Human exposure assessment related to oil activities in Ecuador: from the air quality monitoring to the study of metallic contaminants transfer in the soil-plant continuum

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THÈSE

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Évaluation de l'exposition humaine liée aux activités pétrolières en Équateur: de la surveillance de la qualité de l'air à l'étude du transfert des contaminants métalliques dans le continuum sol-plante

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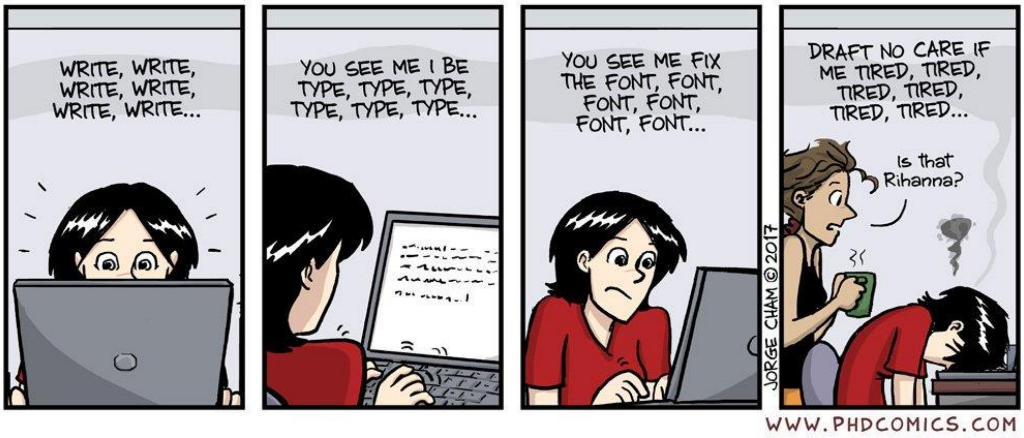
Acknowledgments

⚠**….***before reading this, I warn you, it is going to be a little bit long. So, you have two options, skip to* ***Introduction*** *on page 1 or take a seat, bring a cup a coffee (or a glass of wine) and enjoy.*



I felt silly when months ago, while I was writing my first paper, I made a joke about this comic and nobody understood it. Well, apparently science and pop culture are not compatible.

Science and fashion are not compatible either. Three years ago, after I had finished my Masters in Toulouse while I was living in Peru again (working on a zoo, being the lovely step mother of two monkeys and two wild cats), I found myself on my way to a fashion school in Lima. I had been rejected from a PhD scholarship program 3 months earlier that year. But something happened that day, I received an email, saying that the person who had been selected for the grant instead of me was not interested anymore. Lucky me!

I'm a big fan of the Big Bang Theory, for my friends and family that's how real scientists looks like. So, let's say that for them I don't really fit into that "category". If you don't know, I'm actually a biologist (who hates field work, heat, sweat, mosquitos, spiders and also plants)… to be honest I have tried to be one but I quit.

So, why I decided to do a PhD? I had sent a lot of job applications in Peru without any result. The PhD was my last chance and I really wanted to come back to France, travel as far as I could, meet new people, live….and well, learn more about biogeochemistry. I have spent hours in the clean lab washing savilex, digesting samples, breathing acids…..or in the field, in Ecuador, having nightmares with cacao trees, fighting with ants, spiders and mosquitos, carrying 20 kilos of equipment in a Barbie-pink luggage

or bringing more than 30 kilos of soil and cacao samples (with help, not by myself, just to be clear). However, I have really enjoyed these 3 years….and if someone ask to me, what is biogeochemistry, I would say…..*do you remember how you explain to me that Chiara Ferragni is not a real fashion designer? Well, biogeochemistry is the Chiara Ferragni of science* (copying Sheldon Cooper in my own way), and again…probably nobody would understand the joke.

First, I would like to thank Laurence Maurice, for accepting to be my supervisor even though she didn't know me before. Thank you for giving me the opportunity to join the Monoil Program. Thanks to Gaëlle Uzu, also my supervisor, (who promised to my mom last year in Arequipa that I would finish my thesis on time) for giving me the opportunity to work with her again and to discover the world of aerosols chemistry. I'm very grateful to Eva Schreck. During these last 3 years you were not only my supervisor, but also like a big sister and sometimes a mom. Thank you to the three of you for the long hours reading and correcting my papers, for the company and chatting during field work, for the hours trying to explain to me what is OC/EC, levo, DTT, AA, OP and for the emotional support not only during the manuscript redaction but also in the saddest moment of my life.

Thanks to Innovate Peru program for my PhD scholarship N° 139-Innóvate Perú-BDE-2014.

I extent my sincere thanks to the Monoil team in Ecuador, Valeria Ochoa (for letting me stay at her house), Jenny Ruales (for keeping our samples safe), Gissel Pazmiño and Daniela Racines (love you girls!!!), Martin Ochoa (cacao team), Fausto Lopez (also my officemate in France). Special thanks to Jonathan Prunier and Thibaut Lêvêque, not only for your moral support but also for your assistance during lab work. Thibaut, now I understand what is bioaccessibility and I am able to make fake saliva and gastric juices by myself! Thank you to Antoine Pierart too for the preliminary analysis on Cd bioaccessibility.

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Thanks to the clean lab, ICP-MS and chemistry team for their technical support. Special thanks to Priscia Oliva, Christine Destrigneville, Manuel Henry, Laure Laffont, Stephanie Mounic and Aurélie Marquet.

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Moreover I want to thank the Ecuadorian farmers, especially to the Ramirez and Garcia Family, to Oscar Herrera and to Hungria Hinostroza. Without your help, interest and even enthusiasm (given the hard situation you live) this research would not have be possible.

Now it is time for friends:
Thanks to the international "drinking Friday" PhD/post-doc group: Andrea, Anna, Chris, Martin, and Nang. Special thanks to Cristina a.k.a "Rayito de Sol", you have probably heard us laughing and talking extremely loud in the lab. Thanks to Franzi, Marie and Hannah….it is lunch time!. The "chilenos pal pico" and the "ché": Camila, Carlos, Héctor, "ché" Lucia and Valeria (Vale, a ti te agradezco en español….gracias por haberme hecho escuchar "Despacito" por primera vez, jamás lo olvidare!). The "compatriota" from Peru Pedro, and in the same office thanks to the poor Simon trying to understand Spanish. Thanks a lot for listening to me the last two weeks before the manuscript's submission!
I don't want to thank the person who unplugged my freezer and the mice who ate my chocolate samples!
To Aude, for the long hours on the phone talking about our hard days in the lab, or about how awful the "ego problem" is in the world of science, but also for sharing drinks, laughs, films and TV shows during my countless "séjours" in Grenoble. To Natalia, my French-peruvian best friend ever!!!! You and your craziness… « quelle idée de faire une thèse », « je raccroche, je suis en manips ». To my lovely coloc Isabelle, for the peace and love atmosphere at home.
To my friends in Peru: Gina and Wendy (pony-unicorn group). Viviré en deuda con ustedes por siempre, todo lo que han tenido que soportar durante 3 años por whatsapp. Comparto todos mis éxitos con ustedes, las amo!. Erika, Cecilia and María Luisa, siempre pendientes de mí y de mi bienestar psicológico!

And finally, to my beloved family in Peru, Malaysia, United States and Spain (we are so international!):
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A mi prima Adriana……jamás olvidare nuestra aventura Tomorrowland!

Acknowledgments

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A mi papá, que no está más aquí, por quien decidí venir a Francia: “esponjita-esponjita”….si papá, absorbí todo cuanto pude, espero estés orgulloso!

I just want to write a few lines more….I don’t know if I will keep doing this for the rest of my life, I don’t know if I’m good enough to have a brilliant career. The only thing that I know is that I will to be a humble and respectful scientist.

Bonne lecture à tou(te)s !

Résumé

L'Équateur est le 5ème pays producteur de pétrole en Amérique du Sud. Les étapes d'extraction et de raffinage génèrent des déchets contentant des métaux co-émis avec les hydrocarbures aromatiques polycycliques (HAPs), connus pour leurs effets cancérogènes et toxiques chez l'homme.

Dans ce contexte et dans le cadre du programme ANR MONOIL, le premier objectif de cette thèse était de déterminer la distribution des métaux dans l'environnement sur les provinces d'Orellana et Sucumbíos (zones d'extraction du pétrole en Amazonie, « NAR ») et dans la ville d'Esmeraldas (raffinerie, sur la côte Pacifique,« NPC »). Le 2ème objectif était d'étudier le transfert des métaux depuis le sol ou l'air vers les cacaoyers, largement cultivés en Equateur, et d'en déterminer les impacts sur la santé après ingestion. Enfin, le 3ème objectif était de surveiller la qualité de l'air (PM_{10}) afin d'évaluer l'impact des activités anthropiques et le potentiel oxydatif des particules.

Des sols et des cultures ont été collectés dans 31 fermes entre 2014 et 2016. Des échantillons d'air ont été prélevés mensuellement sur 3 sites, à proximité des plateformes pétrolières et de la raffinerie. La composition chimique (teneurs en métaux, carbone organique et élémentaire, ions, sucres, polyols, HAPs) a été déterminée dans les PM_{10}.

L'évaluation des risques sanitaires a été réalisée via 3 voies d'exposition. Les effets non cancérogènes et cancérogènes ont été quantifiés à l'aide de l'Indice de Risque (HI) et du Risque Total de Cancer (TCR). La bioaccessibilité du Cd par ingestion a été déterminée avec le test BARGE dans les pâtes et fèves de cacao. La formation d'espèces réactives de l'oxygène (ROS) a été quantifiée par spectrophotométrie en utilisant 3 essais acellulaires: DTT, AA et DCFH.

Les résultats montrent que dans 72% des sites, les concentrations en Ba, Co, Cu, Cr, Ni, V et Zn dans les sols dépassent les limites de la législation équatorienne. Pour la plupart des cultures, les concentrations en métaux sont inférieures aux limites de détection, sauf pour le Cd dans le cacao et le Pb dans le manioc, qui dérogent aux normes de qualité alimentaire.

Les cacaoyers accumulent le Cd dans les feuilles, les cabosses et les fèves. Comme la teneur en Cd du pétrole est inférieure à la limite de détection, les produits agrochimiques et les intrants organiques peuvent être incriminés. Près de 100% de la teneur totale en Cd dans les pâtes et fèves de cacao est bioaccessible par ingestion. Le risque pour la santé après consommation de chocolat oscille entre faible et élevé, selon la quantité ingérée.

Dans les PM_{10}, le Ba et le Mo, traceurs spécifiques des activités pétrolières, ont montré des valeurs beaucoup plus élevées que celles enregistrées dans les sites industriels. Les ratios OC/EC étaient plus élevés en Amazonie, suggérant des émissions biogéniques plus importantes. La teneur en HAPs est

Résumé

plus élevée sur la côte, cependant les niveaux de BaP étaient inférieurs aux limites de l'UE. Les résultats du potentiel oxydatif ont montré que les composés émis par l'industrie pétrolière (HAPs, Ba, Ni, Zn), par la combustion de la biomasse (sucres) dans le NAR ainsi que les traceurs industriels dans le NPC (As, Ba, Ni, NH_4^+) corrèlent avec la génération de ROS.

Les principales voies d'exposition aux métaux sont l’ingestion et l'inhalation et, dans une moindre mesure, le contact dermique avec le sol. Les enfants tout comme les adultes sont vulnérables aux effets toxiques de ces composés.

Enfin, d'autres facteurs comme la déforestation, l'agriculture et les émissions naturelles (volcans) dans le NAR mais aussi les industries dans le NPC contribuent également aux dégâts environnementaux constatés et aux effets néfastes sur la santé.

Mots-clés: Equateur; Activités pétrolières; métaux et metalloïdes; Continuum sol-plante; qualité de l'air; Évaluation des risques pour la santé.

Abstract

Ecuador is the 5th crude oil producer country in South America. Oil extraction and refining generate toxic waste containing metals co-emitted with polycyclic aromatic hydrocarbons (PAHs), naturally present in crude oil or added during production, and known for their carcinogenic and toxic effects in humans.

In this context and as a part of the ANR MONOIL program, the first aim of this PhD research was to determine the distribution of metal(loid)s in the environment in the provinces of Orellana and Sucumbíos (oil extraction, North Amazon « NAR ») and the city of Esmeraldas (oil refining, North Pacific Coast, « NPC »). The second aim was to determine if there was a transfer of toxic metals, such as Cd, from soil or air to cacao crops, widely cultivated in Ecuador, and if the consumption of cacao-based products could imply risks for human health. Finally, the third aim was to monitor air quality over 2 years in both areas to assess the impact of anthropogenic activities and the oxidative burden of particulate matter (PM_{10}).

Soils and local crops were collected in 31 small-scale farms, between 2014 and 2016. Monthly PM_{10} samples were collected in 3 sites, in the vicinity of oil platforms close to the refinery.

PM_{10} chemical composition (contents in metals, organic and elementary carbon (OC, EC), ions, sugars, polyols, PAHs) was determined. Human health risk assessment was performed taking into account ingestion, inhalation and dermal contact. Non carcinogenic and carcinogenic effects were quantified using the Hazard Index (HI) and the Total Cancer Risk (TCR). Bioaccessibility of Cd after ingestion was determined by the BARGE *in vitro* test in cacao beans and liquors. In aerosols, reactive oxygen species (ROS) formation was quantified using 3 acellular assays: DTT (dithiothreitol), AA (ascorbic acid) and DCFH (2',7'-dichlorodihydrofluorescein).

Ba, Co, Cu, Cr, Ni, V and Zn concentrations in 72% of the study soils exceeded the limits of the Ecuadorian legislation. For most of the crops, elements were below the limits of detection but, Cd in cacao and Pb in manioc were above the international standards for food quality. Cacao trees accumulate Cd in leaves, pod husks and beans. Because Cd contents in crude oil were below the detection limits, agrochemical products and natural inputs may also be important sources. Almost 100% of the total Cd content in cacao beans and liquor was bioaccessible by ingestion. The health risk after chocolate consumption was low to high, depending on the ingestion rate and the cacao liquor percentage.

In PM_{10}, As, Cd, Ni and Pb were below the EU thresholds. However, Ba and Mo, specific tracers of oil activities, used as weighting agents or catalyzers during drilling and refining, showed values much

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higher than those recorded in other urban-industrialized sites. PM_{10} composition depended on oil activity. OC/EC ratios were higher in the NAR (oil extraction) than in the NPC (oil refining), suggesting that biogenic emissions were more important in the Amazon area. PAHs contents were higher in the NPC, but levels of benzo-a-pyrene were below the EU limits. Oxidative potential results showed that compounds emitted by oil industry (PAHs, Ba, Ni, Zn) and by biomass burning (sugars) in the NAR as well as industrial tracers in the NPC (As, Ba, Ni, NH_4^+) were correlated with ROS generation.

Regarding residential exposure, the main routes of exposure to metal(loid)s were first ingestion, inhalation and in a small extent soil dermal contact, being both children and adults vulnerable.

Finally, the oil Ecuadorian environment is not only contaminated by oil activities. Other factors like deforestation, agriculture and natural emissions (volcanos) in the NAR and industries in the NPC also contribute to environmental damages and may lead to adverse health effects.

Keywords: Ecuador; oil activities; metal(loid)s; soil-plant continuum; air quality; health risk assessment.

Abbreviations

AA: Ascorbic Acid

BaP: benzo(a)pyrene

BARGE: BioAccessibility Research Group of Europe

BF: Bioaccumulation Factor

CEC: Cation Exchange Capacity

DCFH: 2',7'-dichlorodihydrofluorescein

DTT: Dithiothreitol

EC: Elementary Carbon

EF: Enrichment Factor

GC-MS: Gas chromatography–mass spectrometry

GEF: Global enrichment factor

HRA: Health Risk Assessment

ICP-MS: Induced Coupled Plasma-Mass Spectrometry

MC: Manabí (control area)

MS: Morona Santiago (control area)

NAR: North Amazon Region of Ecuador

Nitro-PAHs: Nitrated Polycyclic Aromatic Hydrocarbons

NPC: North Pacific Coast of Ecuador

OC: Organic Carbon

OM: Organic Matter

OP: Oxidative Potential

Oxy-PAHs: Oxygenated Polycyclic Aromatic Hydrocarbons

PAHs: Polycyclic Aromatic Hydrocarbons

PM: Particulate Matter

PM_{10}: Particulate Matter <10µm

ROS: Reactive Oxygen Species

SEM: Scanning Electron Microscopy

SOTE: Sistema de Oleoducto Transecuatoriano (Trans-Ecuadorian Oil Pipeline System)

THP: Total Hydrocarbons of petroleum

TOC: Total Organic Carbon

UBM: Unified Barge Method

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Introduction

> ***« Si vous voulez gouverner le monde, vous devez contrôler le pétrole. Tout le pétrole. Partout »***
>
> (Michael Collon, *Monopoly*)

Malgré sa petite taille (250 000 km^2) représentant 1,5% de la surface totale de l'Amérique du Sud, l'Equateur est caractérisée par une très grande biodiversité, non seulement en nombre total d'espèces mais aussi en variété d'espèces par unité de surface (Rodrigues et al., 2014; Sierra et al., 2002). La diversité des régions biogéographiques (Figure I) associée à la grande variabilité environnementale de ce pays expliquent pourquoi il héberge 8% des amphibiens, 5% des reptiles, 8% des mammifères et 16% des espèces d'oiseaux répertoriées au niveau mondial (INEC, 2014). Au niveau environnemental, la variabilité des climats, le relief, les sols et les régimes d'inondation donnent lieu à des paysages naturels différents mais également à des menaces permanentes en raison de la pression continue exercée par l'exploitation des ressources naturelles depuis plusieurs décennies (MAE, 2012).

La principale menace pour la conservation de la biodiversité en Équateur est la déforestation (Mena, 2008; Messina et al., 2006; Sierra, 2000). La collecte de bois à brûler, l'expansion urbaine, l'exploration et l'exploitation pétrolière, l'agriculture, les activités minières, la pauvreté, les migrations humaines et le développement du tourisme sont d'autres facteurs importants contribuant à la détérioration de la richesse biologique du pays (CBD, 2017).

C'est à l'aube du 20ème siècle que le premier puits a été foré dans la péninsule de Santa Elena afin de confirmer l'existence de pétrole brut en Équateur. Cinquante ans plus tard, en 1967, un premier puits a été foré dans la région amazonienne, connue aussi sous le nom de région "Oriente", par la compagnie pétrolière Texaco Gulf (EP Petroecuador, 2013). En juin 1972, la Société Pétrolière Equatorienne (plus tard appelée EP Petroecuador) a transporté pour la première fois un baril de pétrole par l'Oléoduc Trans-Équatorien (Figure II), de Lago Agrio, dans la région amazonienne, à Balao, sur la Côte Pacifique.

Après quatre décennies d'exploitation et d'exportation d'hydrocarbures en Equateur, le pétrole reste l'une des principales sources de revenus économiques du pays, et reste considéré comme un secteur totalement stratégique pour son développement. En 28 ans de présence en Equateur, Texaco a foré 339 puits, construit 18 stations de production, mais a également déversé 19,3 milliards de gallons d'eaux de formation dans l'environnement et a abandonné entre 800 et 1000 puits de déchets toxiques. En outre, il a été estimé que les dégâts environnementaux en Équateur pendant les opérations de Texaco ont dépassé de 30 fois les déversements d'hydrocarbures provoqués par l'échouage du pétrolier Exxon Valdez en Alaska en 1986 (Almeida and Proaño, 2008).

Plus globalement, l'histoire de l'exploitation du pétrole n'est pas seulement ternie par la violence et l'interventionnisme de l'Etat ou de l'armée, mais aussi par des conflits sociaux et environnementaux (Figure III), notamment du fait que cet «or noir » est inégalement réparti à la surface du globe (Arora and Gupta, 2013).

En Equateur, aujourd'hui, 20% des réserves de pétrole brut (846 millions de barils) sont situées dans le bloc Ishpingo-Tambococha-Tiputini (ITT), dans le Parc National Yasuní, une zone protégée d'environ 2000 km^2 dans la région amazonienne, qui abrite également des communautés autochtones, dont une partie vit en isolement total (Vallejo et al., 2015).

L'Équateur dispose également de trois raffineries de pétrole: La Libertad, Shushufindi (situées toutes deux en «Oriente») et Esmeraldas (sur la côte Pacifique), d'une capacité totale d'environ 176 000 barils par jour (EIA, 2015). Récemment, la raffinerie nationale d'Esmeraldas a subi une rénovation complète afin d'atteindre un fonctionnement optimal et de traiter du pétrole lourd. Le système de l'oléoduc Trans-Equatorien, ou «SOTE» (figure IV), transporte aujourd'hui 306 000 barils par jour sur 497 km de longueur (Ministère d'Hydrocarbures de l'Equateur, 2016). D'après le journal "El Comercio" (2013), depuis 1972, l'inauguration du SOTE, un total de 725 655 barils (provenant de 72 ruptures de l'oléoduc) ont été déversés dans l'environnement.

Comme le rapportent Alaa El-Din et al. (2017) and Chaudhuri (2011), le pétrole brut est un mélange de différents constituants: des hydrocarbures (HAP), des composés organiques volatils (COV: benzène, toluène, éthylène, xylène, appelés "BTEX"), du soufre, de l'azote, de l'oxygène, et des composés métalliques (en particulier Ni, V, As, Pb Cd, Hg, Mn et Fe). Cependant les opérations d'extraction pétrolière et de raffinage peuvent enrichir les gaz, eaux ou boues de rejets en Ba et Mo.

De plus amples informations concernant la formation et la production du pétrole sont rapportées dans la section A.1, en Annexe A.

En Equateur, les déversements de pétrole brut, les fuites, le brûlage des gaz dans les torchères, les eaux de formation et les boues de forage entreposées dans des puits ouverts ont libéré, dans l'environnement amazonien, un mélange de composés toxiques qui a entraîné une contamination massive de l'air, de l'eau et des sols en hydrocarbures et métaux lourds essentiellement, sans pour autant que cette contamination n'ait été évaluée par aucune étude scientifique ou même par le gouvernement. De plus, les plantes cultivées à proximité des plateformes pétrolières ont pu également être impactées par ces composés (San Sebastián and Hurtig, 2004).

