151 200	>200
B. bajad	≤ 200	201-400	>400
C. anguillaris	≤ 200	201-400	>400
S. intermedius	≤ 150	151 - 200	>200
L. niloticus	≤ 500	_	_
S. membranaceus	≤ 150	151- 200	>200

Table S3.3 Quality of analytical results of metal(loïd) in water and fish tissues. DORM-2, DORM-3, TORT-2 are certified reference materials (CRM) from the National Research Council of Canada.

Element	**TORT-2 (ng.g-1)**				**DORM-2 (Hg), DORM-3 (As,Se) (ng.g-1)**			
	Certified value	Our results	Sample (n)	Recovery (%)	Certified value	Our results	Sample (n)	Recovery (%)
THg	300 ± 15	297,25 ±10	39	99 ± 3	4640 ± 260	3233± 526	29	70 ± 11
MeHg	152 ± 13	160 ± 22	9	105 ± 14	_	_	_	_
TSe	5630 ± 600	5225± 414	17	93 ± 7	3300 ±	3370 ± 230	18	91 ± 7

Table S3.4. Weight -length relationship for selected fish from Burkina Faso showing regression parameter a and b Coefficient a and b used in the $W_s = \log_{10}(a) + b \times \log_{10}(L)$ calculation were in bold characters. Coefficient a = 10^(intercept).

fish	n	Intercept	a	b= slope	r^2	p-value
Loumbila						
O. niloticus	31	**-5.265**	**0.0000054**	**3.384**	**0.82**	**< 0.001**
A. occidentalis	28	**-5.805**	**1.57e-06**	**3.503**	**0.78**	**< 0.001**
C. anguillaris	28	-4.801	1.58e-05	2.917	0.98	< 0.001
S. intermedius	13	-2.13	0.0074	1.681	0.3	> 0.05
Ziga						
O. niloticus	28	-4.708	1.958e-05	3.113	0.94	< 0.001
S. membranaceus	31	**-2.86**	**0.0014**	**2.234**	**0.60**	**< 0.05**
C. anguillaris	31	-4.069	8.53e-05	2.619	0.94	< 0.001
S. intermedius	17	-2.477	0.0033	1.823	0.59	< 0.001
B. bajad	34	**-3.864**	**0.00014**	**2.547**	**0.83**	**< 0.001**
Kompienga						
O. niloticus	20	-3.855	0.00014	2.750	0.97	< 0.001
A. occidentalis	13	-4.974	1.06e-05	3.123	0.78	< 0.001
S. membranaceus						
C. anguillaris	13	**-5.214**	**6.1e-06**	**3.087**	**0.98**	**< 0.001**
S. intermedius	31	**-6.650**	**2.24e-07**	**3.798**	**0.96**	**< 0.001**
B. bajad	10	-2.336	0.0046	2.007	0.77	< 0.001
L. niloticus	5	**-4.006**	**9.863e-05**	**2.76**	**0.99**	**< 0.001**

Table S3.5. Main food items (%) of fish from the three reservoirs during 2010 rainy season. number in parenthesis n is the sample size.

Fish species (n)	**MFI (%)**			
	Detritus	**Plants**	**Invertebrates**	**Fish**
Loumbila				
O. niloticus (34)	**74.4**	23.8	0.4	1.4
A. occidentalis (27)	**56.1**	1.1	42.6	_
S. intermedius (20)	10.9	0.4	22.8	**66.2**
C. anguillaris (31)	12.7	18.8	**19.2**	**49.3**
Ziga				
O. niloticus (30)	**56.6**	19.6	3.7	20.2
S. membranaceus (33)	21.4	4.6	**70.3**	
B. bajad (34)		0.2	**56.0**	41.6
C. anguillaris (33)	2.04	3.0	**62.9**	32.1
Kompienga				
O. niloticus (20)	**92.1**	6.9		1.0
A. occidentalis (13)	36.2	1.2	**62.6**	_
B. bajad (10)	_	8.6	30.4	**60.8**
C. anguillaris (13)	2.9	4.0	21.7	**71.4**
S. intermedius (30)	_	_	**85.7**	14.35