Concernant l'évaluation des risques pour la santé des populations locales vivant à proximité des zones d'exploitation et de raffinage pétrolier, il a été mis en évidence que l'exposition aux métaux et aux composés organiques avait essentiellement lieu par inhalation, ingestion et contact dermique. L'inhalation est généralement la voie d'exposition professionnelle la plus importante, tandis que l'ingestion de contaminants par l'alimentation et l'eau potable est habituellement la principale voie d'exposition pour l'ensemble de la population (Elert et al., 2011). Bien que l'absorption cutanée de composés métalliques soit généralement faible, elle peut s'avérer élevée pour les composés organométalliques et induire à des doses importantes des conséquences parfois mortelles (Nordberg et al., 2015).

Une exposition aigüe au pétrole brut par inhalation et par absorption cutanée peut provoquer des réactions respiratoires, des vomissements, des diarrhées et des réactions allergiques transitoires. Cependant, une exposition faible mais chronique à long terme, peut provoquer des problèmes au niveau du système nerveux central, ou endommager des organes tels que les reins ou le foie. Il existe également une augmentation significative du risque de cancer (Ordinioha and Brisibe, 2013).

Les hydrocarbures aromatiques à faible poids moléculaire, également connus sous le nom de BTEX, peuvent exprimer des effets mutagènes, cancérogènes, embryotoxiques, tératogènes, retardateurs de croissance, métaboliques et neuromodulateurs (Sirotkin et Harrath, 2017). Les hydrocarbures aromatiques polycycliques (HAPs) et leurs dérivés (nitro-HAP et oxy-HAP) sont classés par l'Agence des Substances Toxiques et le registre des maladies (ATSDR, 1995) comme « possibles », « probables » et « connus » cancérogènes chez l'homme (Bandowe and Meusel, 2017).

Même si certains métaux sont nécessaires, en raison de leur activité biologique, au fonctionnement du corps humain (métaux essentiels), d'autres métaux et métalloïdes comme l'As, le Co, le Cd, le Cr, le Ni et le Pb, peuvent générer, même à de faibles doses des dommages multiples aux organes (Karri et al., 2016), en raison du stress oxydatif induit par la formation de radicaux libres (Jaishankar et al., 2014). Les signes indésirables de l'exposition à long terme aux métaux et métalloïdes incluent des effets neurotoxiques, des maladies cardiovasculaires, des effets rénaux (sur la fonction tubulaire rénale), une carcinogenèse (cancer du poumon, cancer de la prostate) ainsi que des effets sur la fonction reproductrice (Nordberg et al., 2015). Les principaux organes cibles des métaux traces non essentiels sont exposés dans la Figure V. Par ailleurs, des informations plus détaillées sur la toxicité des métaux et métalloïdes sont données dans le tableau B.1 de l'Annexe B.

A la fin des années 90, des études sur l'exposition humaine à long terme aux émissions de composés issus de l'exploitation pétrolière ont été menées dans des communautés et des villages de l'Amazonie équatorienne. Selon San Sebastián et al. (2001a), les résidents des zones situées à proximité des infrastructures pétrolières (p. ex. plateformes pétrolières, puits de forage, torchères, etc.) ont développé plus de maladies que les communautés non exposées. Parmi ces maladies sont répertoriés l'anémie, la malnutrition, les infections de la peau, la gastrite, l'irritation des yeux, les maux de tête et des fausses couches chez les femmes. Ces résultats se basent sur la détermination des hydrocarbures polycycliques totaux (HPT) dans des échantillons d'eau consommée par les populations locales, ainsi que sur des enquêtes de santé menées dans les mêmes sites d'étude. Sur un total de 20 rivières, 18 ont des concentrations en HPT 10 fois plus élevées que les niveaux établis et autorisés par les législations internationales. En outre, l'incidence des cancers a augmenté dans le village de San Carlos, entouré de plus de 30 puits de pétrole et de 4 torchères puissantes (San Sebastián et al., 2001b). Paz-y-Miño et al. (2008) ont effectué différents essais sur des échantillons de sang des ouvriers exposés aux hydrocarbures et des habitants de la ville de San Carlos, et les ont comparés à une population témoin. Les résultats ont montré que les individus exposés présentaient une plus grande quantité de dommages à la fois à l'ADN et aux niveaux chromosomiques. Ces résultats indiquent que l'inhalation d'air et la consommation d'eau devraient être considérées comme des voies d'exposition importantes aux contaminants issus de l'industrie pétrolière.

Cependant, jusqu'au début du programme MONOIL « Monitoring environnemental, Santé, Société et Pétrole en Equateur » en 2011, aucune information fiable n'était disponible sur la qualité de l'eau, de l'air, des sols ou des cultures, ni dans l'Oriente (région Amazonienne), ni sur la côte du Pacifique, et en particulier près de la Raffinerie Nationale d'Esmeraldas. D'autre part, les risques sur la santé des métaux et des métalloïdes, naturellement trouvés dans le pétrole brut, n'avaient jamais été étudiés.

Ce manque d'information fiable sur les impacts environnementaux et sanitaires des polluants organiques et inorganiques a été utilisé comme argument par Texaco pour éviter toute responsabilité jusqu'en 1995 où ils ont accepté une part de 37,5% de dégâts et ont démarré la remédiation de certaines zones affectées. Quelques années plus tôt, en 1993, environ 30 000 agriculteurs indigènes et locaux ont intenté un procès contre Texaco, affirmant que la compagnie pétrolière avait causé des dommages irréparables à la forêt tropicale (Buccina et al., 2013). Après plus de 20 ans de tractations juridiques internationales aux États-Unis, en Équateur et jusqu'au tribunal de La Haye, le plus grand procès environnemental de tous les temps (de 9 milliards de dollars en dernière instance) contre Chevron-Texaco est toujours en cours.

Mais le développement incontrôlé des activités pétrolières en Équateur a également causé des impacts sociétaux tels que des conflits réguliers entre les communautés locales et le gouvernement et les entreprises pétrolières, des pertes culturelles, la destruction des ressources naturelles utilisées quotidiennement par les populations locales, la violation des droits des populations indigènes et des territoires ancestraux ainsi que la perte de cohésion sociale (Becerra et al., 2013).

Au total, 9 nationalités indigènes existent dans la région amazonienne équatorienne: Achuar, A'I Cofán, Huaorani, Kichwa, Secoya, Shiwiar, Shuar, Siona et Zápara (SIDENPE, 2005). Avant les années 1970, la plupart de ces communautés autochtones ne disposaient pas de contacts avec le monde extérieur, mais vivaient dans un isolement relatif sur des terres presque entièrement couvertes par la forêt. Suite au développement de l'exploitation pétrolière, de la colonisation agricole et de l'urbanisation, les communautés indigènes ont été en grande partie déplacées dans la périphérie voire très loin des zones d'exploitation pétrolière (Bozigar et al., 2016).

En termes de ressources naturelles, les zones les plus riches coïncident désormais avec les régions économiquement les plus pauvres et les moins développées. Comme l'ont souligné Becerra et al., (2013), la subsistance des habitants (agriculteurs et indigènes) des zones pétrolières dépend non seulement de la conservation des ressources naturelles, mais aussi de la compensation financière ou du travail qui peut être obtenue après un déversement de pétrole ou l'installation d'infrastructures pétrolières sur leurs terres.

Sur le plan juridique, les pays moins industrialisés comme l'Équateur sont moins susceptibles de protéger les droits environnementaux et sociaux en raison du manque de normes. En bref, la législation équatorienne sur la qualité de l'air ne régule que les concentrations de Cd et de benzène avec des seuils assez élevés alors que l'Union européenne considère en plus l'As, le Ni, le Pb et le BaP (benzo-a-pyrène). Les seuils pour les hydrocarbures restent trop peu clairs : Dans les sols et l'eau, la réglementation considère les hydrocarbures totaux du pétrole ("HTP", sans une définition exacte des composés inclus) et les HAP, mais la liste fait référence à 12 composés au lieu des 16 proposés par l'US-EPA (Cancer Environnement, 2016; TULSMA, 2015). Le gouvernement de l'Équateur a publié 2 législations environnementales, le TULSMA (Texte Unifié de Législation Secondaire Environnementale) et le RAHOE (Régulation Environnementale des Activités Hydrocarburifères). Cette dernière est la plus permissive, elle est dédiée uniquement aux suivis des activités pétrolières.

La quantité de HTP trouvée dans un échantillon est utile comme indicateur général de la contamination pétrolière sur un site. Cependant, la gestion des risques écologiques et de la santé humaine nécessite la compréhension de la toxicologie de chaque contaminant environnemental et la prise en compte des effets combinés sur chaque compartiment (ATSDR, 1999; Pinedo et al., 2013).

Face à cette situation, l'Institut français de Recherche pour le Développement (IRD) a été contacté en 2010 par des institutions publiques équatoriennes et par l’entreprise nationale Petroecuador, et, en collaboration avec des partenaires français et équatoriens, a mis en place le programme MONOIL: "Monitoring environnemental, Santé, Société et Pétrole en Equateur" financé par l'Agence Nationale de Recherche (ANR SOCENV, 2014-2018). L'objectif principal de cette approche interdisciplinaire et transversale entre Environnement, Santé et Population et axée sur l'écosystème est l'amélioration de la compréhension, du suivi, de la réduction et de la prévention de la contamination pétrolière et de ses impacts sur l'environnement et les populations locales, ainsi que ses vulnérabilités (MONOIL, 2017). La zone d'étude comprend les villages de Dayuma (province d'Orellana) et Pacayacu (province de Sucumbíos) au nord de la région amazonienne, et la ville d'Esmeraldas (où se trouve la principale raffinerie de pétrole) sur la côte Nord de l’Océan Pacifique.

Le programme MONOIL (Monitoring Environnemental, Santé, Société et Pétrole en Equateur) portait initialement sur l'impact des HAPs et des métaux lourds sur l'eau (et la chaîne trophique, c'est-à-dire les poissons et les mollusques), l'air et les sols. Cependant, l'existence de petites fermes (ou «fincas») à proximité des installations pétrolières a suscité l'intérêt d’une l'étude sur le continuum sol-plante et son possible impact sur la santé humaine à travers l'ingestion de végétaux contaminés (fruits ou légumes).

En effet, une étude préliminaire dans le cadre du programme MONOIL, réalisée lors du stage de Master de F. López (2014), a montré que les sols prélevés dans la zone touchée par l'extraction du pétrole avaient des concentrations en V, Cr, Co, Cu, Zn et Ba au-dessus des limites établies par la législation équatorienne. En outre, les fèves de cacaos de la même zone ont montré des concentrations en Cd allant de 2,2 à 5,3 mg kg^{-1}, c’est-à-dire des teneurs 3 à 6 fois plus élevées que celles fixées par la directive européenne sur le Cd dans les produits à base de cacao (chocolat noir). Selon Chachane et al. (2015) et Grujić et al. (2004), le pétrole brut et les déchets générés par les sites d'extraction, de production et de raffinage peuvent présenter des niveaux élevés en Cd. De plus, le cacao et d’autres produits qui en sont issus, comme la pâte et le beurre de cacao, ont été classés quatrième dans la catégorie des exportations non pétrolières de l'Équateur de janvier à avril 2017 (ProEcuador, 2017).

En ce qui concerne la surveillance de l'environnement et la comparaison des compartiments impactés (à savoir l'air, l'eau et le sol) aux normes de qualité, la procédure classique consiste à déterminer d'abord la concentration totale de chaque contaminant. Cependant, en vue d'une évaluation des risques sanitaires impliqués pour l'Homme, on devrait également tenir compte de la fraction bioaccessible des contaminants dans des matrices spécifiques.

La bioaccessibilité d'un élément est définie comme la fraction de cet élément qui se libère d'un aliment pendant la digestion ou par contact avec les fluides pulmonaires après inhalation de matière particulaire (PM) (Carbonell-Capella et al., 2014; Guney et al., 2016) et pouvant alors atteindre la circulation systémique, devenant ainsi biodisponible. Le contenu bioaccessible est toujours égal ou supérieur au contenu biodisponible et, lorsqu'il est considéré comme un proxy pour ce dernier, il génère le pire scénario concernant le contaminant (Cardoso et al., 2015).

Plusieurs tests acellulaires ont alors été développés au cours des dernières années afin de déterminer cette fraction bioaccessible. Le principe est d'utiliser des solutions synthétiques pour imiter au mieux *in vitro* les processus physiologiques (Mendoza et al., 2017; Wiseman, 2015). Les avantages de ces tests sont leur coût relativement faible et la vitesse à laquelle ils sont exécutés en laboratoire.

Notre objectif était dès lors d'utiliser quatre de ces tests *in vitro*: (i) le test BARGE (BioAccessibility Research Group of Europe) pour l'étude de la bioaccessibilité gastrique humaine des contaminants dans les sols développé par Caboche (2009) et adapté ensuite dans les fruits et végétaux (Xiong et al., 2014). (ii) Les tests DTT (dithiothréitol), AA (acide ascorbique) et DCFH (dichlorofluorescéine) selon Cho et al. (2005), Wang and Joseph (1999), pour mesurer le potentiel oxydatif des aérosols en prenant en compte leur bioaccessibilité pulmonaire, afin de quantifier la génération d'espèces réactives de l'oxygène (ROS) induites par ces matières particulaires après une mise en contact avec des fluides pulmonaires.

Ainsi, réalisée dans le cadre du programme de recherche ANR-MONOIL, les objectifs globaux de cette thèse étaient les suivants:

1. Évaluer les niveaux de contamination en métaux et métalloïdes dans les sols, les cultures et l'air mais aussi les risques impliqués pour la santé humaine (selon la voie d'exposition considérée) dans 3 zones d'étude impactées par les activités pétrolières: les provinces d'Orellana et Sucumbíos, dans le nord de la région amazonienne, et dans la ville d'Esmeraldas, sur la côte Pacifique.

2. Déterminer si le Cd, un métal non essentiel et toxique, est facilement absorbé par les cultures de cacaoyers dans les sites contaminés par les activités pétrolières, et si cela implique également des risques pour la santé humaine par ingestion de produits à base de cacao.
3. Estimer si le cocktail de métaux, metalloïdes et HAPs présents dans l'air (fraction PM_{10}) induit la formation d'espèces réactives d'oxygène dans les poumons, qui pourrait contribuer à l'exposition par inhalation des populations vivant dans ces zones impactées.

Les objectifs spécifiques, qui seront décrits dans chaque chapitre de la thèse, sont brièvement présentés dans la figure VI et énumérés ci-dessous:

- Identifier les sources naturelles et anthropiques possibles de métaux et métalloïdes dans les zones de production du pétrole.
- Déterminer quelles sont les principales voies d'exposition humaine aux métaux et metalloïdes trouvés dans les échantillons de sols, de cultures et d'air.
- Estimer quelle est la fraction bioaccessible du Cd dans les fèves de cacao et dans les produits à base de cacao lors de la digestion (phase gastrique).
- Comparer la composition chimique des aérosols PM_{10} émis dans les sites d'extraction et ceux de raffinage du pétrole.

Le présent manuscrit est alors organisé en 3 chapitres, sous format de publications scientifiques. Des informations supplémentaires (définitions de mots-clés ou données non publiées) seront incluses au début et à la fin de chaque chapitre. La section Matériels et Méthodes sera décrite dans chaque publication. Enfin, des informations complémentaires générales seront incluses en Annexes.

Le **Chapitre 1** est un article ***Accepté*** intitulé «Distribution, contents and health risk assessment of metal(loid)s in small-scale farms in the Ecuadorian Amazon: an insight into the impacts of oil activities». Dans ce chapitre, les concentrations en métaux et métalloïdes dans l'eau potable, les cultures locales, les sols et l'air (PM_{10}) ont été comparées aux seuils équatoriens et internationaux. L'évaluation des risques pour la santé humaine tient compte de deux types de population (adultes et enfants) et de 3 voies d'exposition (ingestion, inhalation et contact dermique).
Les données spécifiques d'Esmeraldas sont présentées à la fin de ce premier chapitre.

Chapitre 2. Le Cd est l'un des métaux non essentiels trouvés à des concentrations élevées dans les fèves de cacao, un des produits phare d'exportation en Equateur. Mais les activités pétrolières n'en sont pas la principale source. L'article ***publié***, intitulé «Cadmium bioaccumulation and gastric

bioaccessibility in cacao: A field study in areas impacted by oil activities in Ecuador» propose une première approche du contenu en Cd dans les fèves de cacao (et les produits à base de liqueur de cacao) pouvant être potentiellement absorbé par l'épithélium gastrique, atteindre le système circulatoire et être dangereux pour les consommateurs.
Les concentrations en autres métaux (supérieurs aux limites de détection) dans les échantillons de poudres et de pâtes de cacao sont présentées à la fin de cette section et comparées aux rares données disponibles dans la littérature.

Le **Chapitre 3** est spécialement dédié au compartiment atmosphérique Dans ce chapitre, présenté comme un article ***en préparation*** dont le titre est : «Chemical composition of PM_{10} and ROS generation in the vicinity of gas flares in oil extraction and refining areas in Ecuador», des résultats de métaux, de métalloïdes mais aussi les principaux HAPs sont présentés. La spéciation chimique des aérosols permet de déterminer si la production de ROS dans les fluides pulmonaires (après avoir été en contact avec les PM atmosphériques) est spécifique pour un groupe de composés donné et leur source d'émission. Cet article montre également que les émissions biogéniques, telles que la combustion de la biomasse et les microorganismes vivant dans le sol, constituent une source importante des aérosols dans la région amazonienne, qui pourrait masquer les émissions par torchères des activités pétrolières.

Enfin, les **Conclusions générales** sont présentées à la fin du manuscrit tandis que les **Perspectives** ouvrent notre travail sur une réflexion plus globale dans l’idée de poursuivre les études amorcées et d’étendre leur impact.

La version en anglais avec les figures est exposée ci-dessous.

Introduction

> ***"If you want to rule the world, you need to control the oil. All the oil. Anywhere"***
>
> (Michael Collon, *Monopoly*)

One of the most diverse countries, both measured as the absolute number of species and as the number of species per unit of area, is Ecuador, despite its small size, 250,000 km^2, representing only 1.5% of the total South America surface area (Rodrigues et al., 2014; Sierra et al., 2002). The confluence of several biogeographic regions (Figure I) and the environmental variability within each one explains why 8% of amphibian, 5% of reptile, 8% of mammal and 16% of bird species in the world are found there (INEC, 2014). The interactions of several environmental variables, such as bio-climates, relief, soil and flood regimes, give rise to different natural landscapes and also to permanent threats due to the continuous and permanent pressure on the exploitation of natural resources (MAE, 2012).

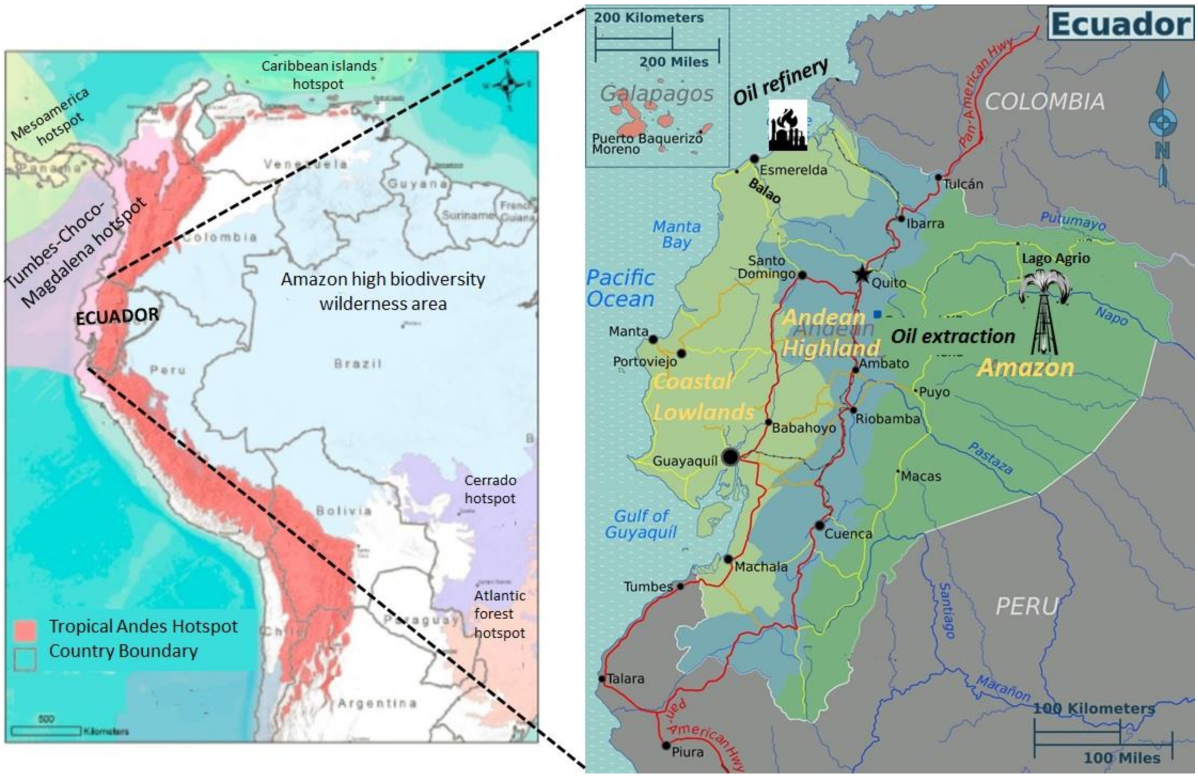

Figure I. South America biodiversity hotspots (Modified from: NatureServe and EcoDecisión, 2015) and Ecuador Natural Regions and main cities (Modified from: PCL, 2017).

The main threat to biodiversity conservation in Ecuador is deforestation (Mena, 2008; Messina et al., 2006; Sierra, 2000). Firewood collection, urban expansion, petroleum exploration and exploitation, agriculture, mining, poverty, human migrations and tourism development are other important aspects contributing to the deterioration of the country's biological richness (CBD, 2017).

It was hardly the beginning of the 20th century when the first well was drilled in the peninsula of Santa Elena to confirm the existence of crude oil in Ecuador. Fifty years later, in 1967, the first well was drilled in the Amazon region, known as the "Oriente," by the Texaco Gulf Oil Company (EP Petroecuador, 2013). On June 1972 (Figure II), the Ecuadorian Oil State Corporation (later called EP Petroecuador) transported an oil barrel for the first time through the Trans-Ecuadorian Oil Pipeline, from Lago Agrio, in the Amazon region, to Puerto Balao, in the Pacific Coast (Figure I). After 4 decades, since Ecuador became an oil exporting country, petroleum is still one of the main sources of economic income and is a strategic sector for its development.