Annexe 4. Données supplémentaires du chapitre 4

4.1 Figures supplementaires du chapitre 4

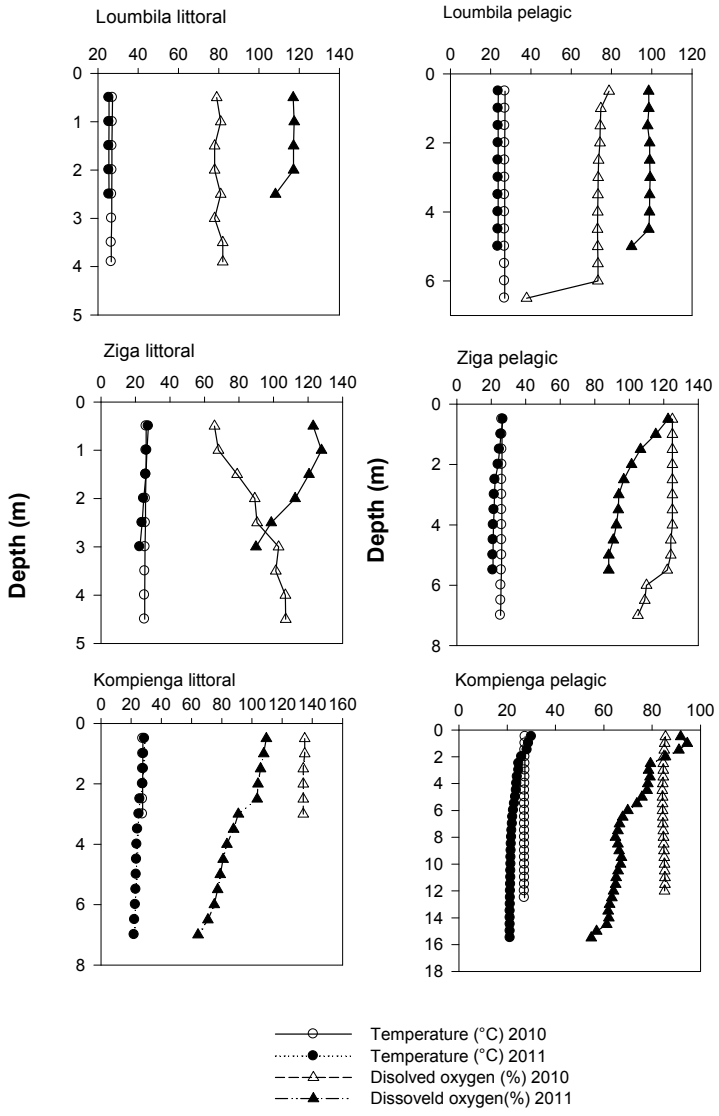

Figure S4.1 Temperature and dissolved oxygen profiles in littoral and pelagic zones of three freshwater reservoirs from Burkina Faso at both rainy season 2010 and dry season 2011.

4.2. Tables supplémentaires du chapitre 4.

Table S4.1. Study sites water quality variables, metal(loïds) concentrations in water and sediment (ng/g d.w.). Metalloid values are presented as mean ± standard deviation of 6 replicates samples for 2010 rainy and 2011 dry seasons.