21 Páginas
2 Secciones
6529 EL COMERCIO
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DIARIO INDEPENDIENTE

Gobierno contratará estudio sobre la reserva petrolera

Se inició la explotación petrolera

102 perforaciones totalizan más de un millón de pies

No podemos esperar todo del petróleo

2 graves problemas petrolíferos estudia desde ayer la OPEP

Delegado del Consorcio destacó que Ecuador será en breve segundo país exportador en Latinoamérica

Es un recurso fundamental, pero no el único, dijo Presidente de la República, en el acto de ayer

Nueva estructuración del Gobierno anuncia

Figure II. Front page of "El Comercio" journal (June 27th, 1972) announcing: the interest of the government on determine the crude oil reserves of the country (pink border), the beginning of oil exploitation (violet border), and the most attractive highlight ***"we cannot expect all from petroleum"***. (Source: El Comercio. June 26th, 2012)

Over almost 28 years, Texaco drilled 339 wells, built 18 production stations, but also released 19.3 billion gallons of oil brine into the environment and abandoned between 800 and 1000 unlined earthen waste pits (Buccina et al., 2013). Furthermore, it has been estimated that environmental damage in Ecuador during Texaco operations was 30 times greater than the oil spill caused by Exxon Valdez in Alaska in 1986 (Almeida and Proaño, 2008). More globally, oil history is not only tarnished with violence and interventionism, but also with social and environmental conflicts (Figure III), due to the fact that this valuable resource is not found uniformly across the world (Arora and Gupta, 2013). In Ecuador, 20% of the proven crude oil reserves (846 million barrels) are located in the Ishpingo-Tambococha-Tiputini (ITT) block in the Yasuní National Park, a protected area of around 2000 km^2 in the Amazon region which also houses indigenous communities, a small part of them living in isolation (Vallejo et al., 2015).

Ecuador has three commercial oil refineries: La Libertad, Shushufindi (both in the Oriente) and Esmeraldas (on the Pacific Coast) with a total capacity of about 176 000 barrels per day (EIA, 2015). Recently, the Esmeraldas National Refinery has been upgraded to reach its full capacity and to process heavy oils. The Trans-Ecuadorian oil pipeline system, or "SOTE" (Figure IV), transports 306 000 barrels per day over a total distance of 497 km (Ministry of Hydrocarbons of Ecuador, 2016). According to the

"El Comercio" (2013) journal, since 1972, a total of 725 655 barrels (from 72 pipeline ruptures) have been spilled since the inauguration of the SOTE.

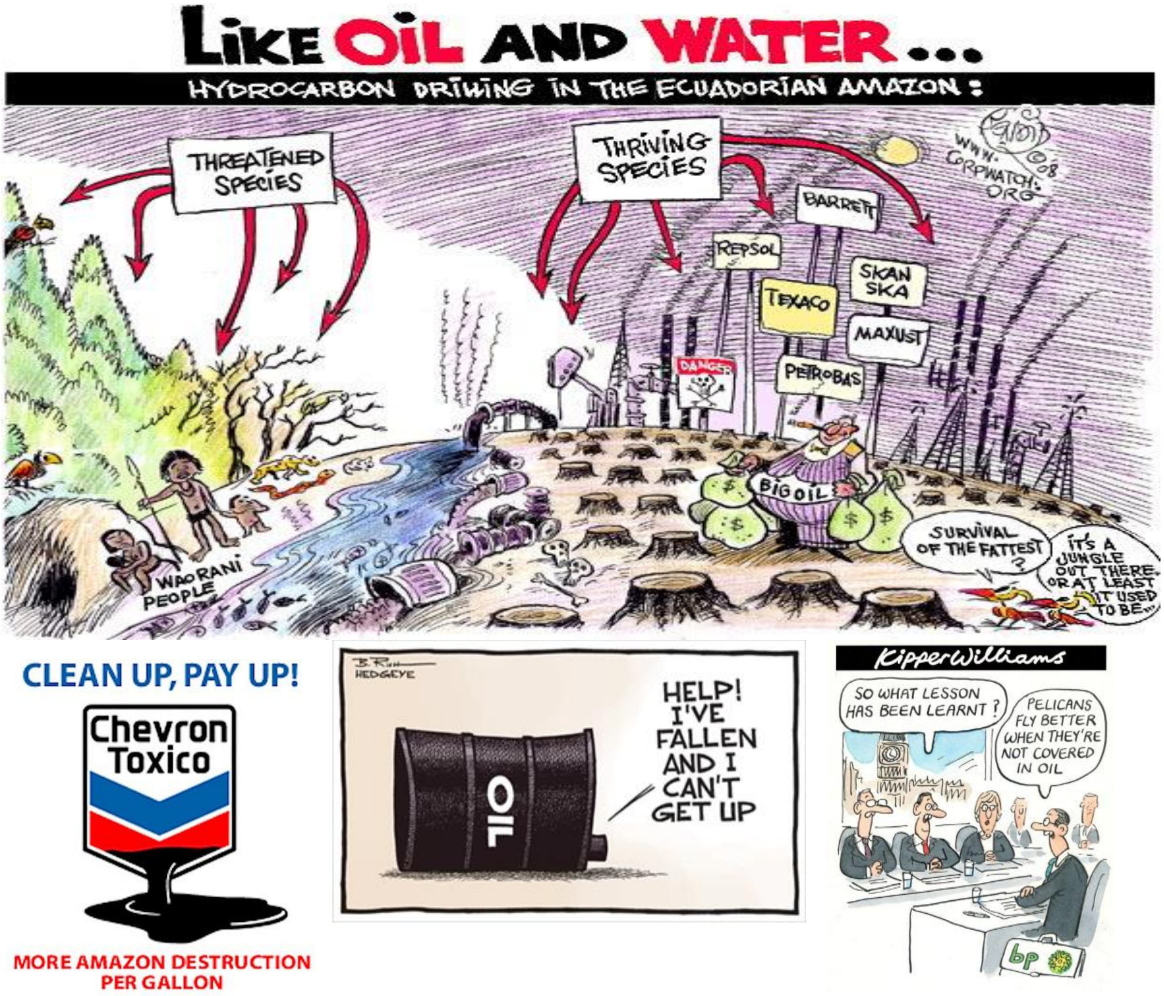

Figure III. Cartoons making allusion to (starting from the top, left to right): social and environmental conflicts in the Ecuadorian Amazon (by Khalil Bendib, 2008), Ecuador trial against Chevron-Texaco (by Laura Beans, in: www.ecowatch.com, 2014), oil price crisis (by B.R.Hedgeye, 2015) and one of the biggest environmental disasters of the decade, the marine oil spill in the Gulf of Mexico by the British Petroleum (BP) company in 2010 (by Kipper Williams, 2010).

As reported by Alaa El-Din et al. (2017) and Chaudhuri (2011), crude oil is a mixture of a wide variety of constituents: hydrocarbons, Volatile Organic Compounds (VOCs: benzene, toluene, ethylene, xylene usually called “BTEX” components), sulfur, nitrogen, oxygen, organometallic compounds (Ni, V, As, Pb) and other trace metals (i.e., Cr, Cd, Hg, Mn, Fe). Information about petroleum formation and production are available in Section A.1, Appendix A.

Oil spills, oil leaks, gas open flares and formation waters with drilling muds deposited in open pits have released into the Ecuadorian Amazon environment a mixture of toxic compounds resulting in the massive contamination of air, water and soils with hydrocarbons and trace metals. Besides, food crops cultivated in the vicinity of oil platforms may also be impacted by these compounds (San Sebastián and Hurtig, 2004).

Figure IV. Oil transport and storage system in Ecuador. **A.** Trajectory of the Trans-Ecuadorian oil pipeline system, from the North Amazon region (NAR) to the North Pacific Coast (NPC). **B.** Polyducts, fuel terminals, tanks and marine terminals. (Modified from: EP Petroecuador, 2016)

Regarding the human health risk assessment of the local population living in oil exploitation and refining areas, exposure to metals and organic compounds may take place by inhalation, ingestion and skin penetration. Inhalation is usually the most important occupational exposure route while ingestion of contaminants via food and drinking water is ordinarily the main pathway of exposure for the overall population (Elert et al., 2011). Despite the fact that dermal uptake of inorganic metallic compounds is usually low, for organometallic compounds it can be high and cause substantial, sometimes lethal, doses (Nordberg et al., 2015). Acute contact with crude oil via inhalation and skin transfer can cause transitory respiratory, vomiting, diarrhea and skin reactions. However, long-term exposure, which can be a matter of months or years, can cause central nervous system problems or damage organs such as the kidneys or liver. There is also a significant increase in the risk of cancer (Ordinioha and Brisibe, 2013). The petroleum low-weight aromatic hydrocarbons, also known as BTEX, can express mutagenic, carcinogenic, embryotoxic, teratogenic, growth retarding, metabolic and neuromodulatory effects (Sirotkin and Harrath, 2017). Polycyclic Aromatic Hydrocarbons (PAHs) and their derivatives (nitro-PAHs and oxy-PAHs) are classified as possible, probable and known carcinogens for humans (Bandowe and Meusel, 2017) by the Agency for Toxic Substances and Disease Registry (ATSDR, 1995).

Even if some metals are required for human life (essential metals) because of their biological function, exposure to other metal(loid)s—such as As, Co, Cd, Cr, Ni and Pb, even at low levels (trace elements)—can induce damage to multiple organs (Karri et al., 2016) due to the oxidative stress induced by the formation of free radical compounds (Jaishankar et al., 2014). Adverse human effects from long-term exposure to metal(loid)s include neurotoxic effects, cardiovascular diseases, kidney effects (on renal tubular function), carcinogenesis (lung cancer, prostatic cancer) and reproductive effects (Nordberg et al., 2015). The main target organs for non-essential trace metals are shown in Figure V. Detailed information about toxicity is given in Table B.1, Appendix B.

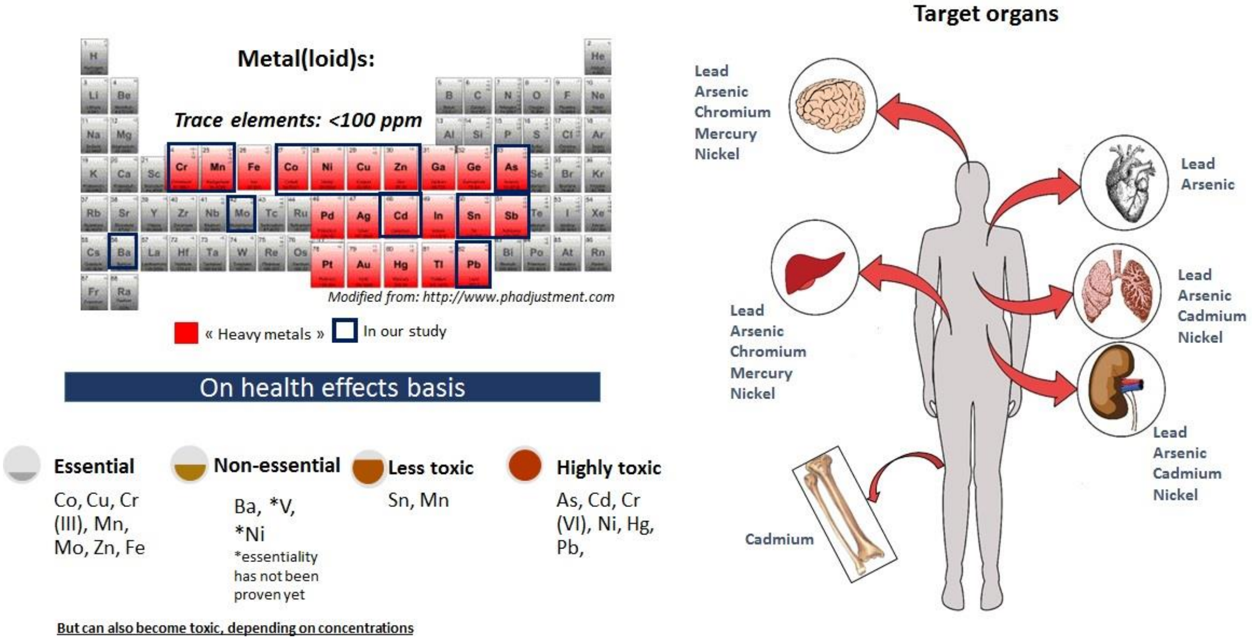

Figure V. Metal(loid)s importance and toxicity for humans (Modified from: IGEM, 2015 and completed by information given by Cobbina et al., 2015; Exley, 2016; Fraga, 2005; Goyer, 2004; Lim et al., 2016).

Previous studies of long-term human exposure to oil contamination has been conducted in communities and villages in the Ecuadorian Amazon region. According to San Sebastián et al. (2001a), inhabitants living close to oil infrastructures (i.e., oil platforms and pits) developed more diseases and illnesses, such as anemia, malnutrition, skin infections, gastritis, eye irritation, headaches and miscarriages, than non-exposed communities. These results were based on the determination of Total Polycyclic Hydrocarbons (TPHs) in water samples and on a health survey made in the area. From a total of 20 rivers, 18 had concentrations 10 times higher the permissible levels established by international legislations. Besides, cancer incidence increased in the village of San Carlos, surrounded by more than 30 oil wells and 4 powerful gas open flares (San Sebastián et al., 2001b).

Paz-y-Miño et al. (2008) conducted different tests on blood samples of oil workers exposed to hydrocarbons and local people living in the village of San Carlos and compared them to a control population. Their results showed that exposed individuals presented a greater amount of damage both at the DNA and chromosomal levels. These findings indicate that air inhalation and water consumption should be considered as important exposure pathways to contaminants emitted by the oil industry. However, till the beginning of the MONOIL (Environmental Monitoring, Health, Society and Oil in Ecuador) Program, no data was available about water, air, soils or crop quality in the Oriente (Amazon region), nor in the Pacific coast and especially not near the National Refinery in Esmeraldas. On the other hand, the health impacts of metal(loid)s naturally found in crude oil have not been studied yet.

The lack of reliable and available information about environmental and sanitary impacts has been used as an argument by Texaco to avoid all responsibility until 1995 when they accepted 37.5% of the damage and agreed to cleanup. A few years earlier, in 1993, about 30,000 indigenous and local farmers filed a lawsuit against Texaco, claiming that the oil company had caused irreparable damage to the rain forest (Buccina et al., 2013). After more than 20 years of international trials in the United States, Ecuador and The Hague, the 9-billion-dollar legal battle against Chevron-Texaco is still ongoing.

The uncontrolled development of oil activities in Ecuador for three decades has also caused social impacts such as regular conflicts, cultural losses, destruction of natural resources used daily by local populations, violation of indigenous rights and ancestral territories and loss of social cohesion (Becerra et al., 2013). A total of nine indigenous nationalities exist in the Ecuadorian Amazon region: the Achuar, A'I Cofán, Huaorani, Kichwa, Secoya, Shiwiar, Shuar, Siona and Zápara (SIDENPE, 2005). Before the 1970’s, most of these indigenous communities experienced some contact with the outside world but lived in relative isolation on lands almost entirely covered by forests. As a result of oil development, immigration from the Andes, agricultural colonization and increasing urbanization, indigenous peoples have largely been displaced to the periphery of the oil exploitation area (Bozigar et al., 2016).

In terms of natural resources, the richest areas coincide with the economically poorest and least developed regions. As highlighted by Becerra et al. (2013), the subsistence of the inhabitants of oil areas (farmers and indigenous people) depends not only on the conservation of natural resources but also on the financial compensation or jobs that can be obtained after an oil spill or during the installation of oil infrastructure on their land.

In the legal plan, non-industrialized nations like Ecuador are less likely to safeguard environmental and social rights because of a lack of norms and standards. To cite briefly, Ecuadorian legislation about air quality only regulates Cd and benzene concentrations, whereas the European Union considers in addition As, Ni, Pb and BaP (benzo-a-pyrene). Hydrocarbon thresholds are quite confusing. In soils and water, they consider the "TPH," Total Petroleum Hydrocarbons (without an exact definition of which compounds are included), and PAHs, but the list makes reference to 12 compounds instead of the 16 proposed by the US-EPA. (Cancer Environnement, 2016; TULSMA, 2015). The Ecuadorian government published two environmental legislations, TULSMA (Unified Text of Secondary Environmental Legislation) and RAHOE (Environmental Regulation of Hydrocarbon Activities), the latest one and the most permissible dedicated only to oil activities impacts.

The amount of TPH found in a sample is useful as a general indicator of petroleum contamination at one site. However, managing ecological and human health risks requires an understanding of the toxicology of each environmental contaminant and the combined effects on the respective compartment (ATSDR, 1999; Pinedo et al., 2013). Faced with this situation, the French "Institut de Recherche pour le Développement" (IRD) in collaboration with French and Ecuadorian partners launched the MONOIL program: "Monitoring of oil activities in Ecuador: a cross-disciplinary approach between Environment, Health and People" funded by the French National Research Agency (ANR, 2014-2018). The main objective of this cross-disciplinary and ecosystem-focused approach is the improvement of the understanding, monitoring, reduction and prevention of oil contamination and its impacts on the environment and the local people (MONOIL, 2017). The study area includes the villages of Dayuma (province of Orellana) and Pacayacu (province of Sucumbíos) in the North Amazon region and the city of Esmeraldas (surroundings of the oil refinery) on the North Pacific Coast.

Although the MONOIL program was initially focused on the impact of PAHs and heavy metals on water (and the trophic chain, i.e., fish and mollusks) and air, the existence of small-scale farms (or "fincas") in the vicinity of oil facilities has aroused interest in the study of the soil-plant continuum and its possible impact on human health through crop ingestion. Indeed, in a preliminary study within the MONOIL program, López (2014) showed that soils collected within the area impacted by oil extraction had concentrations of V, Cr, Co, Cu, Zn and Ba above the limits established by the Ecuadorian legislation. In addition, cacao pods from the same area showed Cd concentrations ranging from 2.2 to 5.3 mg kg^{-1}, between three and six times higher than the European directive on Cd in cacao-based products (dark chocolate). According to Chachane et al. (2015) and Grujić et al. (2004), crude oil and wastes generated from extraction, production and refining sites may have elevated levels of Cd. Besides, cacao and cacao-based products, such as cocoa liquor and cocoa butter, have been ranked fourth in the category of non-oil exports of Ecuador in January-April 2017. (ProEcuador, 2017).

In terms of environmental monitoring and in order to compare impacted compartments (i.e., air, water, soil) to quality standards, we first determine the total concentration of each contaminant. However, for human health exposure and risk assessment purposes we should also consider the bioaccessible fraction of the contaminants in specific matrices. Bioaccessibility of an element is defined as the fraction which is released from the food during digestion or by contact with lung fluids after inhalation (ambient particulate matter, PM) that can be absorbed (Carbonell-Capella et al., 2014; Guney et al., 2016) and probably reach the systemic circulation. The bioaccessible content is always equal to or higher than the bioavailable content and, whenever considered as a proxy for the latter, it generates a worst case scenario regarding the contaminant (Cardoso et al., 2015).

Several acellular tests have been developed in the last years in order to determine this fraction. The principle is to use synthetic solutions to mimic the physiological processes (Mendoza et al., 2017; Wiseman, 2015). The advantages of these assays are the relatively low cost and the speed at which they are run in the lab. The MONOIL program proposes to use four of these assays: the BARGE (BioAccessibility Research Group of Europe) test for studying human gastric bioaccessibility of contaminants in soils and vegetables developed by Caboche (2009) and the DTT (dithiothreitol), AA (acid ascorbic) and DCFH (dichlorofluorescein) tests according to Cho et al. (2005) and Wang and Joseph (1999) to measure the oxidative potential of PM, which means the generation of reactive oxygen species (ROS) directly correlated with lung inflammation and respiratory diseases.

Thus, as a part of the MONOIL program, the global aims of this study were to:

1. Assess the levels of contamination of metal(loid)s in soils, crops and air and the associated human health risks (depending on the exposure pathway) in three study areas impacted by oil activities: Orellana and Sucumbíos provinces, the North Amazon Region, and Esmeraldas city on the North Pacific Coast.

2. Determine if Cd, a non-essential and toxic metal, is easily taken up by cacao crops growing in oil contaminated sites and if it also implies health risks by the ingestion of cacao-based products.

3. Estimate if the cocktail of metal(loid)s and PAHs in the air (PM_{10} fraction) induce the formation of ROS in the lungs.

Specific aims for each chapter are briefly presented in Figure VI and listed below:

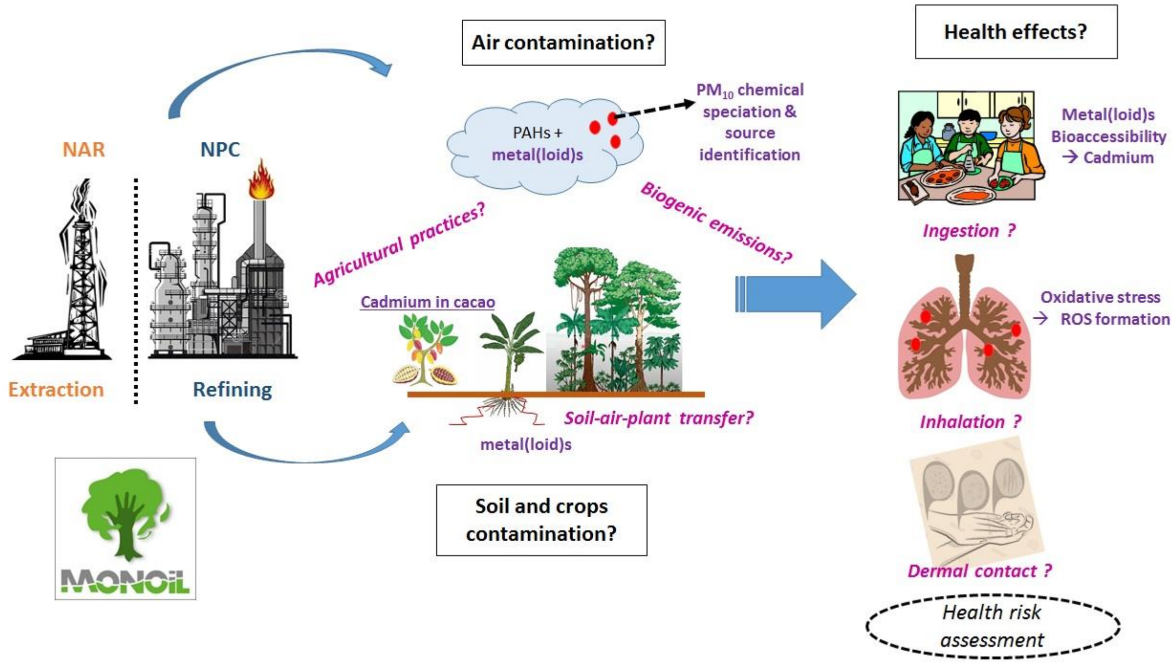

Figure VI. Graphical abstract of the main and secondary aims of this study. NAR: North Amazon Region, NPC: North Pacific Coast, PAHs: Polycyclic aromatic hydrocarbons, PM_{10}: particulate matter <10µm, ROS: reactive oxygen species.

- Identify other possible natural and anthropogenic sources of metal(loid)s within the oil production areas.
- Determine which are the main exposure pathways to metal(loid)s found in crops, air and soil samples.
- Estimate the bioaccessible fraction of Cd found in beans and cocoa-based products during digestion (gastric phase).
- Compare PM_{10} chemical composition from oil extraction and oil refining emissions

The present manuscript is organized in three chapters in scientific publication format. Additional information about key definitions or unpublished data is included at the beginning and at the end of each chapter. The Materials and Methods sections are also described according to each publication. General supplementary information is included in the Appendix.

Chapter 1 is an ***Accepted*** article entitled «Distribution, contents and health risk assessment of metal(loid)s in small-scale farms in the Ecuadorian Amazon: An insight into the impacts of oil activities.» In this chapter, drinking water, local crops, soil and air concentrations (PM_{10} fraction) were compared to Ecuadorian and international thresholds for metal(loid)s. A health risk assessment considers two population categories (adults and children) and three exposure pathways (ingestion, inhalation and dermal contact). Data from Esmeraldas are shown at the end of this first chapter.

Chapter 2. One of the non-essential metals found in higher concentrations in crops, such as cacao, is Cd, but oil activities are not the main source. The ***published*** article, entitled «Cadmium bioaccumulation and gastric bioaccessibility in cacao: A field study in areas impacted by oil activities in Ecuador» is a first approach in which the percentage of Cd contained in cacao beans (and cacao-based products) can be potentially absorbed by the intestinal epithelium, reach the circulatory system and be dangerous for consumers. Concentrations of other metal(loid)s above the detection limit in cacao and cocoa liquor samples are reported and the end of this section and compared to available data.

Chapter 3 is especially dedicated to the air compartment. In this chapter, presented as an ***In prep*** article entitled «Chemical composition of PM_{10} and ROS generation in the vicinity of gas flares in oil extraction and refining areas in Ecuador,» not only metal(loid) but also PAH concentrations are shown. Aerosol chemical speciation will determine if ROS production in lung fluids (after being in contact with PM) is specific for a group of compounds and their emission source. It also shows that biogenic emissions, such as biomass burning and microorganisms living in the soil, are an important source of PM in the Amazon region that somehow may hide the emissions from oil activities.

Finally, **General Conclusions** and **Perspectives** are presented at the end of the manuscript.

Chapter 1. Distribution of metal(loid)s in oil areas and human exposure associated with petroleum activities

Foreword

What does an area impacted by oil activities look like? Figure VII illustrates some oil infrastructure located in the North Ecuadorian Amazon Region (NAR) and its environmental impacts. The pictures were taken close to the districts of Dayuma (Orellana province) and Pacayacu (Sucumbíos province). Farms are usually located next to the oil platforms. Some of the inhabitants arrived at the beginning of the extraction operations, some of them later.

Figure VII. North Ecuadorian Amazon Region. **A.** Gas open flare. **B.** Reinjection well. **C.** Pineapples growing close to an abandoned oil waste pool. **D.** Abandoned oil pool. **E.** Crude oil emerging from a remediated oil waste pool. (Pictures taken by: Fiorella Barraza, Fausto Lopez and Alexandra Ter Halle, MONOIL field work).

The reasons why people live under these conditions have been briefly explained in the general Introduction. Two important events have marked the migration to the Amazon region: the land reform (1964, 1973) followed by the discovery of oil reserves (1967) (Holland et al., 2014; Lu et al., 2010; San

Sebastián and Córdoba, 1999). As a consequence, farmers form the highland and coastal Ecuador colonized the traditional homelands of Amerindian populations, looking for productive lands.

Most agricultural production has remained small-scale, with an emphasis on a mix of subsistence food crops, livestock for domestic consumption and some cash crops such as coffee and cacao. (Becerra et al., 2013; Welford and Yarbrough, 2015). However, regarding the environmental impacts, other questions arise: which compartments concentrate the highest levels of metal(loid)s? Do these metals come only from oil production activities? Is the terrestrial environment the most contaminated? In this case, what is the risk of consuming fruits and vegetables cultivated on these sites?

It is a well-known fact that oil spills have been of major concern and are regarded as one of the most critical forms of pollution of water bodies, causing serious impacts to wildlife and their habitats. But what about the terrestrial environment?

The mud resulting from drilling activity has harmful effects on soil properties due to its high content of heavy metals, additives and salts (Samuel et al., 2016). These contaminants reduce aeration by blocking air spaces between soil particles, creating favourable conditions for anaerobiosis, reducing plant growth rates, nutrient availability (especially N and P) and photosynthetic rate and inhibiting crop germination (Tanee and Albert, 2015). Although indigenous microorganisms in the soil can degrade a wide range of chemical compounds, their population and activity are generally directly affected by toxic molecules (Adesina and Adelasoye, 2014; Xu and Lu, 2010). Moreover, the uptake of metal(loid)s by plants and its accumulation in the food chain is a serious threat to both animal and human health.

To answer some of the questions listed above, we have to bear in mind that the amount of a toxic compound found in a specific compartment doesn’t give us information about the possible health effects. At the same time, the greater the amount of a substance a person is exposed to, the more likely that health effects will occur. However, physiological reactions are not the same in each exposed individual. Duration of exposure is a key factor that affects a person’s sensitivity to a chemical compound. Other factors to consider are age, gender, diet and illness. For these reasons, we have proposed a health risk assessment (HRA) calculated for people living in the Oil Amazon region of Ecuador over several decades.

According to the United States Environmental Protection Agency (U.S. EPA, 2016), HRA is, “the process to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media, now or in the future.” HRA includes four basic steps shown in Figure VIII.

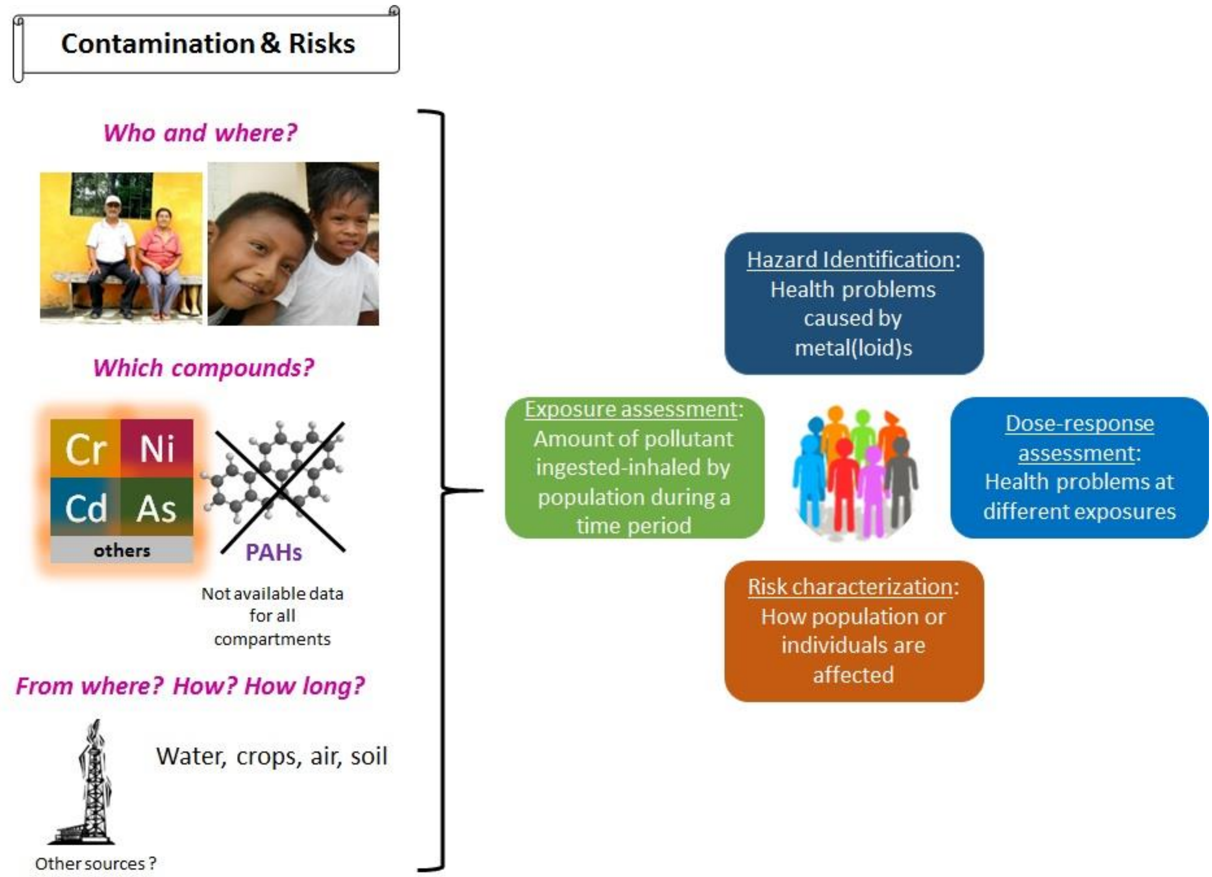

Figure VIII. Planning of HRA in areas impacted by oil activities and the four basic steps. (Adapted from: U.S EPA, 2016).

Human exposure of small-scale farmers living in the NAR can result from direct contact with the soil and water or after transfer, such as consumption of fruit from trees growing on impacted soils. The effects on health can vary from imperceptible symptoms (skin reaction, headaches) to chronic diseases such as cancer and even death (Swartjes and Cornelis, 2011).

The HRA described below takes into account two population groups (adults and children) and a cocktail of metal(loid)s originating from oil production that are present in water, crops, soils and air, that enter the body by ingestion, inhalation and dermal contact and that probably have non-carcinogenic or carcinogenic effects on health. The complete HRA data set is available in Appendix C. The results for Esmeraldas (North Pacific Coast) are presented separately after the following accepted article in Science of the Total Environment journal.

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I. Distribution, contents and health risk assessment of metal(loid)s in small-scale farms in the Ecuadorian Amazon: an insight into impacts of oil activities

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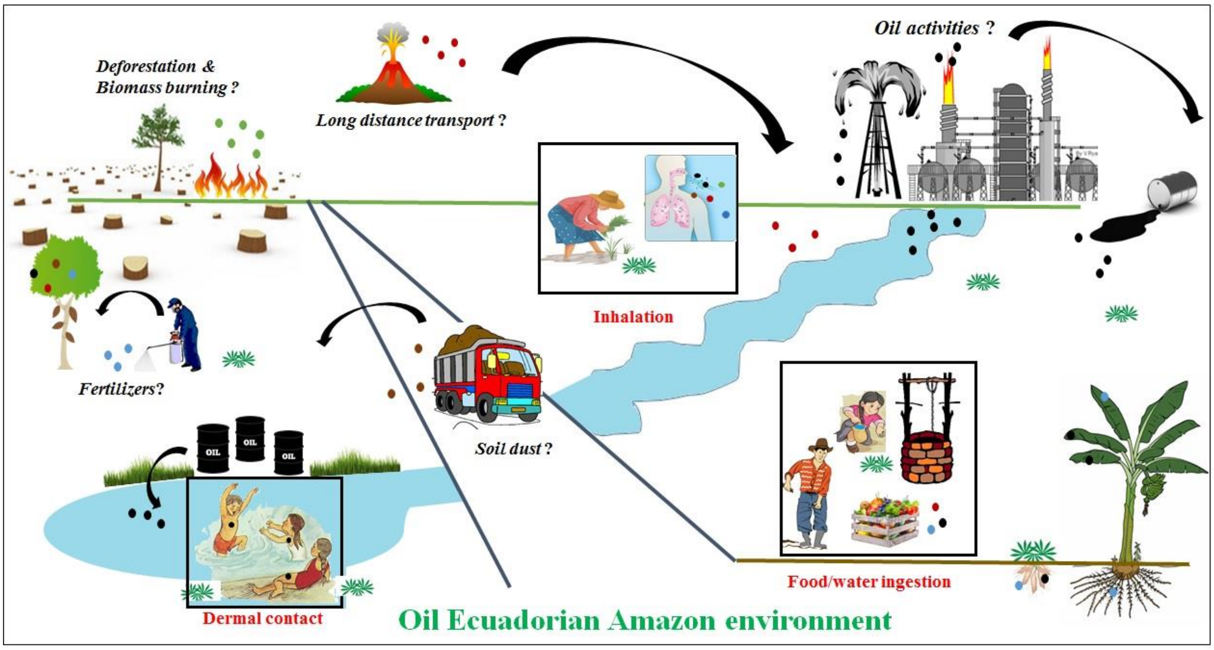
Keywords: metal(loid)s; oil activities; human exposure; health risk; Ecuadorian Amazon

Graphical abstract:



Abstract:

In the last 50 years, oil extraction activities in the Northeast Amazonian Region (NAR) of Ecuador impacted ecosystems and may still affect the local population health. To our knowledge, no previous studies have determined the concentrations of metal(loid)s in the oil Ecuadorian Amazon environment. A total of 15 small farms, located in the Orellana and Sucumbíos provinces, were sampled in order to determine the concentrations of As, Ba, Co, Cu, Cd, Cr, Mn, Mo, Ni, Pb, V and Zn in soils, crops, drinking water and air (PM_{10}). Among non-essential metal(loid)s, Ba concentrations in soils exceeded the Ecuadorian limits of 200 mg kg^{-1} in 53 % of the sampling sites. In crops, Cd concentrations in cacao and Pb in manioc exceeded the FAO/WHO recommendations. In drinking water and PM_{10}, regulated metal(loid)s did not exceed the international thresholds. Nevertheless metals such as Ba and Mo showed the highest annual mean concentrations in PM_{10} in both sampling sites. Natural (bedrock, volcanic ashes) and anthropogenic (oil activities, agrochemical products) sources could explain the high content of some meta(loid)s in the environment. According to the hazard quotient and cancer risk indexes, crops and water ingestion represent 71% and 88% of the exposure pathways for non-carcinogenic elements in adults and children respectively while inhalation is the main exposure pathways for carcinogenic elements for the whole population. Both indexes were 2 to 13 times higher than the US EPA recommended values. However, estimates of exposure pathways should be considered taking into account the risk perception of residents: they may be overestimated for people who are able to change their dietary and/or agricultural practices to limit their exposure, or

underestimated in the case of persons who are socio-economically vulnerable and who cannot leave the impacted areas.

1. Introduction

Crude oil, despite being a non-renewable energy source, is a key strategic resource for the development of countries (E et al., 2017) especially in Latin America where the economy and government's budget are mainly based on the exploitation of raw materials (Arsel et al., 2016). In South America, Ecuador has the 3rd largest oil reserves as well as being ranked as the 4th largest oil producing country (equivalent to 549 thousand barrels per day), according to the Organization of the Petroleum Exporting Countries (OPEC, 2016). Most of the extractive activities have been developed in the Northeast Amazonian region (NAR), known as the "Oriente", for more than 50 years (Lessmann et al., 2016).

The Amazon basin also houses millions of hectares of tropical rainforest and near to 500 000 inhabitants including eight groups of indigenous people (30 % of the total regional population) and rural population from Ecuador's coastal and highland regions (Lu et al., 2010; San Sebastián and Hurtig, 2004). Concerns have been raised over the environmental issues, specifically impacting on the Amazon biodiversity (Arellano et al., 2017) from burning large volumes of gas and whether reserves and conventional oil production have the capacity to meet the growing demands (Owen et al., 2010). For example, the clearing of lowlands forests for agricultural crops and by the opening of roads due to oil extraction and forest exploitation (Mosandl et al., 2008) has decreased prime Amazonian forest by approximately 44% (Welford and Yarbrough, 2015).

Crude oil is essentially formed by a complex mixture of hydrocarbons, sulfur, nitrogen, oxygen , organo-metallic compounds of Ni, V, and others trace metals (Speight and Özüm, 2002; Zhuang et al., 2016). Inorganic salts such as magnesium chloride ($MgCl2$), sodium chloride (NaCl) and other mineral compounds are also present naturally, in formation waters (Moquet et al., 2014), or added as a part of chemicals injected during drilling and production operations (i.e. weighting agents like Barite, $BaSO4$), as reported by Oskarsson (2015) and Speight and Özüm (2002). Outdated practices and technologies used during oil production in Ecuador, initiated by Texaco-Chevron, have released into the environment an estimated of 19.3 billion gallons of oil and left between 800 and 1000 unlined earthen waste pits (Buccina et al., 2013).

Polycyclic Aromatic Hydrocarbons (PAHs), Volatile Organic Compounds (VOCs), metals and metalloids are the main contaminants generated by oil production or refining activities (Baltrenas et al., 2011; Sarma et al., 2016) . The intentional or accidental release of such contaminants have resulted in a long-term pollution of air, soil, plants, rivers, streams and groundwater (Finer et al., 2008).

Some metals such as Co, Cr (III), Cu, Mn, Mo and Zn (Cobbina et al., 2015) are considered as essential compounds for humans at low concentrations because they are usually involved in metabolic functions. However, an excess may lead to adverse health effects (Crichton, 2016). Other elements like As, Ba, Cd, Pb and Sb are toxic and have no beneficial effects. Nickel and V seem to have biological functions in plants and some animals, but their importance in humans is yet to be shown (Kabata-Pendias and Szteke, 2015). According to the International Agency for Research on Cancer (IARC), As, Cd, Cr hexavalent compounds and Ni are classified as carcinogenic, whereas Co, Pb and Sb trioxide are considered as possibly carcinogenic (Mulware, 2013). Diseases such as skin and lung lesions, bladder, hepatic or renal cancers, damage of the central and peripheral nervous systems and adverse effects on fetuses and infants have been described in populations exposed to these toxic metals (Masto et al., 2017; Nordberg and Nordberg, 2016).

The three main exposure pathways to contaminants are: water and crops ingestion, inhalation of particulate matter (PM) and dermal contact (Huang et al., 2016; Swartjes and Cornelis, 2011).

As a general pattern, once the contaminant is transferred from a mobile phase (i.e. pore water soil) into a contact medium (i.e. fruits), it enters the body through an intake pathway; one part can be carried by the blood stream to target organs, and finally the remaining part is excreted. For dermal contact, direct intake can pass through the skin (e.g. shower with contaminated water) (Elert et al., 2011). The consumption of vegetables and fruits is one of the main exposure routes to metal(loid)s (McLaughlin et al., 2011) especially for people who do not frequently eat marine or freshwater fish. Contaminant concentrations in the edible parts of crops are the result of their capacity to uptake (by roots or leaves), transport, accumulate and even degrade bioavailable metal species (Elert et al., 2011). Atmospheric particulate matter (PM), depending on their diameter and emission sources, also carry an important source of metal(loid)s that can be absorbed into the lung tissues while breathing (Jena and Singh, 2017; Mousavian et al., 2017). While dermal contact usually accounts for a small percentage within the whole risk assessment evaluation (WHO, 2014), this pathway is relevant under certain conditions, especially for young children in the NAR, where swimming and bathing in contaminated waters or playing outside are common activities. Soil ingestion is also considered as a significant exposure pathway towards children due to specific age-related activities such as the hand-to-mouth behavior (Tepanosyan et al., 2017).

As recommended by the Agency for Toxic Substances and Disease Registry (ATSDR, 2005), the evaluation of these exposure pathways must consider past, current, and future exposure conditions.

The potential or proven impact of oil activities on the environment and on human health is still poorly documented in Ecuador. A few studies suggest the presence of cancer, birth defects, miscarriages, and other diseases in the local population (Maddela et al., 2016; San Sebastián et al., 2002). San Sebastián et al. (2001) previously showed that women living close to oil wells and platforms in the Orellana Province, in Sachas and Shushufindi villages (NAR), regularly suffered from fungal skin infections, nose, eye and throat irritations, fatigue, headaches, earaches, diarrhea or gastritis. These results were based on the correlation between water samples collected in the main rivers of the area, with levels of Total Petroleum Hydrocarbons (TPH) above Ecuadorian thresholds, and epidemiological studies.

In Ecuador, the environmental policy which characterizes a populations' vulnerability to oil contamination is based on the distance of the source (i.e. oil pools, spills or pits), the residence time in the contaminated area, the density of oil infrastructures and the potential impacts of oil activities in the water resources (MAE-PRAS, 2010). In accordance with these policies, a large national program dedicated to repair social and environmental impacts was established in the NAR. In the absence of remediation, families were moved into social housing and kept away from contaminated sources (Becerra et al., 2016).

However, there is a lack of information regarding metal(loid)s co-emitted with PAHs in the other environmental compartments: the soil-crop continuum and the air (PM), as well as their harmful effects on NAR population. Most of the families living in the "Oriente" region consume a large amount of the products they cultivate in their own small-scale farms, usually located in the proximity of oil facilities. At each facility, over 16.3 million liters of liquid wastes are generated every day and discharged into pits. Roughly 1.5 million cubic meters of "waste" gas from the separation process is burned daily with very basic temperature or emissions controls (San Sebastián and Hurtig, 2004).