Compartiment	**Variable**	**Loumbila**		**Ziga**		**Kompienga**	
		Rainy season	Dry season	Rainy season	Dry season	Rainy season	Dry season
Water							
	Depth (m)	**6**	**5**	**6.5**	**5.5**	**12**	**15.5**
	NO3- (mg/L)	1.03 ± 0.01	0.024	0.42 ± 0.12	0.15 ± 0.08	0.4 ± 0.07	0.12 ± 0.06
	SO42-(mg/L)	0.057 ± 0.024	0.048	0.75 ± 0.02	0.78 ± 0.05	0.71 ± 0.02	0.58 ± 0.05
	DOC (mg/L)	**2.06 ± 0.66**	**3.10 ± 0.23**	**1.94 ± 0.63**	**2.84 ± 0.85**	**1.85 ± 0.3**	**2.92 ± 0.03**
	Condu (µS/cm)	81.5 ± 0.7	86.5 ± 7.7	106 ± 4.2	115 ± 1.4	134	110.5 ± 0.7
	T (° C)	26.8 ± 0.16	24.6 ± 1.3	25.46 ± 0.14	22.4 ± 2	27.4 ±0.3	21.8 ± 0.84
	DO (%)	73.15 ± 0.21	108 ± 13	101.4 ± 7.7	93.4 ± 7.5	86.65 ± 2.2	63.9 ± 9.7
	pH	7.25	7.4 ± 0.14	7.68 ± 0.14	8.1 ± 0.4	7.2 ± 0.2	7.16 ± 0.03
	THg (ng/L)	3.016 ± 0.11	0.738 ± 0.37	5.180± 0.76	2.335 ± 0.12	2.43 ± 0.176	1.23 ± 0.06
	DHg (ng/L)	2.49 ± 0.20	0.634 ± 0.23	1.976 ± 0.14	1.768 ± 0.25	1.55 ± 0.23	0.986 ± 0.10
	PHg (ng/L)	0.704 ± 0.23	0.103 ± 0.2	4.753 ± 0.82	0.567 ± 0.25	0.853 ± 0.144	0.242 ± 0.07
	MeHg (ng/L)	0.074 ± 0.06	0.065 ± 0.052	0.130 ± 0.09	0.146 ± 0.10	0.040 ± 0.02	0.220 ± 0.12
	Tse (ng/L)	69.05 ± 5.16	31.32 ± 1.71	62.5	115.54 ± 53.46	56.6 ± 1.2	46.7 ± 5.2
Sediment (d.w**.)**							
	THg (ng/g)	13.78 ± 8.36	15.75 ± 9	20.5 ± 9.5	21.06 ± 3	23.15 ± 5.7	21.7 ± 1.5
	Tse (ng/g)	158.5 ± 136.4	142.4 ± 110	256.5 ± 111	229 ± 32	157.5 ± 21.9	176 ± 10

Table S4.2. Classification of fish into small (coded as 1), medium (coded as 2) and large (coded as 3) class size according to their total length

Fish	Fish class size (total length) (mm)		
	Small (1)	Medium (2)	Large (3)
O. niloticus	≤ 150	151 - 200	> 200
A. occidentalis	≤ 150	151 200	>200
B. bajad	≤ 200	201-400	>400
C. anguillaris	≤ 200	201-400	>400
S. intermedius	≤ 150	151 - 200	>200
L. niloticus	≤ 500	_	_
S. membranaceus	≤ 150	151- 200	>200

Table S4.3. Quality of analytical results of mercury and selenium in water and fish tissues from 2010 and 2011 samples analyses. TORT-2 and DORM-3 are certified reference materials (CRM) from the National Research Council of Canada. Sample sizes are in parentheses

Element	**TORT-2 (ng/g)**					**DORM-3**				
	Certified value	2010 Our results	% recovery	2011 Our results	% recovery	Certified value	2010 Our result	% recovery	2011 Our result	% recovery
THg	300 ± 15	297 ± 10 (39)	99 ± 3	299 ± 34 (50)	99 ± 11	382 ± 60	357 ± 33 (10)	93.5 ± 0.8	377 ± 15 (8)	98.6 ± 4
MeHg	152 ± 13	160 ± 22 (9)	105 ± 14	150 ± 34 (14)	98 ± 22	_	_	_	_	_
TSe	5630 ± 600	5225 ± 414 (17)	93 ± 7	5730 ±	102 ±	3300	3370 ± 230 (18)	91 ± 7	3324 ± 76 (2)	100 ± 2

Table S4.4. Fish relative weight in the three reservoirs from Burkina Faso at the 2010 rainy and 2011 dry seasons. Sample sizes are in parentheses.

site	Fish species	Relative weight (W_r)	
		2010 rainy season	2011 dry season
Loumbila	*O.niloticus*	46.7 ± 9.2 (32)	57 ± 7.5 (9)
	A.occidentalis	47.2 ± 8.4 (28)	49.5 ± 5.4 (6)
	B. bajad		121±13 (4)
	C.anguillaris	68.7 ± 12 (30)	68 ± 9.6 (9)
	S.intermedius	70.12 ±14.3 (16)	67.8 ± 14 (10)
Ziga	*O.niloticus*	45.43 ± 5.7 (30)	53.3 ± 4.4 (9)
	A. occidentalis		47 ± 6.7 (9)
	S. membranaceus	52.7± 7.07 (33)	
	B. bajad	51.25 ± 8.65 (34)	127.4 ± 26.4 (9)
	C.anguillaris	72.35 ± 9.6 (33)	75 ± 8 (6)
	S.intermedius	59.8± 10 (17)	63.5 ± 7.8 (10)
Kompienga	*O.niloticus*	54.46 ± 5.15 (20)	57 ± 4.7 (14)
	A.occidentalis	37.8 ± 3.3 (13)	49 ± 3.7 (12)
	S.membranaceus	59.15 ± 29 (3)	
	B. bajad	78.12 ± 11 (11)	186.16 ± 29 (3)
	C.anguillaris	70 ± 6.8 (13)	84.4 ± 10 (11)
	S.intermedius	67.3 ± 12.7 (17)	93 ± 40 (8)
	L. niloticus	61.25 ± 3.13 (5)	200.5 ± 33 (3)

Table S4.5. Metal(loids) concentrations in fish of the three freshwater reservoirs from Burkina Faso in 2010 rainy season and 2011 dry season. Sample sizes are in parentheses.

Fish	Size class	THg (µg/g w.w.)		MeHg (µg/g w.w.)		Tse (µg/g w.w.)	
		Rainy season	Dry season	Rainy season	Dry season	Rainy season	Dry season
Loumbila							
O. niloticus	1	0.007 ± 0.003 (22)	0.013 ± 0.006 (21)	0.008 ± 0.003 (3)	0.013 ± 0.01 (4)	0.14 ± 0.02 (22)	0.14 ± 0.04 (4)
	2	0.005 ± 0.003 (10)	0.016 ± 0.008 (2)	0.004 ± 0.002 (2)	0.015 ± 0.006 (2)	0.13 ± 0.03 (10)	0.2± 0.006 (2)
A. occidentalis	1		0.017 ± 0.01 (4)		0.02 ± 0.008 (4)		0.08 ± 0.002 (2)
	2	0.024 ± 0.01 (21)	0.03 ± 0.01 (24)	0.02 ± 0.004 (5)	0.022 ± 0.011 (4)	0.08 ± 0.04 (21)	0.07 ± 0.01 (4)
	3	0.023 ±0.006 (7)	0.030 ± 0.01 (2)			0.112 ± 0.10 (7)	
B. bajad	2		0.050 ± 0.007 (5)		0.03 ± 0.003 (5)		
C. anguillaris	1	0.025 ± 0.01 (1)	0.024 ± 0.01 (4)	0.02 ± 0.008 (3)	0.020 ± 0.01 (3)	0.18 ± 0.04 (7)	0.12 ± 0.01 (3)
	2	0.06 ± 0.05 (19)	0.043 ± 0.02 (16)	0.05 ± 0.038 (6)	0.045 ± 0.02 (3)	0.17 ± 0.07 (19)	0.140 ± 0.06 (3)
	3	0.187 ± 0.05 (2)	0.048 ± 0.02 (13)		0.042 ± 0.02 (4)	0.23 ± 0.003 (2)	0.105 ± 0.01 (3)
S. intermedius	1	0.22 ± 0.1(11)	0.08 ± 0.06 (22)	0.14 ± 0.035 (2)	0.092 ± 0.06 (10)	0.18 ± 0.02 (11)	0.10 ± 0.01 (10)
	2	0.21 ± 0.1 (4)	0.143 ± 0.13 (7)	0.137 (1)	0.109 ± 0.08 (3)	0.23 ± 0.04 (4)	0.156 ± 0.04 (3)
Zooplankton		0.02	_	_	_	_	_

Table S4.5. (continued)