Thus, as a part of the transdisciplinary MONOIL research program about environmental monitoring, health, society and oil in Ecuador (MONOIL, 2017), this field study aims to determine for the first time, the distribution and the level of contamination by metals and metalloids in the crops, soils, water and air (PM10 fraction), in the oil Ecuadorian Amazon provinces. In addition, we propose a health risk assessment using the multicomponent model developed by the United States Environmental Protection Agency (US EPA), considering different routes of metal(loid) absorption (ingestion, inhalation and dermal contact). Hazard quotient (HQ), hazard index (HI), and total cancer risk (TCR) were calculated for adults and children and were compared to current standards and regulations. Finally, a social approach integrating the "risk culture" and thoughts about local politics, people vulnerability and capacities were discussed and proposed for the future to better assess the health risk due to environmental contamination by anthropogenic activities.

2. Materials and Methods

2.1 Study area

The sampling sites were selected within the areas influenced by oil activities as described by the Ecuadorian Ministry of Environment (MAE-PRAS, 2010). According to the Multidimensional Subsystem of Socio-Environmental Statistics of Productive Activities in Ecuador, this area is associated with inadequate environmental practices such as oil spills, construction of oil pools without technical design, dumping of formation waters and gas burning in open flares (SIESAP, 2015). A total of 181 open gas flares are located within this area (see Fig SI-1, Supplementary Information).

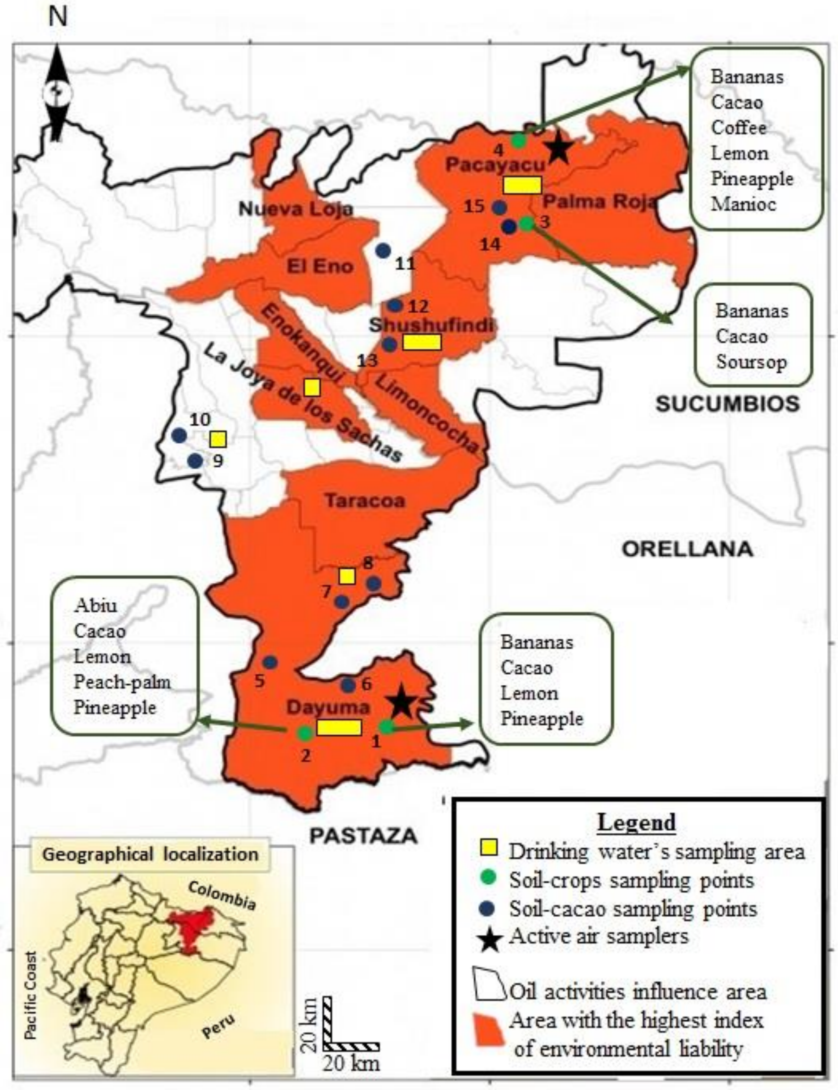


Fig.1. Sampling sites localization in the Northeast Amazon Region (NAR) of Ecuador. Sampling points are presented as green (for soils and crops sampling) or dark blue (for soil and cacao tree sampling) and associated to a code number. Water samples are indicated by yellow rectangles. Active air samplers' localization is depicted by black stars. The black border area represents the zone impacted by the oil production activities and the red area covers the highest index of environmental liability region (oil infrastructures, contamination sources, social and environmental vulnerability) according to the Ecuadorian Ministry of the Environment (SIPAS, 2012).

Soils and crop samples were collected in December 2014 and on February 2016 (only cacao) from 15 small-scale farms located in the Ecuadorian provinces of Orellana and Sucumbíos, in the Northeast Amazon Region, NAR (Fig.1). These small-scale farms were chosen according to their vicinity to oil activities, their accessibility and the statements reported by the farmers about the supposed contamination. The experimental design of sample collection was determined in order to propose a comprehensive characterization of the contamination distribution, taking into account all these field parameters.

Water samples were collected in the same area, during 5 field campaigns between 2012 and 2016. Air samples were collected over one year, from December 2014 until December 2015, in the vicinity of the Auca Sur (S 0° 42’ 16.49’’, W 76° 53’ 15.96’’, village of Dayuma, Orellana) and Shuara 9 (N 0° 3’ 33.26’’, W 76° 33’ 37.01’’, village of Pacayacu, Sucumbios) oil platforms.

Precipitation in the NAR is evenly spread throughout the year, being less pronounced from December to February. Total annual precipitation in this area can reach more than 3000 mm, ranging between 200 to 500 mm per month. The annual mean temperature is around 25°C, varying from 13°C to 38°C (FAO, 2006; INAMHI, 2014; Pourrut, 1995). The predominant wind direction in the Orellana Province is NE (Northeast) whereas in Sucumbíos Province is SW (Southwest) (INAMHI, 2015).

2.2 Sampling and pre-treatment of crops, soils, waters and atmospheric aerosols

A large variety of crops produced locally, including abiu, banana, different cacao varieties, coffee, lemon, manioc, peach-palm, pineapple and soursop, were collected depending on their availability, in 4 small-scale farms in Dayuma and Pacayacu (represented by green points in Fig.1). For each type of crop, between 3 and 5 fruits, grains or roots were randomly selected (from different plants), together with the associated soils (to 20 cm of depth to focus on the soil layer directly colonized and influenced by the roots).

For the rest of the sampling sites (dark blue points Fig.1), only cacao and soil samples were collected based on the preliminary results obtained for the samples collected on 2014. These samples represented a part of a specific study focused on cacao trace elements contents and are described elsewhere (Barraza et al., 2017).

All crop samples were freeze-dried prior to and during transportation, prior to analysis. Peel and / or shells were separated and discarded from fruit samples before being lyophilized and ground with liquid nitrogen. Soil samples were air-dried under laminar flow hood for two weeks, sieved at 2 mm and ground with agate ring mills (Vibratory Disc Mill RS200®).

As the main objective of this study was to assess the overall range of contamination distributed in the different environmental compartments as a first approach, and not to perform robust statistical treatment, composite samples were collected. Crops samples from the same species and the same sampling point were ground together, whereas soil samples were mixed together during sampling Water samples were collected according to the EPA-1638 method (US EPA, 1996) in Teflon bottles previously decontaminated and acidified using bi-distilled HNO3. A total of 70 drinking water samples were collected in the whole Ecuadorian Amazon basin with a special focus on the Northern oil producting region (yellow squares, Fig.1), where 16 were from deep (groundwater) and 7 from shallow private wells, 9 from rainfall stored in plastic or concrete water tanks, 10 from small rivers, 18 from springs, 5 from streams, and 5 were from the local drinking water network. Air samples (PM10) were collected monthly on pre-calcined Millipore quartz fiber filters using low volume air samplers (3 L min^{-1}, two Microvol® devices in parallel on each site) located on the top of two houses located 300 m from the gas burning open flares.

All samples were pre-treated under clean conditions and collected to avoid direct contact.

2.3 Soil physico-chemical properties

Soil classification within the "Inceptisols" category were determined according to the United States Soil Taxonomy (USDA, 1999) and to the Soil Maps of the Ecuadorian Ministry of Agriculture, Livestock, Aquaculture and Fisheries (MAGAP and CGSIN, 2012), following the morpho-pedological characterization performed in the field. Soil pH (pHwater) was measured with a 744 Metrohm pH meter preparing a 1:5 dilution (w/v). Cation exchange capacity (CEC) was measured by spectrocolorimetry using cobalt hexamine trichloride (Fallavier et al., 1985) at 475 nm. Total organic carbon (TOC) was determined by dry-combustion using a HORIBA EMIA-320V carbon / sulfur analyzer.

2.4 Metals and metalloids concentrations analysis

For soil and plant samples, 0.1 and 0.2 g respectively were mineralized with a mixture of bi-distilled and suprapur acids (Aqua Regia solution and HF) and H_2O_2 (only for plants) using a Mars 5-CEM microwave. Aerosol samples on the quartz fiber filters were digested on a hotplate using an Aqua Regia solution, H_2O_2 and HF. Field blank filters were included on each batch of analysis in order to subtract the quartz matrix composition from the aerosols.

Mineralization solutions were then evaporated at 60°C; dry residues were re-suspended in acid nitric and to make up a final volume of 10 or 20 mL of bi-distilled water for aerosols and for plants or soil samples respectively.

For soil and plant solubilized samples as well as for the raw drinking water samples, total As, Ba, Co, Cu, Cd, Cr, Mn, Mo, Ni, Pb, V and Zn contents were measured by ICP-MS (Agilent 7500®) after being diluted with 0.37 N bi-distilled HNO_3.

Limits of detection (LD) and quantification (LQ) were calculated as 3 and 10 times the standard deviation (SD) of laboratory blank samples. Depending on the matrix, some of the metal(loid)s were under these limits (Table SI-1a, Supplementary Information).

Certified reference materials, SRM1515 “Apple leaves”, SRM 2709 “San Joaquin Soil” and SRM1648a “Urban Particulate Matter” were used to validate the mineralization protocols for plants, soils and air, respectively. Recoveries of 70-100 % were calculated for all the metal(loid)s mentioned above and the measured values are shown in Table SI-1b, in Supplementary Information

Composite samples (crops and soils) were analyzed in triplicate. Values are presented as mean ± Standard Deviation (SD) and expressed as annual mean concentrations for PM_{10}.

Finally, metal and metalloid concentrations in the different compartments (soil, crops edible parts, water and PM_{10}) were compared to the maximum levels established by international legislation and listed in Table SI-2 (in Supplementary Information).

2.5 Health Risk assessment

The potential for non-cancer and cancer risks were determined for the populations living in the study area to assess the human exposure to metals and metalloids listed below. The multicomponent model developed by the United States Environmental Protection Agency (US EPA, 2016) was used taking into account 3 exposure routes: ingestion, inhalation and dermal contact. All the equations, parameters and values employed in this study are described in Table 1. Fruits and vegetables ingestion rates were obtained using data from the Ecuadorian Health and Nutrition Survey of 2011-2013 (Freire et al., 2013) combined with information collected during the field work and Ecuador’s FAO statistics. Consumption values (g per person per day) were as follow for children and adults, respectively: 70-100 g of bananas, 10-15 g of manioc, 11 g of pineapples, 15-20 g of lemon, 8 g of peach palm, 17-25 g of abiu, 18 g of soursop and 1 g of cacao.

For drinking water, a mean consumption rate of 3.5 liters per person per day was obtained from surveys about food inquiries realized during the sampling fieldwork.

Hazard Quotients (HQ) for non-carcinogenic effects were estimated by the ratio of the potential ingested dose of a metal or metalloid (Lifetime Average Daily Dose: LADD) with respect to the Reference Dose (RfD) at which no adverse effects are expected. The sum of all HQ for a specific exposure pathway was likewise expressed as the Hazard Index (HI). If HI is greater than 1, it is considered that adverse health effects over a lifetime are more likely to occur. An HI below 1 suggests the contrary, as reported by the US EPA (2001).

Potential carcinogenic health effect (cancer risk, denoted CR) was calculated for As, Cd, Cr and Ni multiplying the LADD by the cancer Slope Factor (SF). This factor is an estimation of the probability that a person will develop cancer if exposed to a chemical for a lifetime.

Table 1. Exposure assessment equations by crops and water ingestion, inhalation and dermal contact of metallic elements (USEPA, 2016), health risk effects equations and parameters values.

Exposure route	Compartment	Equations
Ingestion	Soil and crops	$ADD_{s/v} = \frac{Cf/s \times IngRs/v \times EF \times ED}{BW \times AT} \times i1$
	Water	$ADD_{w} = \frac{Cw \times IwR \times EF \times ED}{BW \times AT}$
Inhalation	Air (PM_{10})	$ADD_{inh} = \frac{Cair \times InhR \times ET \times EF \times ED}{BW \times AT} \times i2$
Dermal contact	Soil	$ADD_{ds} = \frac{Cs \times SA \times AF \times ABS \times EF \times ED}{BW \times AT} \times i3$
	Water	$ADD_{dw} = \frac{Cw \times Kp \times ET \times SA \times EF \times ED}{BW \times AT} \times i4$
Health risk effects through the main exposure routes	Cancer risk (As, Cd, Cr, Ni)	$CR = LAAD \times SF$
	Hazard quotient (non-carcinogenic)	$HQ = \frac{ADD}{Rfd \text{ or } Rfc}$

Symbol	Default value	Data source
ABS	As: 0.03, other metals: 0.001	(US EPA, 2011)
AF	0.02 (children) - 0.07 (adults)	(Liu et al., 2013); (US EPA, 2011)
BW	65 kg (adults), 15 kg (children)	(Freire et al., 2013)
ED	30 years (adults), 6 years (children)	(Peña-Fernández et al., 2014)
EF	350 days year^{-1} / x events year^{-1}	(US EPA, 2011)
ET	24 hours day^{-1} *shower: 20 min day^{-1}	(US EPA, 2011) Information collected on field
IngRs	adult: 0.1 g day^{-1}, children: 0.2 g day^{-1}	(Li et al., 2015)
IngRv	Adults: 190 g day^{-1}, children: 158 g day^{-1}	Freire et al. (2013); FAOSTAT (2017)
InhR	adult: 1.44 m^{3} hour^{-1}, children: 1.24 m^{3} hour^{-1}	Adapted to our study from (US EPA, 2009)
IwR	3.5 L day^{-1}	Information collected on field
Kp	1x10^{-3} (As, Ba, Cu, Mn, V), 1.1x10^{-3} (Cd), 2x10^{-3} (Cr), 2x10^{-4} (Ni), 1.3x10^{-4} (Pb), 6x10^{-4} (Zn)	(US EPA, 2004)
RfD (oral)	As : 3x10^{-4}, Ba : 2x10^{-1}, Co : 2x10^{-2}, Cd : 1x10^{-3}, Cr : 3x10^{-3}, Mo: 5x10^{-3}, Mn: 1.4x10^{-1}, Ni: 2x10^{-2}, Pb: 3.5x10^{-3}, V: 9x10^{-3}, Zn: 3x10^{-1}	(IRIS, 2016; Sun et al., 2017)
RfC (inhalation)	As: 5x10^{-5}, Ba : 1.43x10^{-4}, Cr: 8x10^{-6}, Co: 5.71x10^{-6}, Mn: 5x10^{-5}, Pb: 3.52x10^{-3}, Sb: 2x10^{-4}	Ferreira-Baptista and De Miguel (2005); Zheng et al. (2010); IRIS (2016)
RfD (dermal)	As : 3x10^{-4}, Ba : 2x10^{-1}, Cd : 5x10^{-4}, Cr : 3x10^{-3}, Mo : 5x10^{-3}, Mn : 1.40x10^{-1}, Ni : 2x10^{-2}, Pb: 3.5x10^{-3}, V : 9x10^{-3}, Zn : 3x10^{-1}	
SA	5700 cm^{2} (adults), 2800 cm^{2} (children)	(US EPA, 2004, 1992)
SF	As: 1.5 (oral), 15.1 (inhalation), 3.66 (dermal) Cd: 6.1 (inhalation), Cr: 47.6 (inhalation), Ni: 0.84 (inhalation)	Zheng et al. (2010); IRIS (2016)

Notes: ABS= dermal absorption fraction; ADD=Average daily potential dose (mg kg^{-1} BW day^{-1}); AF= adherence factor (mg cm-2 events-1); AT= averaging time (ED×365 days year^{-1}); BW= body weight (kg); Cair= concentration in air (ng m^{-3}); Cf/s=concentration in soil or crops (mg kg^{-1}); Cw=concentration in water (mg L^{-1}); ED= life exposure duration (years); EF= annual exposure frequency (days per year); ET= daily exposure frequency (hours); InRs/v = soil/vegetable-fruits ingestion rate (g day^{-1});InhR= inhalation rate (m^{3} hour^{-1}); IwR= ingestion water rate (L day^{-1}); i1,2,3,4= units conversion factor (10^{-3} kg g^{-1}, 10^{-6} mg ng^{-1}, 10^{-6} kg mg^{-1}, 10^{-3} L cm^{-3})Kp= permeability coefficient (cm hour-1); LAAD= lifetime average daily dose (mg kg $^{-1}$ BW day^{-1}, LT= 70 years, instead of AT); Rfc=reference concentration for inhalation (mg m^{-3});Rfd=oral reference dose (mg kg^{-1} day^{-1}), SA=exposure skin area (cm^{2}); SF=slope factor of hazardous substances (mg^{-1} kg day)

All CR values were then summed for each exposure route, expressed as a total cancer risk (TCR) and compared to the acceptable reference values within the range of $10^{-6} – 10^{-4}$ (US EPA, 2001).

3. Results

3.1 Distribution of metal(loid)s concentrations in soils and crops

Soil physico-chemical properties in relation to the specific site descriptions (infrastructure and oil activities) are presented in Table SI-3 (in Supplementary Information).

Correlations between these parameters and metal(loid)s content in soils are show in Table SI-4 (in Supplementary Information). Ba, Co, Cu and Mn were moderate to strongly correlated (r^2 = 0.5-0.8) with pH, whereas Ba and Cr has a moderate positive and negative correlation, respectively with TOC (r^2 > 0.5 and -0.5). CEC has a negative correlation with Zn and Ni (r^2 > -0.5).

High levels of Ba, Co, Cu, Cr, Ni, V and Zn were found in most of the soils (Table 2). These concentrations were above the Ecuadorian Legislation limits but below the European Union Legislation (Table SI-2). Ba concentrations varied from 8 ± 0.3 mg kg^{-1} at site 4b, in a covered oil waste pool, to 785 ± 139 mg kg^{-1} at site 15a which is close to reinjection wells in Pacayacu. Arsenic and Mo did not exceed the regulation: concentrations remained low, varying between 3.1 ± 0.2 and 11.6 ± 0.5 mg kg^{-1} and between 1,225.6 ± 184.6 and 4,481.8 ± 363.7 µg kg^{-1}, respectively. Cadmium concentrations were below 500 µg kg^{-1}, except for the sampling points 5 and 14 associated with cacao crops, which registered concentrations of 1,254.6 ± 1.5 and 1,508.2 ± 4.8 µg kg^{-1} respectively. Strongly correlations (r^2 > 0.7) were found between the contents of some metals in soils: Zn-Cu, Ni-Cu, Cu-Co, Co-Mn and V-Ni. (Table SI-4).

Important SD values observed in some crop samples can be due to the heterogeneity of samples after being ground. Among the analyzed metals, Mn, Ba, Zn and Cu were quantified in all crop samples, with high concentrations of Mn in some cacao beans, bananas and pineapple samples (around 300, 196 and 109 mg kg^{-1} respectively), Ba in lemon fruits (153 mg kg^{-1}), Zn in cacao beans (111 mg kg^{-1}) and Cu also in cacao beans (41 mg kg^{-1}) and coffee grains (21mg kg^{-1}). Arsenic and Cr concentrations remained under the LQ for all fruits and vegetables. Cobalt, Ni, Mo, Pb and V were also below the LQ, except for Co, in lemon fruits (0.11 mg kg^{-1}) and cacao beans (between 0.27 and 3.56 mg kg^{-1},), Ni in cacao beans (4.0 mg kg^{-1}) and Mo in bananas (545.4 µg kg^{-1}), cacao beans (62.4 – 1329.5 µg kg^{-1}), peach palm (140.2 µg kg^{-1}) and soursop (1083.5 µg kg^{-1}). Cadmium contents in crop samples varied from 1.2 ± 0.4 µg kg^{-1} in bananas (300 m far from oil pumping station, in Dayuma) to 1,515.1 ± 80.3 µg kg^{-1} in cacao beans (close to reinjection wells and waste pools, in Pacayacu).

Table 2. Total metal(loid)s concentration (mean ± standard deviation, lab triplicates) in soils and consumed crops in the NAR and compared with the Ecuadorian thresholds for soils. Mn, Ba, V, Zn, Cr, Cu, Ni, Co, Pb and As are expressed in mg kg^{-1} whereas Mo and Cd are expressed in µg kg^{-1} (denoted by the * sign).