Fish	Size class	THg (µg/g w.w.)		MeHg (µg/g w.w.)		Tse (µg/g w.w.)	
		Rainy season	Dry season	Rainy season	Dry season	Rainy season	Dry season
Loumbila							
Unionidae (2)		0.024 ± 0.005	0.014 ± 0.017 (2)	0.025±0.001	_	0.39 ± 0.005	0.28 ± 0.26
Gastropoda(3)		0.026 ± 0.015	0.1 ± 0.08 (2)	0.013 ± 0.004	0.004 ± 0.001 (2)	0.19 ± 0.04	0.17 ± 0.10
Sediment		0.014 ± 0.008	0.015 ± 0.007	_	0.004 ± 0.001 (4)	0.16 ± 0.14	0.14 ± 0.09 (6)
Ziga							
O. niloticus	1	0.010 ± 0.008 (16)	0.013 ± 0.006 (23)	0.008 ± 0.003 (3)	0.013 ± 0.004 (6)	0.26 ± 0.03 (16)	0.3 ± 0.04 (4)
	2	0.014 ± 0.006 (12)	0.016 ± 0.008 (15)	0.01 (1)	0.019 (1)	0.3 ± 0.04 (12)	0.27 (1)
	3	0.01 ± 0.001 (2)		0.01 ± 0.0003 (2)		0.34 ± 0.04 (2)	
A. occidentalis	1		0.03 ± 0.007 (2)		0.035 (1)		0.12 ± 0.04 (2)
	2		0.03 ± 0.01 (8)		0.03 ± 0.001 (2)		0.16 ± 0.005 (2)
	3		0.05 ± 0.03 (9)		0.045 ± 0.013 (5)		
S. membranaceus	2	0.18 ± 0.10 (2)		0.1 (1)		0.24 ± 0.02 (2)	
	3	0.17 ± 0.04 (30)		0.15 ± 0.04 (5)		0.22 ± 0.06 (30)	
B. bajad	1		0.10 ± 0.04 (5)		0.10 ± 0.06		0.30 ± 0.07
	2	0.10 ± 0.06 (34)	0.14 ± 0.05 (16)	0.094 ± 0.05 (6)	0.04 ± 0.03 (6)	0.35 ± 0.06 (34)	0.325 ± 0.06 (6)

Table S4.5. (continued)

Fish	Size class	THg (µg/g w.w.)		MeHg (µg/g w.w.)		Tse (µg/g w.w.)	
		Rainy season	Dry season	Rainy season	Dry season	Rainy season	Dry season
Ziga							
C. anguillaris	2	0.114 ± 0.09 (30)	0.078 ± 0.04 (14)	0.156 ± 0.1(4)	0.06 ± 0.04 (5)	0.25 ± 0.08 (30)	0.26 ± 0.12 (5)
	3	0.24 (1)	0.06 ± 0.01 (2)	0.2 (1)	0.05 (1)	0.2 (1)	0.3 (1)
S. intermedius	1	0.10 ± 0.07 (12)	0.09 ± 0.04 (4)	0.24 (1)	0.06 ± 0.02 (3)	0.20 ± 0.05 (12)	0.155 ± 0.02 (2)
	2	0.115 ± 0.03 (5)	0.10 ± 0.06 (27)	0.16 (1)	0.08 ± 0.07 (5)	0.20 ± 0.06 (5)	0.15 ± 0.02 (3)
Zooplankton		0.024		_	_	_	_
Unionidae (2)		0.04 ± 0.03	0.04 ± 0.03	0.006 ± 0.0001	0.02 ± 0.02	0.04 ± 0.06	0.405 ± 0.07
Gastropod (3)		0.1 ± 0.07	0.10 ± 0.07	0.032 ± 0.006	0.03 ± 0.006	0.3 ± 0.08	0.29 ± 0.07
Sediment		0.02 ± 0.009	0.021 ± 0.003	_	_	0.256 ± 0.11	0.21 ± 0.10
Kompienga							
O. niloticus	1		0.01 ± 0.004 (4)		0.004 (1)		0.02 (1)
	2	0.006 ± 0.03 (10)	0.013 ± 0.004 (5)				
	3	0.02 ± 0.012 (10)	0.02 ± 0.007 (21)	0.002 (1)	0.02 ± 0.007 (8)	0.1 ± 0.01 (10)	0.09 ± 0.01 (8)
A. occidentalis	3	0.08 ± 0.05 (12)	0.04 ± 0.02 (26)	0.06 ± 0.03 (8)	0.03 ± 0.008 (6)	0.12 ± 0.04 (12)	0.06 ± 0.02 (6)

Table S4.5. (end)

Fish	Size class	THg (µg/g w.w.)		MeHg (µg/g w.w.)		Tse (µg/g w.w.)	
		Rainy season	Dry season	Rainy season	Dry season	Rainy season	Dry season
Kompienga							
S. membramaceus	2	0.78 (1)				0.96 (1)	
	3	0.05 ± 0.005 (2)		0.04 ± 0.004 (3)		0.2 ± 0.016 (2)	
B. bajad	2	0.11 (1)	0.11 ± 0.014 (2)	0.14 ± 0.06 (2)		0.21 (1)	
	3	0.21 ± 0.08 (10)	0.24 ± 0.13 (6)	0.19 ± 0.05 (8)	0.12 ± 0.04 (4)	0.17 ± 0.03 (10)	0.17 ± 0.02 (4)
C. anguillaris	2	0.15 ± 0.09 (5)	0.07 ± 0.04 (18)	0.07 ± 0.016 (3)	0.07 ± 0.05 (5)	0.17 ± 0.05 (5)	0.16 ± 0.023 (5)
	3	0.23 ± 0.07 (8)	0.12 ± 0.06 (11)	0.13 ± 0.08 (7)	0.08 ± 0.04 (5)	0.23 ± 0.06 (8)	0.16 ± 0.01 (5)
L. niloticus	1	0.19 ± 0.08 (5)	0.1 ± 0.06 (7)	0.15 ± 0.065 (5)	0.11 ± 0.05 (3)	0.2 ± 0.02 (5)	0.17 ± 0.01 (3)
S. intermedius	1	0.11 ± 0.06 (30)	0.105 ± 0.04 (30)	0.13 ± 0.05 (3)	0.075 ± 0.035 (4)	0.15 ± 0.05 (30)	0.10 ± 0.003 (4)
Zooplankton		0.008	_	0.024	0.004 ± 0.0001	_	0.156
Gastropod (3)			0.02 ± 0.001	0.005± 0.002	0.004 ± 0.01		0.300 ± 0.04
Sediment		0.02 ± 0.006	0.02 ± 0.002	_	_	0.16 ± 0.02	0.17 ± 0.05

Table S4.6. Comparison of the slopes of regression log10-metal(loïd) – adjusted.δ15N in the three reservoirs among 2010 rainy and 2011 dry seasons. t_c is the calculated t-value and $t_{2\alpha,v}$ is the value of t in the Student.t table. v is the degree of freedom (n_1+ n_2 - 2). n_1 and n_2 were the sample size used in the regression for rainy season of 2010 and dry season of 2011 respectively.

	(n)		**TMF**		**t-test**		
Loumbila	n_1	n_2	2010	2011	t_c	$t_{2\alpha,v}$ (α = 0.05)	observations
THg	27	33	2.87 ± 1.4	2.87 ± 1.4	0	1.96	$\|t_c\| < t_{2\alpha,v,}$ difference not significant
MeHg	22	31	3.10 ± 1.4	2.39 ± 1.4	1.93	1.67	$\|t_c\| > t_{2\alpha,v,}$ TMF 2010 > 2011
TSe	22	31	1	1	0	1.68	$\|t_c\| < t_{2\alpha,v,}$ difference not significant
Ziga							
THg	25	39	3.29 ±1.5	4.73 ± 1.3	3.94	1.67	$\|t_c\| > t_{2\alpha,v,}$ TMF 2011 > TMF 2010
MeHg	25	39	3.10 ± 1.1	3.57 ± 1.3	1.55	1.67	$\|t_c\| < t_{2\alpha,v,}$ difference not significant
TSe	25	30	1	1.46 ± 1.2	9.58	1.68	$\|t_c\| > t_{2\alpha,v,}$ TMF 2011 > TMF 2010
Kompienga							
THg	49	42	6.54 ± 1.4	3.78 ± 1.2	10.12	1.67	$\|t_c\| > t_{2\alpha,v,}$ TMF 2010 > 2011
MeHg	49	42	6.54 ± 1.3	2.76 ± 1.2	14.41	1.67	$\|t_c\| > t_{2\alpha,v,}$ TMF 2010 > 2011
TSe	49	40	1.34 ± 1.2	1.42 ± 1.1	0.32	1.67	$\|t_c\| < t_{2\alpha,v,}$ difference not significant