Code	Sample type	Mn	Ba	V	Zn	Cr	Cu	Ni	Co	Pb	As	Mo*	Cd*
	Max.levels in soils [a]	--	200	76	60	54	25	19	10	19	12	5000	500
1a	Soil (0-20 cm)	1690±7	113±6	113±15	68.4±3.4	51.6±1.6	19.8±0.6	14.3±1.5	17.0±3.9	16.9±2.3	3.9±0.2	1555.6±229.3	100.5±6.4
	Bananas	58.4±1.2	11.0±0.6	<LQ	8.8±0.2	<LQ	3.5±0.02	<LQ	<LQ	<LQ	<LQ	<LQ	1.2±0.4
	Lemon	8.3±0.7	152.7±1.5	<LQ	7.0±0.5	<LQ	3.6±0.2	<LQ	<LQ	<LQ	<LQ	<LQ	3.7±1.7
1b	Pineapple	93.9±7.0	2.9±1.0	<LQ	7.0±0.5	<LQ	5.2±0.4	<LQ	<LQ	<LQ	<LQ	<LQ	5.8±0.4
	Soil (0-20 cm)	214±19	42±2	170±12	72.7±4.2	56.1±3.4	20.3±1.4	22.3±1.3	6.6±1.5	7.8±1.3	3.4±0.2	1225.6±184.6	103.5±6.6
	Cacao	81.1±2.0	31.9±2.6	<LQ	52.0±4.7	<LQ	36.6±4.4	4.0±0.3	0.5±0.1	<LQ	<LQ	66.4±12.5	288.1±135.1
2a	Cacao	300.2±2.0	59.8±2.7	<LQ	60.6±4.7	<LQ	23.8±4.4	<LQ	3.6±0.1	<LQ	<LQ	62.4±12.5	581.6±135.1
	Soil (0-20 cm)	466±20	251±25	212±12	165.1±4.2	84.7±3.4	49.9±1.4	42.3±1.3	21.5±1.5	23.6±1.3	11.3±0.5	2404.1±184.6	488.6±31.2
	Abiu	34.4±1.6	16.8±1.8	<LQ	22.2±0.2	<LQ	8.8±0.2	<LQ	<LQ	<LQ	<LQ	<LQ	156.9±17.6
	Peach-palm	76.6±0.7	37.6±1.5	<LQ	22.5±0.6	<LQ	10.2±0.4	<LQ	<LQ	<LQ	<LQ	140.2±17.7	150.1±7.0
	Lemon	9.2±0.7	117.45±1.5	0.2±0.002	7.9±0.5	<LQ	3.8±0.2	<LQ	0.11±0.05	<LQ	<LQ	<LQ	6.1±1.7
2b	Pineapple	109.0±7.0	6.0±1.0	<LQ	8.4±0.5	<LQ	6.5±0.4	<LQ	<LQ	<LQ	<LQ	<LQ	7.4±0.4
	Soil (0-20 cm)	177±7	165±12	148±13	67.9±10.0	56.5±6.1	18.8±1.9	19.5±1.1	6.1±0.2	15.0±1.5	6.0±0.3	1631.1±430.0	114.9±7.3
3	Cacao	10.6±1.2	10.1±0.6	<LQ	49.0±2.0	<LQ	17.9±10.1	LQ	0.8±0.4	<LQ		216.6±57.5	1188.2±321.1
	Soil (0-20 cm)	1060±45	751 ±71	116±2	106.1±1.1	32.6±0.1	56.5±1.2	22.5±1.8	18.3±0.4	12.7±0.6	3.5±0.3	1600.6±20.5	467.5±6.3
	Bananas	15.3±0.7	3.4±1.5	<LQ	10.5±0.6	<LQ	5.8±0.2	<LQ	<LQ	<LQ	<LQ	545.4±17.7	<LQ
	Cacao	50.7±3.0	41.6±0.9	<LQ	110.9±10.2	<LQ	41.0±2.4	<LQ	0.5±0.04	<LQ	<LQ	1329.5±35.6	1515.1±80.3
4a	Soursop	6.3±0.4	9.22±0.6	<LQ	8.8±0.2	<LQ	9.4±0.6	<LQ	<LQ	<LQ	<LQ	1083.5±136.5	21.6±1.4
	Soil (0-20 cm)	78±6	14±0.4	183±3	126.4±10.5	71.5±1.3	26.2±1.8	24.3±0.1	5.2±0.7	18.4±0.8	8.6±0.36	4112.4±123.7	57.1±5.6
4b	Grains of coffee	17.2±0.5	5.3±0.1	<LQ	10.3±0.7	<LQ	21.3±0.03	<LQ	<LQ	<LQ	<LQ	<LQ	2.1±0.2
	Soil (0-20 cm)	48±7	8±0.3	190±7	47.1±10.2	76.6±2.7	14.9±2.3	28.8±0.8	6.1±1.4	4.4±0.2	8.4±0.3	4481.8±363.7	50.6±1.1
4c	Lemon	36.7±0.7	10.0±1.0	<LQ	18.2±0.2	<LQ	12.2±0.2	<LQ	<LQ	<LQ	<LQ	<LQ	2.9±1.7
	Soil (0-20 cm)	68±4	14±0.3	157±4	53.8±3.3	64.4±1.4	18.9±0.2	19.9±0.1	4.7±0.4	3.8±0.24	8.7±0.4	4384.0±175.6	51.8±4.8
	Bananas	195.8±5.9	18.8±1.5	0.5±0.002	18.8±0.6	<LQ	7.4±0.2	<LQ	<LQ	<LQ	<LQ	<LQ	1.9±0.4
	Cacao	172.9±2.0	34.0±2.7	<LQ	63.8±4.7	<LQ	23.3±4.4	<LQ	0.6±0.1	<LQ	<LQ	<LQ	116.6±21.9
	Cacao	42.9±2.0	8.2±2.7	<LQ	40.3±4.7	<LQ	29.2±4.4	<LQ	0.3±0.1	<LQ	<LQ	<LQ	116.9±11.9
	Manioc	24.6±0.4	15.6±4.0	0.4±0.3	11.7±1.4	<LQ	5.6±0.9	<LQ	<LQ	1.3±0.5	<LQ	<LQ	13.3±4.1

Notes: LQ = Limits of Quantification

The order of metal(loid) content was chosen according to their global abundance in soil samples

(continued on next page)

Table 2. Total metal(loid)s concentration (mean ± standard deviation, lab triplicates) in soils and consumed crops in the NAR and compared with the Ecuadorian thresholds for soils. Mn, Ba, V, Zn, Cr, Cu, Ni, Co, Pb and As are expressed in mg kg^{-1} whereas Mo and Cd are expressed in µg kg^{-1} (denoted by the * sign).

Code	Sample type	Mn	Ba	V	Zn	Cr	Cu	Ni	Co	Pb	As	Mo*	Cd*
	Max.levels in soils [a]	--	200	76	60	54	25	19	10	19	12	5000	500
5	Soil (0-20 cm)	1300±102	310±103	166±2	118.2±4.7	67.1±2.0	32.7±1.0	33.8±1.1	19.8±0.9	19.2±1.1	5.2±0.3	1517.4±32.1	1508.2±4.8
6	Soil (0-20 cm)	384±23	81±14	86±4	47.6±3.0	127.9±3.8	12.3±0.7	8.1±0.4	4.1±0.2	16.0±1.5	3.6±0.2	1862.1±50.1	294.0±2.4
7	Soil (0-20 cm)	1370±82	91±16	147±4	105.7±6.6	44.5±1.3	24.5±1.4	19.2±1.0	13.6±0.8	15.9±1.5	7.4±0.4	2479.5±66.7	471.4±4.2
8	Soil (0-20 cm)	1127±68	119±21	179±4	67.1±4.2	65.7±2.0	24.7±1.4	19.6±1.0	14.6±0.9	13.0±1.2	9.3±0.5	1656.7±44.6	297.7±2.3
9	Soil (0-20 cm)	131±8	39±7	200±4	54.9±3.5	53.4±1.6	24.7±1.4	21.4±1.1	8.0±0.5	10.2±0.9	6.7±0.3	3397.7±91.4	324.1±4.4
10	Soil (0-20 cm)	725±44	99±18	196±4	93.5±5.9	61.6±1.8	25.6±1.5	17.5±0.9	14.8±0.9	14.5±1.3	6.2±0.4	3195.1±85.9	478.0±4.3
11a	Soil (0-20 cm)	1951±117	400±71	220±4	130.0±8.2	66.6±2.0	88.6±5.0	30.7±1.5	37.0±2.2	16.5±1.5	4.9±0.3	1659.9±44.7	442.0±1.9
11b	Soil (0-20 cm)	2017±92	190±45	285±11	139.6±4.4	98.1±4.7	63.4±1.7	37.0±1.1	39.0±2.1	14.3±0.8	4.8±0.2	1739.9±24.3	496.1±2.2
12	Soil (0-20 cm)	1703±102	84±15	240±4	149.0±9.4	44.6±1.3	91.6±5.2	30.9±1.5	31.2±1.9	8.6±0.8	6.5±0.4	2240.6±60.3	740.0±2.7
13a	Soil (0-20 cm)	230±16	127±21	200±5	108.2±3.7	65.9±3.6	29.1±1.4	28.6±1.7	9.0±0.3	13.0±0.6	11.6±0.5	3264.4±96.7	380.2±2.5
13b	Soil (0-20 cm)	1009±61	652±115	137±4	88.9±5.6	31.5±0.9	51.9±2.9	21.3±1.1	19.5±1.2	13.1±1.2	3.1±0.2	1581.0±42.5	501.9±1.6
14	Soil (0-20 cm)	1197±72	691±122	123±4	117.3±7.4	30.8±0.9	56.4±3.2	23.1±1.2	20.2±1.2	12.7±1.2	3.7±0.2	1545.3±41.6	1254.6±1.5
15a	Soil (0-20 cm)	960±58	785±139	134±4	94.2±5.9	30.6±0.9	64.2±3.6	24.4±1.2	20.6±1.2	13.3±1.0	4.0±0.2	1844.2±49.6	470.0±1.6
15b	Soil (0-20 cm)	979±59	701±124	122±4	97.2±6.1	29.0±0.9	58.3±3.3	22.4±1.1	18.9±1.1	12.5±0.8	3.6±0.2	1641.3±44.2	650.4±1.6

Notes: *The order of metal(loid) content was chosen according to their global abundance in soil samples*

When comparing this concentrations to the available data about international legislation on trace metals in fruits and vegetables (Table SI-2), Pb concentrations of 1.3 ± 0.5 mg kg^{-1} in manioc exceeded the European limit of 0.10 mg kg^{-1}. Cd concentrations in abiu and peach palm were 3 times higher than the limit established by the European Union of 50 µg kg^{-1}. In addition, 50 % of the cacao beans collected in Orellana (sampling sites 1b and 2b, close to oil well drilling and gas open flares, respectively) and in Sucumbíos (sampling site 3, close to reinjection wells and waste pools) presented Cd concentrations higher than the critical level of 0.8 mg kg^{-1} established by the European Community for chocolate products (containing up to 70% of cacao total solids). In 3 cacao beans samples (1b, 3a and 4c), concentrations of Cu also exceeded the CODEX reference value of 20 to 35 mg kg^{-1} fixed for derived products (Table SI-2, in Supplementary Information).

Even if we assume that food quality standards for dark chocolate cannot be directly applied to raw cacao beans, a previous study in the Pacayacu area showed that Cd contents in cacao beans were very similar to those measured in cocoa mass (Barraza et al., 2017).

3.2 Distribution of metal(loid)s concentrations in drinking waters and aerosols

Metal(loid) concentrations measured in drinking waters from both Orellana and Sucumbíos Provinces are shown in Table SI-5 (Supplementary Information). The highest concentrations of carcinogenic metals: Cr (10.57 µg L^{-1}), As (4.35 µg L^{-1}) and Ni (3.41 µg L^{-1}) were measured in Dayuma, in the drinking water network, in a deep well located in the stadium and in a shallow private well in Auca oil camp, respectively, while the highest concentration of Cd (0.23 µg L^{-1}) was measured in Pacayacu's groundwater, in a private shallow well. The concentration of these metal(loid)s did not exceed the international regulations for drinking waters.

PM_{10} (Fig.2), showed high concentrations of Mo, Zn, Ba, Cr and Mn, (between 9.7 and 59.4 ng m^{-3}), and low concentrations of Ni, V, Cu, Pb, As, Co, Sb and Cd (from 0.11 to 3.5 ng m^{-3}) for both sites. Important temporal variations were observed at Shuara site 9 (Sucumbíos) which were related to higher concentrations of Zn and Pb, and also for Mo, Ni, Cu and Cd, in October and November 2015 and also at Auca Sur (Orellana) for Zn, Ba, Mn, Pb, Co and V between September and October of the same year. Ar, Cd, Ni and Pb levels were below the recommendations established by the European and Ecuadorian legislation for total annual mean concentrations of metal(loid)s in PM_{10} (Table SI-2, Supplementary Information).

3.3 Health risk assessment depending on main exposure pathways

Risk assessment calculations are presented as a global mean value, assuming that sampling sites are homogenous since calculations made separately for Orellana and Sucumbíos Provinces resulted in very similar values.

Crop ingestion represented 51.8 and 61.2 % of the total HI for adults and children respectively (Fig. 3A), these values being 2.19 to 7.59 times higher than the reference value of 1. Water ingestion was the second main exposure pathway for children whereas for adults the second pathway was inhalation followed by water ingestion. Soil dermal contact and water dermal contact were not considered as relevant in the present study because HI values were below 0.02 (Table 3).

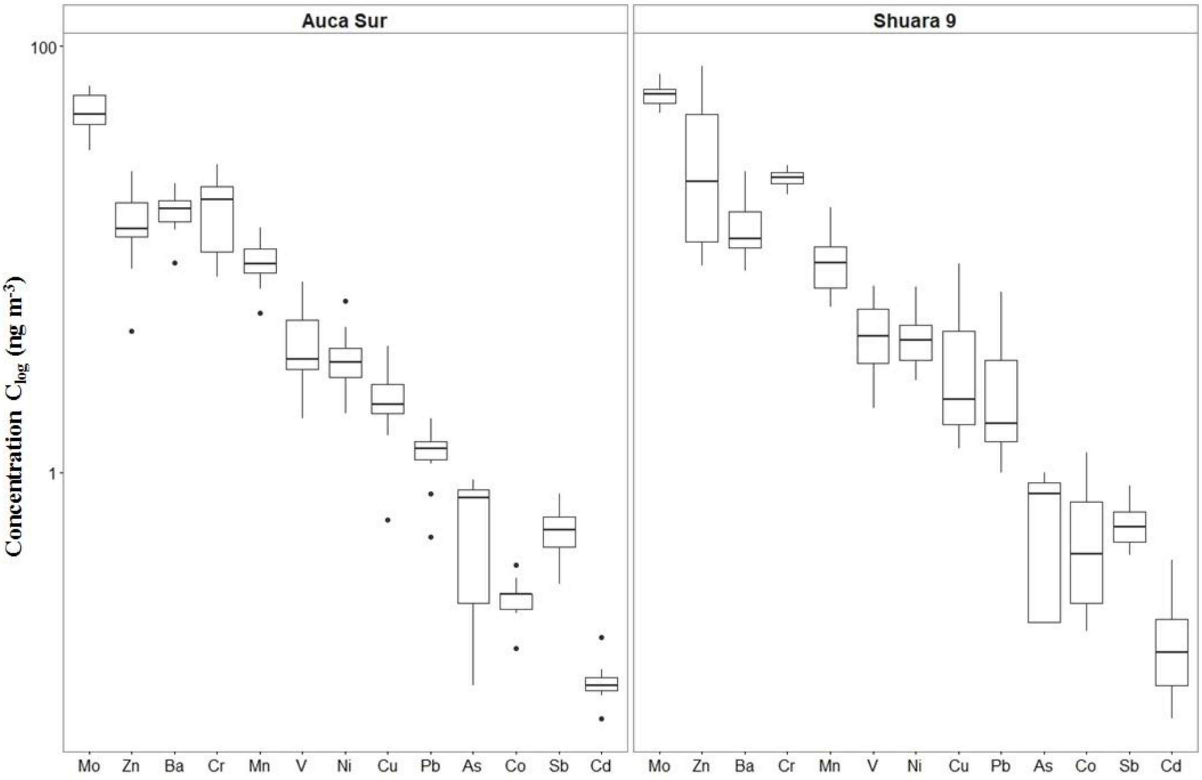


Fig. 2. Trace metal(loid)s concentration (in ng m^{-3}) in the air (PM$_{10}$) expressed in log-scale in the Northern Amazon Region of Ecuador settled in the vicinity of the Auca Sur oil platform, in the province of Orellana, and Shuara 9 oil platform, in the province of Sucumbíos. Metal(loid)s are presented according to their order of abundance in aerosols.

Metals with the highest hazard quotient were: Mn and Ba (crops ingestion), Zn (water ingestion) and As (water and soil ingestion). Taking into account all the exposure routes, HQ for each metal(loid) had the following tendency, in descending order: Mn>Cr>As>Ba>Zn>Mo>V>Pb>Cd>Co>Ni>Sb (for adults) and Mn>As>Ba>Zn>Mo>Cr>V>Pb>Cd>Ni>Co>Sb (for children).

Table 4 shows the TCR for a chronic exposition to As, Cr, Ni and Cd on each sampled compartment. Extremely low concentrations of As, Cr and Ni found in fruits and vegetables (Table 2) restricted the calculations for the "crop ingestion" exposure route. For the inhalation exposure route, Cr exhibited the highest CR value and Ni the lowest. Children and adult population were equally exposed, with a TCR 2.38 to 2.78 times higher than the reference value (US EPA, 2001).

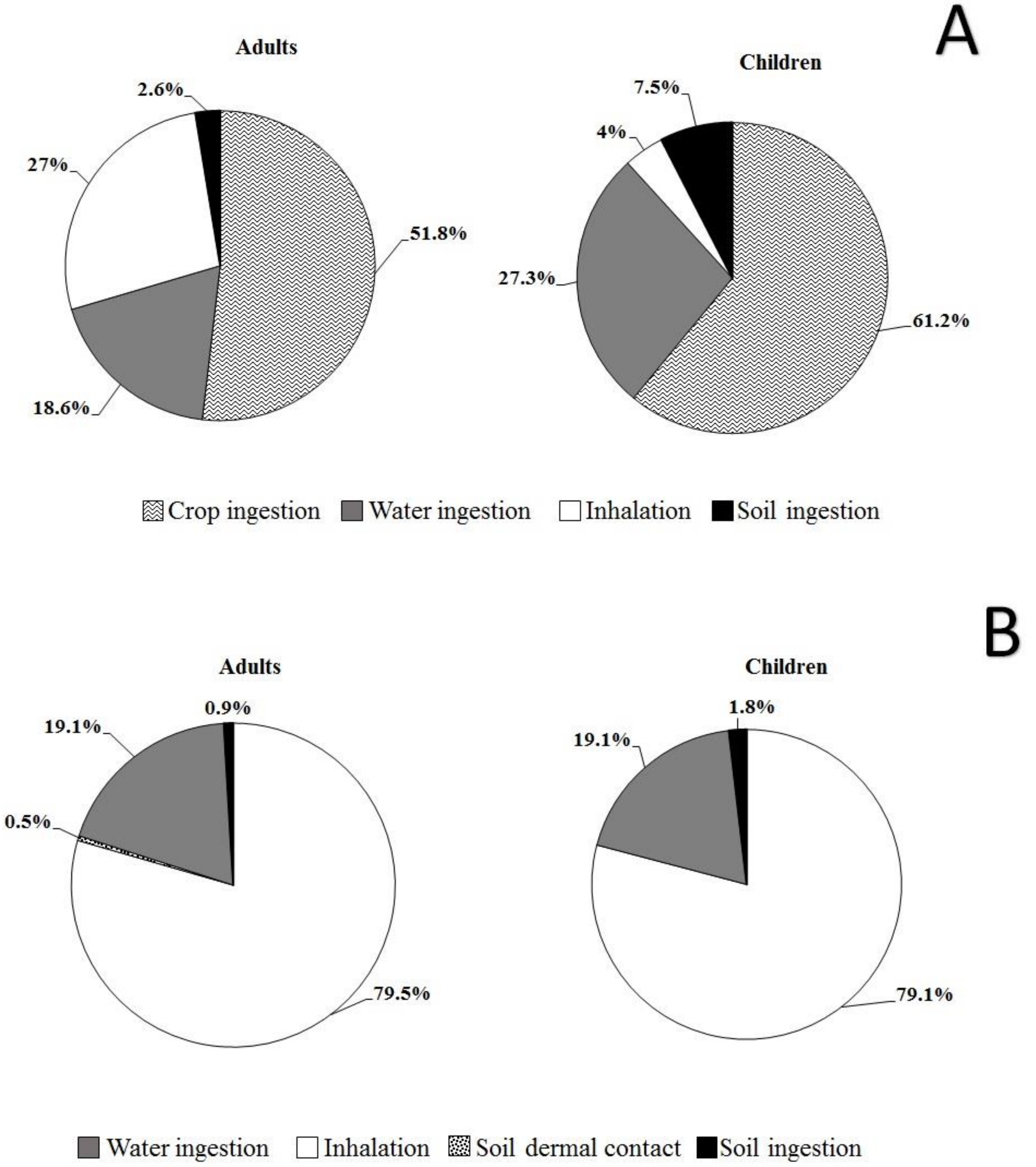


Fig. 3. Hazard Index (HI) for non-carcinogenic health effects of metal(loid)s (**A)** and Total Cancer Risk (TCR) (**B)** considering all exposure routes for adults and children living in the Northern Amazon Region of Ecuador.

Soil dermal contact in children and water dermal contact in both groups were extremely low (in the order of 10^{-7}-10^{-8}) therefore, these values were negligible for individual contributions to the TCR represented in Fig.3B. Main exposure pathways in adults were inhalation and soil dermal contact, counting for 46.7 and 42% of the TCR respectively, whereas in children the main routes were inhalation and water ingestion, with a contribution of 79.1 and 19.1%, respectively.

Table 3. Hazard Index (HI) for each exposure pathway for adults and children from the Northern Amazon Region of Ecuador.

Exposure route/ Population	Crops ingestion	Water ingestion	Soil ingestion	Inhalation	Soil dermal contact	Water dermal contact	TOTAL
Adults	2.19	0.78	0.11	1.14	0.02	0.001	4.24
Children	7.59	3.40	0.94	0.52	0.004	0.0004	13.04

Table 4. Total cancer risk (TCR) for each exposure pathway for adults and children from the Northern Amazon Region of Ecuador.

Exposure route/ Population	Crops ingestion	Water ingestion	Soil ingestion	Inhalation	Soil dermal contact	Water dermal contact	TOTAL
Adults	--	$5.21x10^{-5}$	$2.52x10^{-6}$	$2.16x10^{-4}$	$1.36x10^{-6}$	$7.76x10^{-8}$	$2.72\ x10^{-4}$
Children	--	$4.52x10^{-5}$	$4.36x10^{-6}$	$1.87x10^{-4}$	$4.98x10^{-7}$	$6.90x10^{-8}$	$2.38x10^{-4}$

4. Discussion

4.1 Distribution of essential and non-essential metal(loid)s in the environment

Metal(loid)s in the four environmental compartments considered in this study were compared to the current legislation.

In soils

Metal(loid) sorption and desorption in soils depend on different parameters such as soil texture, pH, CEC and OM (Kabata-Pendias, 2011). Tropical soils generally have low pH, low CEC (due to low exchange activity clays as kaolinite) and moderate OM content (usually lower than in temperate soils) resulting in a high mobility and bioavailability of metals (Rieuwerts, 2007). This soil description fits well with the physico-chemical properties of the soil samples collected in Orellana and Sucumbíos Provinces. For instance, one of the most polluted soils compared to the Ecuadorian thresholds (sampling point 2a) exhibited low values of pH (4.74), TOC (4.43 g kg^{-1}) and CEC (14.8 cmol kg^{-1}) and high concentrations of Ba, V, Zn, Cr, Cu, Ni, Co and Pb (Table 2). In addition, in five of the 15 farms, concentrations of Ba, V, Zn, Cr, Cu, Ni and Co in the soil exceeded the Ecuadorian limits. For the rest of the sampling sites, at least 2 of the quantified metals were also above the legislation.

The low pH of these tropical soils, are significantly correlated with Ba, Cu, Co and Mn concentrations (Table SI-4). However, for these same metals (except for Ba), no correlation was observed with TOC and/or CEC.

Spatial variation of metal(loid) contents in soil was significant in the NAR even if all the collected soils belonged to the same category (*Inceptisols* according to the USDA soil taxonomy). Additionally, there was a high variability in the soils samples with some sampling locations, which could be due in part, to the natural occurrence of these metals within the soils.

Regarding metal(loid) concentrations in the soil compartment, Fu et al. (2014) studied soils polluted by crude oil in Gudao (China) and reported that Cd, Cu, Ni and Zn contents were greater than the local background values, reaching mean concentrations of 0.185, 27.63, 33.80 and 67.12 mg kg^{-1}, respectively while Cr, Mn and V concentrations were below the background levels, in the order of 61.66, 524.2 and 75.10 mg kg^{-1} respectively. Sarma et al. (2016) found that levels of Cr, Cd, Cu, Mn, Ni and Pb in soils collected from different crude oil drilling sites in Assam (India) were 158.66, 15.47, 11.86, 627.83, 58.97 and 73.62 mg kg^{-1} respectively, and showed significant spatial variation with respect to the sampling sites. Concentrations of Cu, Ni and Cr in the NAR were close to values reported by Fu et al. (2014) and Sarma et al.(2016), except for Cd (0.29 mg kg^{-1}) and Pb (13.36 mg kg^{-1}).

In crops

Metal(loid) mobility in soil and their phytoavailability depend on many factors: soil texture, pH, organic matter composition, as well as metal chemical species and contents (Amari et al., 2017; Kabata-Pendias, 2011). After root uptake, the main and well described mechanism (Shahid et al., 2017), metal(loid)s can be efficiently retained and stored in the roots, or exported to the shoots, and sometimes even bio-accumulated in edible parts, thereby entering the food chain (Osuji and Onojake, 2004).

Among the crop samples from the small-scale farms in the NAR, bananas, pineapples and cacao beans showed higher concentrations of essential metals than those found in the literature. Usually the mean Mn levels in fruits and roots are between 12 and 15 mg kg^{-1}, respectively (Kabata-Pendias, 2011). In cacao beans from South America, Mn mean concentration was 24.7 ± 5.5 mg kg^{-1} (Bertoldi et al., 2016), compared to a mean concentration of 109.7 ± 99.0 mg kg^{-1} in our samples. Sultana et al. (2017) reported Mn concentrations less than 25 mg kg^{-1} in bananas, whereas our samples showed a mean concentration 4-fold higher. Kabata-Pendias and Szteke (2015) reported that Mn concentrations in plants vary depending on the tissue and within the vegetative period. However, Mn fluctuations can be also related to a parasitism by fungi, bacteria and insects, being lower in tissues susceptible to the attack and more enhanced in areas around the infection sites (Huber and Wilhelm, 1988). Some of our samples displayed signs of infectious diseases, which is common in tropical regions crops (Ghini et al., 2011). Thus, Mn could be likely associated to a defense mechanism of the plant more than to oil contamination.

The mean values of Co, Cu and Zn in our cacao beans samples (1.03 ± 1.14, 28.6 ± 8.0 and 62.8 ± 22.8 mg kg^{-1}, respectively) were also higher than those published by Bertoldi et al. (2016) from 23 countries of East and West Africa, Asia and Central and South America: 0.48 ± 0.03, 20.8 ± 3.0 and 47.4 ± 3.5 mg kg^{-1}, respectively. We believe there was an increased contribution to the SD in our results due to sampling from different varieties of cacao samples in the field.

Non-essential metals for plants were represented by high concentrations of Ba, followed by Pb and Cd. As described in section 3.2, Cd contents in cacao beans, abiu and peach palm ranged from 120 to up to 1,000 µg kg^{-1}. Nie et al. (2016) found Cd levels of 2.5 ± 0. 9 µg kg^{-1} for deciduous fruits in China (apple, pear, peach, grapes and jujube). In contrast, Cd in vegetables (radish, spinach and cowpea) ranged from 500 to 1,900 µg kg^{-1} according to Khillare et al. (2012). A Cd mean concentration of 634.4 ± 538.7 µg kg^{-1} was found in our cacao beans samples, lower than those referred by Chavez et al. (2015) in Ecuador and Bertoldi et al. (2016) in South America, who reported values between 940 and 1,388 µg kg^{-1}, respectively. However, Cd mean values for Nacional and CCN-51 varieties collected in oil production areas of Ecuador were around 900 and 1,200 µg kg^{-1}, respectively (Barraza et al., 2017). Special attention was given to Cd because it is easily absorbed by plants and it is extremely toxic to animals and humans (Castro et al., 2015; Mulware, 2013); where it enters the body by the ingestion of contaminated food (for non-smokers), and is mainly retained in the kidney and accumulated in the body over a lifetime (Clemens et al., 2013).

While Pb is quite mobile within the plant, it is not readily translocated to the edible portions (Kabata-Pendias, 2011), except in the case of high and direct atmospheric pollution leading to foliar absorption (Schreck et al., 2012; Uzu et al., 2010). Lead concentrations in manioc samples collected in Jamaica (Antoine et al., 2017) and French Guiana (Rimbaud et al., 2017) reached a mean content of 0.1 mg kg^{-1}, 10 times lower than the concentration found in our samples. In a study about the bioaccumulation of heavy metals in manioc tubers growing in soils contaminated by oil production, Idodo-Umeh and Ogbeibu (2010) reported Pb concentrations of 1.75 mg kg^{-1}, similar to our results for samples collected in a small farm in front of an oil platform but protected by a forest. By contrast, in an area without any petroleum activity (in the same study), Pb concentration in manioc was 0.58 mg kg^{-1}.

Ba mean concentration in plants samples from the NAR were also higher than those referred to by Kabata-Pendias and Szteke (2015): between 2 and 13 mg kg^{-1} in comparison to 3 and 153 mg kg^{-1} found in bananas and lemons, respectively.

Despite the fact that concentrations of metal(loid)s in our topsoil samples were high, their translocation to fruit and vegetable edible parts might be limited in some cases by the capacity of plants to detoxify their tissues or retain metals in the roots (Kumar et al., 2016). Nevertheless, heavy metals can also be absorbed and accumulated in plant leaves and fruits by foliar transfer and after atmospheric deposition onto the leaf surface (Gajbhiye et al., 2016; Shahid et al., 2017). In order to consider this pathway, concentrations of metal(loid)s in air have to be extremely high, otherwise they can be neglected (Smolders, 2001).

In drinking water

Developing countries are mostly affected by metal(loid)s in drinking water (Chowdhury et al., 2016). As this study area lacks an official water distribution system, the main drinking water sources are private wells, springs and rainwater.

Metal(loid)s were found in low concentrations in the majority of the drinking waters analyzed in the NAR except for Mn (up to 1,000 µg L^{-1}) which exceeded WHO limits in main groundwater and spring water samples. Amazonian tropical soils are very rich in Al, Fe, and Mn which could explain the high concentrations of these metals measured in groundwater. In our study, the higher concentrations of Pb (7.83 µg L^{-1}) and As (1.9 µg L^{-1}) were still below the guidelines established by the US EPA. Chennaiaha et al. (2014) reported average concentrations of 39.9, 147.5, 13.4, 14.4, and 1,286.1 µg L^{-1} for Cr, Mn, Ni, Pb, and Zn, respectively in drinking waters in rural areas of Bhongiri, India. Significant fractions of the samples collected in Bhongiri exceeded WHO guidelines of 50, 70, and 10 µg L^{-1} for Cr, Ni, and Pb, respectively. In our study, none of these metals exceeded the WHO (2011) guidelines.

Essential elements such as Zn in drinking waters samples taken in the Ecuadorian NAR reached 3.15 mg L^{-1} in private water tanks. This high concentration of Zn can be explained by the fact that water is collected from rainfall streaming down from zinc roofs. In this tropical region characterized by abundant precipitation, the acid rainwater can leach, dissolve, and transport the Zn directly to the water tanks.

In the air

Metal(loid)s composition and concentrations of PM_{10} from Dayuma and Pacayacu sites exhibit levels more comparable to urban than rural sites, even if they did not exceed regulatory limits. Artaxo et al. (2013) worked in two sampling sites in the Brazilian Amazon, one near to pristine forest and the other close to a city highly impacted by land use change and biomass burning. They reported that concentrations of trace elements associated to industrial and fossil fuel emissions in coarse mode (PM_{2-10}), such as Cr, Mn, Ni, Pb and V were less than 1 ng m^{-3} at the forest site, while in the impacted site they were higher, reaching values of 4.0 ± 5.1, 2.7 ± 3.4, 1.2 ± 2.0, 5.9 ± 8.9 and 1.5 ± 2.0ng m^{-3} respectively. In our study, these elements were found in concentrations up to 2.5 to 5 times higher, except for Pb.

Other metals in PM_{10} in the NAR, such as Ba and Mo, showed mean concentrations of 16 and 54 ng m^{-3}, respectively. However, these two metals are not commonly reported in the literature. Ledoux et al. (2017) found Ba mean concentrations in $PM_{2.5}$ of 0.67 ng m^{-3} in the urban site of Saint-Omer in France, but it was not strongly correlated with other metals emitted from industrial sources. Mo concentrations in total suspended particles in two cities of India and China, with intense emissions from biomass burning, dust, coal burning and waste incineration, were 10 and 90 ng m^{-3} respectively (Li et al., 2014). A recent study conducted in Quito, Ecuador, showed that Ba and Mo concentrations in outdoor $PM_{2.5}$ residential areas varied from 7.52 to 11.17 ng m^{-3} and from 0.19 to 0.22 ng m^{-3} respectively (Raysoni et al., 2017).

4.2 Potential sources of metal(loid)s in the NAR environment

Toxic metal(loid)s can come from natural sources such as geochemical bedrock composition or atmospheric depositions due to volcanic activity (Mather, 2015). Refractory metal(loid)s can persist for a longer period of time in soil while others are quickly leached by flooding and water runoff (Sarma et al., 2016), which could probably occur due to intense precipitations occurring in the NAR all year long.

Thus, the natural bedrock material seems to be the first way of metal inputs in this soil ecosystem. Actually the geology of the Amazon basin is characterized by the presence of alluvial sandstones, basalt, clay minerals, mainly kaolinite and feldspar (Baby et al., 2004). These minerals showed V concentrations ranging from 130 to 220 mg kg^{-1} (Aubert and Pinta, 1977) in basic rocks (basalt) and in certain sedimentary rocks, such as clays and loam. Ni mean concentrations in soils worldwide vary between 13 to 37 mg kg^{-1}, associated with Fe and Mn oxides also present in clay minerals (Kabata-Pendias and Szteke, 2015).

Mineral dust from crustal erosion and volcanic emissions are also natural sources of metal(loid)s, carried in the atmosphere as gases and PM (Bradl, 2005; Buccolieri et al., 2006). Volcanic activity possibly followed by long distance transport and deposition could also contribute to metal's accumulation, such as Cd, V and Ba in soils (Mather, 2015; Parelho et al., 2014). In Ecuador, intense volcanic activity has been observed in the last 2,000-3,000 years from the Cotopaxi and the Tungurahua volcanoes (Mothes and Vallance, 2015; Troncoso et al., 2017).

Nevertheless, anthropogenic activities and especially oil production may be considered as important sources of metal(loid)s in the environment (Sarma et al., 2016). Yang et al. (2017) reported that natural crude oil exhibits a unique chemical fingerprint as the result of geological conditions of the exploitation area and ages under which it was formed. It has been well documented that the predominant anthropogenic sources of V and Ni emissions into the environment are the combustion of crude oils

(Assem and Oskarsson, 2015) and fossil fuels (Klein and Costa, 2015). These metals (V and Ni) in crude oil were reported to be mostly present in the form of porphyrins (Huang et al., 2015; Speight and Özüm, 2002; Zhao et al., 2013). In this study, crude oil samples collected from the National Pipeline, the Auca 3 oil camp and an abandoned oil waste pool Auca 8 showed high levels of V (158 ± 0.3, 278 ± 11 and 305 ± 36 mg kg^{-1}, respectively) and Ni (58 ± 2, 87 ± 4 and 99 ± 11 mg kg^{-1}, respectively). Mn and Ba concentrations of 34 ± 4 and 133 ± 5 mg kg^{-1} respectively were also found in the Auca 8 sample. For this particular sample, the concentrations of As, Cu, Mo and Pb were below 4 mg kg^{-1} whereas Zn reached a concentration of 13.7 ± 0.2 mg kg^{-1}. High concentrations of Ba in soils could be explained by the usage of additives during oil extraction such as $BaSO_4$, a common weighting agent (Siddique et al., 2017)

Metals such as Cr, Co and Mn are emitted by fossil fuel combustion (Kabata-Pendias and Szteke, 2015; Lison, 2015; Lucchini et al., 2015). Mo can be also associated to the emissions of gas open flares due to the high concentrations found in PM_{10} in the NAR. Mo is commonly used as a catalyst in oil refinery processes (Chaudhuri, 2011) or added with drilling muds (Max, 1966). Other metals like Cu, Cr, Mn and Zn could also be released during oil operations, by tubing and other metallic parts liable to be corroded and degraded (Lienemann et al., 2007).

In addition, the high levels of Mn, Ba, Z, Co, Mo and Cd in soils for some sampling points (i.e. sites 5 and 11) and the lack of oil infrastructure at the sampling sites, suggest the presence of other anthropogenic sources other than oil extraction. Actually, the traffic on roads connecting oil camps and main villages is continuous and intense, especially in the area of oil operations. In the interval of 1 hour in Auca Sur (air sampling site in Dayuma), we recorded a total of 15 trucks (or heavy machinery) passing, maneuvering, or parking in the entrance of the oil pumping station. In Shuara 9 (air sampling site in Pacayacu), situated in an isolated area with less vehicular traffic, frequency was reduced to 5 trucks per hour. Finally, chemical products widely spread in agricultural areas could also be responsible for high metal(loid) contents in soils and in air particles (Covelo et al., 2007). Herbicides, fungicides and pesticides usually contain Cu, Zn and Mn compounds (Aikpokpodion et al., 2013; He et al., 2005; Lucchini et al., 2015), whereas Co and Cd are commonly present in phosphate fertilizers (Khan et al., 2017; Lison, 2015). According to the information collected in the field, farmers in the NAR have used at least one of these products once or twice per year

Finally, vegetation in tropical forest plays a major role in airborne particle concentration being as well a natural source of Zn, Pb and Cu (Artaxo et al., 1998). Biomass burning, including wildfires and prescribed burning (of forest and agricultural waste), is also a major source of trace metals (Ahmed et al., 2016; Cheng et al., 2013).

4.3 Evidence of impacts of metal(loid)s on human health

The pollution levels of the main environmental compartments (such as air, water, soils and crops) are clearly impacted on by the distance from the pollutant emission sources and, for soils and crops, also by the time period of emissions. Consequently, the human exposure of people living in both of these Provinces cannot be expected to be homogeneous over the studied region. The same goes for other types of pollution due to oil activities, their concentrations should be heterogeneously distributed across the studied area.

Thus, any consideration of proportional exposure to different pollution types, such as those presented in Figure 3, will be highly site-specific. This study does not claim to be exhaustive but presents, for the first time, an analysis of the distribution of metal(loid)s and the associated health risks in the oil producing regions of the Amazon, in Ecuador. Obviously, proper spatial analysis of pollution in the studied area is impossible to assess with such a small number of sparsely located samples; most of the samples were randomly chosen in the oil influenced areas as defined by the Ecuadorian Ministry of the Environment and also in small-scale farms where owners were complaining about oil pollution.

Even if the diversity of the local diet could not be entirely taken into account due to the lack of available data, our results showed that crop ingestion is the main exposure pathway to non-carcinogenic effects of metals with 26 % linked to non-essential metals and 74 % to the essential ones. This is in accordance with several studies reported in the literature (Cai et al., 2015; Liu et al., 2013).

With respect to essential metals, the highest Zn concentration measured in drinking waters of the Ecuadorian NAR corresponds to a daily uptake for an average healthy adult of 0.17 mg kg^{-1} of body weight (BW). A provisional maximum tolerable daily intake of Zn is 0.3 mg kg^{-1} BW, above which adverse effects on health have been observed (WHO, 2011). Moreover, excessive Zn intake over the long term can cause major Cu deficiencies.

The metal with the highest HQ for crops ingestion was Mn, followed by Ba, probably due to the regular consumption of bananas, pineapples and lemon fruits. Mn is considered essential for humans, but long-term exposure (associated with inhalation) may cause neurological and behavioral effects and it could also have a negative impacts on the reproductive, cardiovascular, hematological, endocrine and immunological systems (Lucchini et al., 2015). On the other hand, the Ba free ion is readily absorbed by the gastrointestinal tract (mainly through drinking water) and by the lungs (through inhalation), resulting in a number of disorders, including renal intoxication, hypertension, cardiac malfunction, and hearing loss in experimental animals, after a chronic exposure (Oskarsson, 2015). Although Pb concentrations in manioc were above the threshold, its HQ was low (less than 0.1 % of the ingestion route).

Regarding the carcinogenic metals, Cr and As had the highest HQ for inhalation and water and soil ingestion pathways. However, only the hexavalent Cr form has adverse effects including sores in the mouth, diarrhea, stomach ache, indigestion, vomiting (IRIS, 1998). The risk of cancer through soil dermal contact, water and soil ingestion in the NAR is also linked to As, even if its concentrations in the 3 compartments evaluated did not exceed the Ecuadorian environmental legislation. Besides, Cr is the predominant element in the inhalation pathway, accounting for 99 % of the TCR. Arsenic compounds enters the body mainly by water and food ingestion, and inhalation but less frequently by dermal exposure (Fowler et al., 2015) and is likely to cause lung, bladder, liver, lung and skin cancer (Sun et al., 2014).

Dermal pathway indexes should be carefully interpreted, because most of the metals have limited uptake due to their low tendency to partition into skin lipids in comparison to the semi-volatile organic compounds (Elert et al., 2011). However, this pathway only counts for 0.5% and 0.2% of the TCR in adults and children respectively.

While population exposure can be considered as moderate to elevated for risk indexes such as metals, the US EPA, HI and TCR recommend that the approximation of the cumulative effect on a target organ should be considered, since these compounds do not always have the same toxic mechanism. Despite this, health risk assessments are often overestimated in studies that consider the total concentration of metals instead of their bio-accessible fraction which is soluble in the gastrointestinal environment (Liu et al., 2016; Uzu et al., 2014).

4.4 The limits of metal(loid)s regulations : towards social insights

Robust health risk assessments should be done beyond precise calculations of human exposure to toxic compounds. Measuring the impact of contaminants on human health relies on various medical and epidemiological uncertainties, but the risk assessment should also take into account the social dynamics (Turner et al., 2003). At the individual or community scale, the level of natural resource dependence, the knowledge of risks, rights and institutions, the usage and prioritization of environmental issues, are the main factors that control their behavior faced with the risk of the contamination of their main resources. (Fraser et al., 2003)

Health risk assessments concerning environmental contamination are very often reduced to standardized calculations based on quantitative information such as gender, age or body weight, time of exposure, mean food and water ingestion potential (US EPA, 2017). In fact, the differences of diet among ethnic groups are rarely considered. In the Ecuadorian Amazon region, the two communities (indigenous *versus* non indigenous population) have different food habits (Sirén, 2011; Suárez et al., 2009). The same applies to the social perception of contamination risk as a parameter that can influence consumption habits or protection strategies.

Risk assessment calculations proposed by the US EPA are often based on existing environmental norms that are politically constructed (Boudia and Jas, 2013). Indeed, in Ecuador the oil activities represent the primary source of income and, for years, the national government has prioritized oil extraction over environmental protection (Fontaine et al., 2003; Juteau-Martineau et al., 2014). The current norms are therefore strongly impacted by a political background.

For instance, the reported thresholds from the Unified Text of Environmental Legislation in Ecuador (Ministerio del Ambiente de Ecuador, 2015) are higher than those previously established in 2003 (Ministerio del Ambiente de Ecuador, 2003) particularly for Mo, As, Cr and V.

Another example is where air quality only considers Cd total annual mean concentration in PM_{10}, fixed as the same value as the European Union directive, whereas toxic elements such as Pb, Ni, As or PAHs are not mentioned.

If risk assessment has an impact on management decisions, we should go further in the health risk calculations by integrating social variables. As explained in the social amplification of the risk framework (Renn et al., 1992), hazards interact with psychological, social, institutional, and cultural processes amplifying or attenuating public responses. In the “social disposition approach” developed by Becerra et al. (2016), the “risk culture” appears as fundamental. "Risk culture" refers to the willingness or unwillingness of people to think about and to reduce their exposure. Becerra et al (2016) defined it as a set of ways of thinking and acting to face the everyday life risks and to cope with future uncertainty. Thus from a social point of view, this "risk culture" can influence the people’s risk exposure.

As highlighted by Calès, (2016), from a total of 82 farmers interviewed in the cities of Dayuma and Pacayacu in the NAR, family is the key factor of the risk culture. Indeed family appear as the first arena where individuals can learn about contamination, experience it, and learn how to protect themselves. Families also bring a set of values that can have a direct effect on the individual representations of the contamination risks. For instance, children can learn from their parents about contamination, especially when one of them is working for an oil company or accessed to higher education.