Annexe 5. Supplementary information of chapitre 5

5.1 Figures supplémentaires du chapitre 5

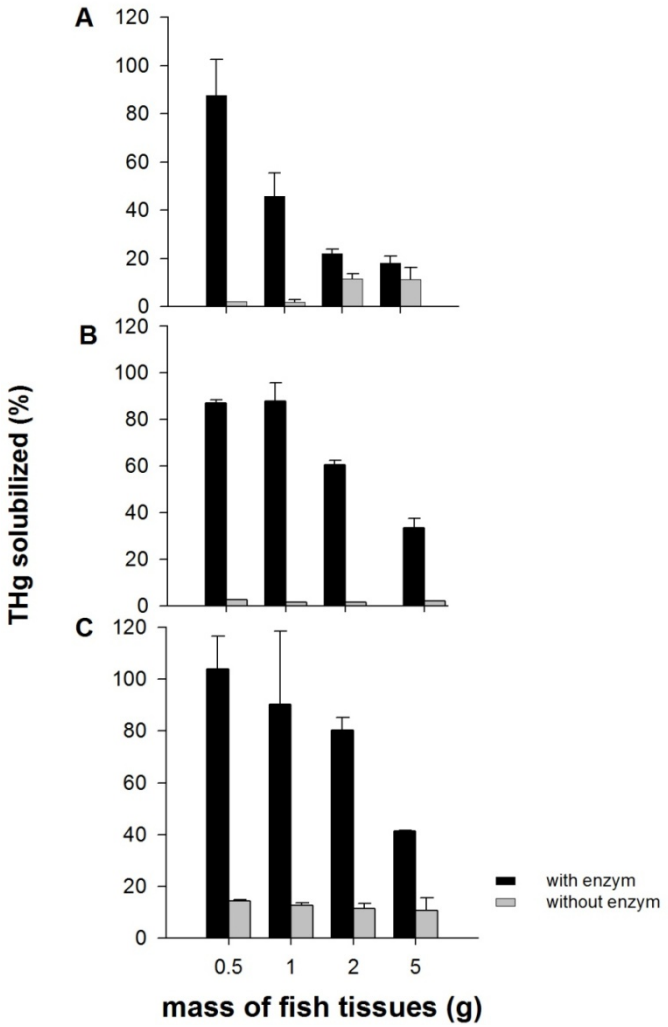

Figure S5.1. THg bioaccessibility from three fish species in vitro digestion among different fish tissues amount **(**0.5; 1; 2 and 5 g wet weight) with enzym (black bars) and without (gray bars) (**A**) from tuna, (**B**) from shark and (**C**) from Spanish mackerel. Values represent means (% THg solubilized) ± standard deviation for 3 replications.

5.2. Tables supplémentaires du chapitre 5

Table S5.1. Kruskal-Wallis signed rank Test. Effect of cooking methods on fish Hg bioaccessibility

Bioaccessibility	**Fish test meal (n =3)**	**Kruskal-Wallis H_{obs} (k=3)**	**P- value**	**H_{α} critique = 7.2 decision**
tuna	Raw/Boiled/Fried	7.2	0.02732	$H \geq H_c$; H_0 rejected
shark	Raw/Boiled/Fried	3.7143	0.1561	$H \leq H_c$; H_0 accepted
mackerel	Raw/Boiled/Fried	9.8462	0.007277	$H \geq H_c$; H_0 rejected

Table S5.2. Kruskal-Wallis rank Test. Effect of food items on fish Hg bioaccessibility

Food item	**Fish test meal (n =3)**	**Kruskal-Wallis H_c (k=5)**	**P- value**	**$X^2_{\alpha = 0.05 \text{ et } v=4} = 9.49$ decision**
Green tea	Tuna	13.3526	0.009676	$H_c > X^2_{\alpha(k-1)}$,H_0 rejected.
	Shark	12.9257	0.01164	$H_c > X^2_{\alpha(k-1)}$, H_0 rejected.
	Mackerel	12.9928	0.01131	$H_c > X^2_{\alpha(k-1)}$, H_0 rejected.
Black tea	Tuna	12.3213	0.01511	$H_c > X^2_{\alpha(k-1)}$, H_0 rejected.
	Shark	9.5012	0.04972	$H_c > X^2_{\alpha(k-1)}$, H_0 rejected.
	Mackerel	11.1799	0.02462	$H_c > X^2_{\alpha(k-1)}$, H_0 rejected.
Coffee	Tuna	12.9928	0.01131	$H_c > X^2_{\alpha(k-1)}$, H_0 rejected
	Shark	13.2333	0.01019	$H_c > X^2_{\alpha(k-1)}$, H_0 rejected
	Mackerel	10.3	0.03567	$H_c > X^2_{\alpha(k-1)}$, H_0 rejected
Corn flour	Tuna	9.8369	.04327	$H_c > X^2_{\alpha(k-1)}$, H_0 rejected.
	Shark	5.976	0.2009	$H_c < X^2_{\alpha(k-1)}$, H_0 accepted
	Mackerel	4.1	0.3926	$H_c < X^2_{\alpha(k-1)}$, H_0 accepted