Then, to assess this risk culture in this specific case of the Ecuadorian NAR, we proposed to take into consideration 3 parameters: i) the oil contamination risks knowledge and perceptions, ii) the behaviors and the social practices to cope with it in everyday life, iii) the capacity to build future projects at both individual and territorial scales iv) individual context that influences risk consciousness. Based on the results of Calès (2016) and Racines (2017), as a part of the MONOIL Research Program, a Risk Culture Index (denoted RCI) hasbeen stablished from qualitative interviews with local populations of the study area, from both indigenous (Shuar and Kichwa) and non-indigenous groups. For instance, the first results demonstrated that the RCI of indigenous groups (6.4/12) is lower than the one of non-indigenous people (8.7/12) and that people working in the oil patch have a significantly higher RCI (9.2/12). Surprisingly, leaving near oil infrastructure does not influence the value of the RCI of the indigenous groups. Finally, a more holistic approach is probably needed in Ecuador: an integrated understanding of the local political dynamics, the social vulnerability and capacities, the forms of socio-spatial organization that enhance exposure, could provide pertinent information on the social dimension of health risk due to environmental contamination in areas impacted by oil activities.

5. Conclusions

This is the first study conducted in the Northern Amazon Region (NAR) of Ecuador, also known as the local influence area of oil extraction activities, to provide evidence of human exposure to carcinogenic and non-carcinogenic metallic contaminants. This study highlighted the spatial heterogeneity of metal(loid)s contents in the environment (air, drinking waters, soils and crops) and the social disparities in exposure of local communities living in the oil Ecuadorian Amazon.

This study does not pretend to be exhaustive. Quantitative results were mainly explained by the high contents of Ba and Mn in specific crops, As and Zn in drinking waters collected from the rainfall and Ba and Mo in atmospheric particles. Even if Ba and Mo seem to be directly linked to oil emissions, the rest of the metal(loid)s can display other anthropogenic or natural sources. Compounds such as PAHs would have been useful to trace more specifically oil emissions into the environment (Tobiszewski and Namieśnik, 2012) and will be further studied. Additional investigations are currently in progress to determine local population’s chronic exposure by considering the metal(loid)-PAHs cocktails in the oil Ecuadorian Amazon.

While the calculated hazard quotients and total cancer risks exceeded the thresholds recommended by the US EPA, these values must be balanced by the spatial heterogeneity of the environmental contamination as well as social factors. At the individual scale, the level of natural resources dependence, the knowledge of contamination risks and rights and the prioritization of environmental issues are the main factors that control human behavior face to the contamination risk of the natural resources.

Finally, these findings help to propose new recommendations based on a holistic approach integrating political dynamics, social vulnerability and socio-spatial organization. We also suggest the use of bioaccessible contents of metal(loid)s instead of total concentrations as a more appropriate tool for health risk assessment.

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Supplementary Information

Table SI-1a. Limits of detection (LD) and quantification (LQ) for metal(loid)s in crops, analyzed by ICP-MS, expressed in mg kg^{-1} for Mn, Ba, V, Zn, Cr, Cu, Ni, Co, Pb and As and in µg kg^{-1} for Mo, Cd and Sb (denoted by the * sign).

Trace element	Crops samples (except cacao)		Cacao samples		Soil samples	
	LD	LQ	LD	LQ	LD	LQ
Mn	6.07	20.22	0.16	0.53	0.08	0.26
Ba	0.05	0.16	0.05	0.16	0.11	0.38
V	0.02	0.07	0.01	0.03	0.03	0.10
Zn	0.28	0.93	0.29	0.97	0.32	1.06
Cr	1.50	4.99	1.15	3.83	0.04	0.12
Cu	0.04	0.12	0.001	0.005	ND	ND
Ni	1.32	4.40	1.02	3.40	0.00	0.01
Co	0.03	0.10	0.02	0.08	0.01	0.05
Pb	0.04	0.14	0.03	0.11	0.00	0.01
As	1.14	3.79	4.67	15.56	0.02	0.06
Mo*	14.87	49.55	13.12	43.74	5.06	16.88
Cd*	0.17	0.57	0.08	0.25	1.05	3.49
Sb*	4.70	15.68	5.31	17.71	4.39	14.63

Note: ND (non detected)

Table SI-1b. Certified and measured concentrations (mean±SD) of metal(loid)s in SRM1515, SRM2709 and SRM1648a, expressed in µg g^{-1}

	SRM1515 « Apple leaves »		SRM2709 « San Joaquin Soil »		SRM1648a « Urban Particulate Matter »	
Metal(loid)	Certified	Measured (N=10)	Certified	Measured (N=20)	Certified	Measured (N=5)
Mn	54±3	51±2	538±17	502±22	790±44	724±37
Ba	49±2	45±0.6	968±40	850±72	--	702±21
V	0.26±0.03	0.22±0.02	112±5	96±8	127±11	121±5.6
Zn	12.5±0.3	11.2±0.9	106±3	97±5	4800±270	3917±228
Cr	0.3*	0.3±0.02	130±4	109±9	402±13	302±85
Cu	5.64±0.24	5.03±0.24	34.6±0.7	28.7±2.4	610±70	563±86
Ni	0.91±0.12	0.85±0.05	88±5	77±3	81.1±6.8	77.7±3.7
Co	0.09*	0.09±0.02	13.4±0.7	10.9±0.7	17.93±0.68	14.65±0.91
Pb	0.470±0.024	0.407±0.008	18.9±0.5	15.8±0.78	6550±330	5307±370
As	0.038±0.007	<LD	17.7±0.8	14.2±0.6	115.5±3.9	105.4±5.1
Mo	0.094±0.013	0.080±0.008	2.0*	1.4±0.1	--	15.7±1.6
Cd	0.013±0.002	0.012±0.001	0.38±0.01	0.38±0.03	73.7±2.3	66.3±1.4
Sb	0.013*	0.013±0.003	7.9±0.6	<LD	45.4±1.4	39.8±1.8

*non certified values

<LD: below limits of detection

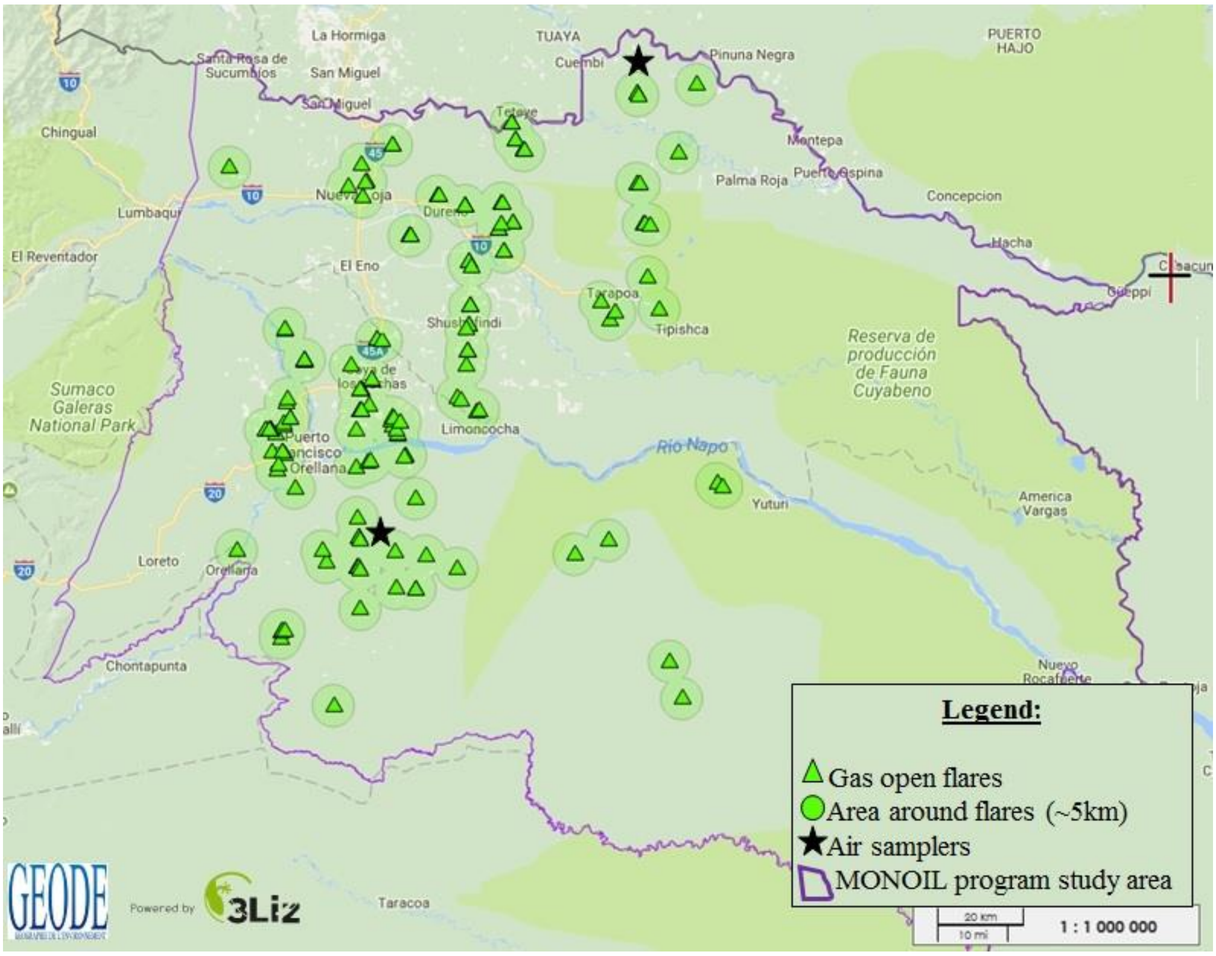

Figure SI-1. Gas open flares (N=181) within study area, North Amazon Region (NAR), of the MONOIL research program.
Elaborated by: Bosque S., Lerigoleur E., Maestripieri N., & M.Saqalli. GEODE Laboratory.

Table SI-2. International legislation on maximum levels of metal(loid)s in soils, crops (including cacao-derived products), drinking water and air (PM_{10}).

Compartment and Category	Mn	Ba	V	Zn	Cr	Cu	Ni	Co	Pb	Mo	Cd	As
Soils (mg kg^{-1})												
• TULSMA[a]	--	200	76	60	54	25	19	10	19	5	0.5	12*
• European Union[b]				150-300		50-140	30-75		50-300		1-3	
Crops (mg kg^{-1})												
• European Union[c.d]												
Fruits	--	--	--	--	--	--	--	--	0.10	--	0.05	--
Abiu. peach-palm. soursop												
Berries and small fruits	--	--	--	--	--	--	--	--	0.20	--	0.05	--
Bananas. coffee. lemon												
Roots and tuber vegetables	--	--	--	--	--	--	--	--	0.10	--	0.10	--
Manioc												
Cacao-based products category[d]												
Chocolate with ≥50% total dry cocoa solids	--	--	--	--	--	--	--	--	--	--	0.8	--
Chocolate with 30-50% total dry cocoa solids	--	--	--	--	--	--	--	--	--	--	0.3	--
• GSCTFF[e]												
Fruits	--	--	--	--	--	--	--	--	0.1	--	--	--
Abiu. peach-palm. soursop												
Berries and small fruits category	--	--	--	--	--	--	--	--	0.1	--	--	--
Bananas. coffee. lemon												
Roots and tubers	--	--	--	--	--	--	--	--	0.1	--	0.1	--
Manioc												
Cacao-based products												
Chocolate [f]	--	--	--	--	--	20	--	--	1	--	--	0.5
Unsweetened chocolate [g]	--	--	--	--	--	35	--	--	2	--	--	1
Chocolate with >70% total dry cocoa solids)[h]	--	--	--	--	--	--	--	--	--	--	0.8	--
Chocolate with 50-70% total dry cocoa solids[i]	--	--	--	--	--	---	--	--	--	--	0.6	--
Drinking water (µg.L^{-1})[j]												
WHO limits	400	700	--	3000	50	2000	70	--	10	70	3.0	10
Air (PM_{10}) (ng m^{-3})												
• Ecuadorian legislation [k]	--	--	--	--	--	--	--	--	--	--	5	--
• European Union [l]	--	--	--	--	--	--	20	--	500**	--	5	6

[a] Texto Unificado de Legislación Secundaria Medio Ambiental del Ecuador. Acuerdo Ministerial No 028 (2015).

[b] European Union Legislation Council Directive 86/278/EEC (1986)

[c] Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs.

[d] Commission Regulation (EU) No 488/2014 as regards maximum levels of Cd in foodstuffs.

[e] General Standard for Contaminants and Toxins in food and Feed (CODEX STAN 193-1995).

[f.g] CODEX Committee on cocoa products and chocolate (1997)

[h.i] CODEX Committee on contaminants in food: Proposed draft maximum levels for Cd in chocolate and cocoa-derived products (2017)

[j] World Health Organization (2011). Guidelines for Drinking-Water Quality

[k] Norma de la Calidad del Aire. Ministerio del Ambiente de Ecuador. Acuerdo Ministerial No 050. Registro Oficial No 464 (2011).

[l] Directive 2004/107/EC relating to As. Cd. Hg. Ni and PAHs in ambient air

*Only for inorganic As

**Limit value entered into force 01/01/2005

Table SI-3. Soil physico-chemical properties, sampling sites location (oil blocks or platforms and wells) and description (presence of a specific oil infrastructure) for all the samples collected in the North Amazon Region of Ecuador.

Code	Sampling site localization	GPS coordinates	Infrastructures	Description of oil activities in the area	Depth (cm)	pH water	TOC (g kg^{-1})	CEC (cmol kg^{-1})
1a	Auca Sur	18M 0289898	Auca Sur Oil Platform	300 m from	0-20	4.68±0.02	4.60	16.4
		9922049		oil pumping station	20-60	4.69±0.01	2.42	16.6
1b		18M 0289883		250 m from	0-20	4.53±0.01	5.65	16.2
		9922064		gas open flares	20-60	4.87±0.02	1.62	15.6
2a	Com. Cristal	18M 0290805	Auca 22 Oil well	10m from an	0-20	5.18±0.12	1.45	3.7
		9917733		oil waste pool	20-60	5.35±0.08	0.52	3.3
2b		18M 0290793		20m from	0-20	4.74±0.02	4.93	14.8
		9917712		Oil well drilling	20-60	5.02±0.05	2.18	14.3
3	Coop.	18M 0324986	Pichincha 2	Reinjection wells	0-20	6.03±0.03	18.50	13.0
	Pacayacu	9996216	Oil well	Oil waste pool	20-60	6.49±0.04	5.80	17.0
4a	Coop. Nuevo Oriental	18N 0326332	Shuara 9 Oil Platform	150 m from	0-20	4.67±0.03	4.99	17.6
		0006536		gas open flares	20-60	4.90±0.02	2.24	17.8
4b		18N 0326456		Oil waste pool	0-20	5.23±0.04	6.09	17.1
		0006653		Gas flares	20-60	5.32±0.03	4.20	8.6
4c		18N 0326531		Small forest	0-20	4.32±0.01	9.07	17.6
		0006760		300m from oil Platform	20-60	4.45±0.01	3.67	18.1
5	Dayuma	18M 0290721	Auca 9	Influence Area	0-5	4.51±0.09	18.12	16.7
		9926366	Oil well	of oil activities	5-20	4.61±0.01	7.62	11.7
					20-60	5.00±0.02	2.76	
6	Auca	18M 0290197	Oil block 61	Influence Area	0-5	5.35±0.04	13.78	13.3
		9924277		of oil activities	5-20	4.87±0.01	2.68	15.1
					20-60	5.04±0.01	2.21	
7	Com.Shuar Tzakim	18M 0298581	Oil block 17	Influence Area	0-5	4.57±0.03	17.38	17.2
		9917079		of oil activities	5-20	4.72±0.01	11.79	18.7
					20-60	4.72±0.01	4.85	
8	Com. Shuar Rio Tiputini	18M 029941	Hormiguero Oil Block	Oil pipelines	0-5	5.49±0.06	18.17	14.1
		9917462			5-20	5.60±0.02	7.81	16.4
					20-60	5.50±0.04	5.26	

Note: CEC measurements were only performed for the topsoil samples (0-20 cm).

(continued on next page)

Table SI-3. (continued). Soil physico-chemical properties, sampling sites location (oil blocks or platforms and wells) and description (presence of a specific oil infrastructure) for all the samples collected in the North Amazon Region of Ecuador.

Code	Sampling site localization	GPS coordinates	Infrastructures	Description of oil activities in the area	Depth (cm)	pH water	TOC (g kg-1)	CEC (cmol kg-1)
9	Com. Kichwa Manduro Yaku	18M 0271565 9945014	Gacela Oil Platform	Influence Area of oil activities	0-5	4.44±0.01	10.4	17.8
					5-20	4.37±0.01	7.54	18.0
					20-60	4.71±0.01	7.25	
10	Com.Flor de Manduro	18M 0270210 9943874	Gacela Oil Platform	Influence Area of oil activities	0-5	5.44±0.02	14.16	16.2
					5-20	5.08±0.08	4.09	18.3
					20-60	5.11±0.01	3.82	
11a	Shushufindi	18M 0314476 9991258	Aguarico 3 Oil Platform	Influence Area of oil activities	0-5	5.99±0.05	15.75	14.0
					5-20	6.09±0.01	8.11	16.0
					20-60	5.95±0.00	6.54	
11b		18M 0314428 9991247	Aguarico 3 Oil Platform	Influence Area of oil activities	0-5	6.00±0.02	10.41	15.5
					5-20	5.91±0.04	6.45	16.6
					20-60	5.54±0.04	3.33	
12	Coop. Nueva Aurora	18M 0319424 9980010	Cobra 1 Oil Platform Well 43	Influence Area of oil activities	0-5	6.15±0.01	4.23	8.8
					5-20	5.48±0.04	25.47	16.9
					20-60	5.39±0.09	8.99	
13a	Coop. Nueva Quevedo	18M 0312284 9979708	Oil Block 57	Influence Area of oil activities	0-5	5.12±0.01	10.55	16.7
					5-20	5.10±0.01	7.22	16.7
					20-60	5.28±0.01	4.13	
13b		18M 031253 9979651		Influence Area of oil activities	0-5	5.86±0.04	20.13	15.7
					5-20	5.93±0.03	15	17.0
					20-60	6.06±0.06	7.35	
14	Coop. Pacayacu	18M 0325025 9996216	Pichincha 2 Oil well	250 m far from oil pools	0-5	6.39±0.01	13.24	14.3
					5-20	6.23±0.04	14.22	15.4
					20-60	6.61±0.03	5.5	
15a	Coop. Pacayacu	18M 0325309 9996156	Pichincha 2 Oil well	250 m from a reinjection well	0-5	6.61±0.07	8.58	17.9
					5-20	6.52±0.10	7.05	18.3
					20-60	6.47±0.11	4.16	
15b		18M 0325405 9996064		50 m far from a reinjection well	0-5	6.65±0.03	10.66	9.0
					5-20	6.32±0.02	28.56	18.0
					20-60	6.42±0.04	7.88	

Note: CEC measurements were only performed for the topsoil samples (0-20 cm)

Table SI-4. Correlation matrix between soil physicochemical parameters and metal(loid)s content in soils (0-20 cm) for the provinces of Orellana and Sucumbíos, in the North Amazon region (NAR) of Ecuador. Bold numbers shown strong correlation (r^2>0.7)

	pH water	TOC	CEC	Mn	Ba	V	Zn	Cr	Cu	Ni	Co	Pb	As	Mo	Cd
pH water	1														
TOC	0.52	1													
CEC	-0.17	0.08	1												
Mn	0.50	0.44	-0.08	1											
Ba	**0.79**	0.57	-0.17	0.34	1										
V	-0.02	-0.23	-0.10	0.20	-0.39	1									
Zn	0.37	0.11	-0.53	0.49	0.26	0.50	1								
Cr	-0.35	-0.54	-0.18	-0.19	-0.59	0.28	-0.07	1							
Cu	**0.76**	0.45	-0.32	0.66	0.58	0.34	**0.71**	-0.34	1						
Ni	0.20	-0.12	-0.50	0.22	0.08	**0.72**	**0.74**	0.08	0.52	1					
Co	0.63	0.32	-0.29	**0.87**	0.41	0.48	**0.70**	-0.12	**0.87**	0.57	1				
Pb	0.01	-0.18	-0.48	0.28	0.15	-0.03	0.53	0.22	0.10	0.22	0.27	1			
As	-0.39	-0.40	-0.19	-0.40	-0.53	0.45	0.15	0.29	-0.26	0.37	-0.26	0.05	1		
Mo	-0.42	-0.33	0.29	-0.59	-0.53	0.26	-0.20	0.22	-0.38	0.04	-0.47	-0.36	0.65	1	
Cd	0.36	0.50	-0.26	0.47	0.48	-0.01	0.50	-0.25	0.44	0.35	0.47	0.29	-0.26	-0.41	1

Table SI-5. Metal(loid)s concentrations (μg L^{-1}) in waters in the North Amazon Region (NAR) of Ecuador.

	Mn	Ba	V	Zn	Cr	Cu	Ni	Co	Pb	As	Mo	Cd
Mean	11.4	47.39	2.12	325.93	0.87	4.73	0.73	0.11	0.68	0.62	1.55	0.03
Min	0.15	2.22	0.034	0.224	0.014	0.029	0.033	0.0003	0.004	0.019	0.004	0.001
Max	108.9	270.19	12.89	3148.87	10.57	46.62	3.41	0.80	5.55	4.35	19.77	0.18

Note: the term “waters” includes small streams, rain and groundwater daily used by local population as drinking waters.

End of the Manuscript

What about Esmeraldas?

II. Distribution, contents and health risk assessment of metal(loid)s in small-scale farms located in front of the main national refinery

The previous research article manuscript highlighted that soils, crops and air in the Northeast Amazon Region (NAR) of Ecuador showed high concentrations of metal(loid)s related to natural and anthropogenic sources. Depending on the population category, crop and water ingestion and inhalation seemed to be the main human exposure pathways for these toxic compounds. However, the impacts of oil activities are certainly not limited to the Amazon region, as refining activities are also developed near the Pacific coast. Actually, the main oil refinery of the country is located in the province of Esmeraldas, on the North Pacific Coast (NPC) (Figure IX) in front of a thermal power plant burning non-commercial oil byproducts. After five years of construction, the refinery started its operations in 1977 with a processing capacity of 55 615 barrels of crude oil with a range of 27.9 to 28.3 API (American Petroleum Institute) degrees and "medium" weight (EP Petroecuador, 2013) per day.

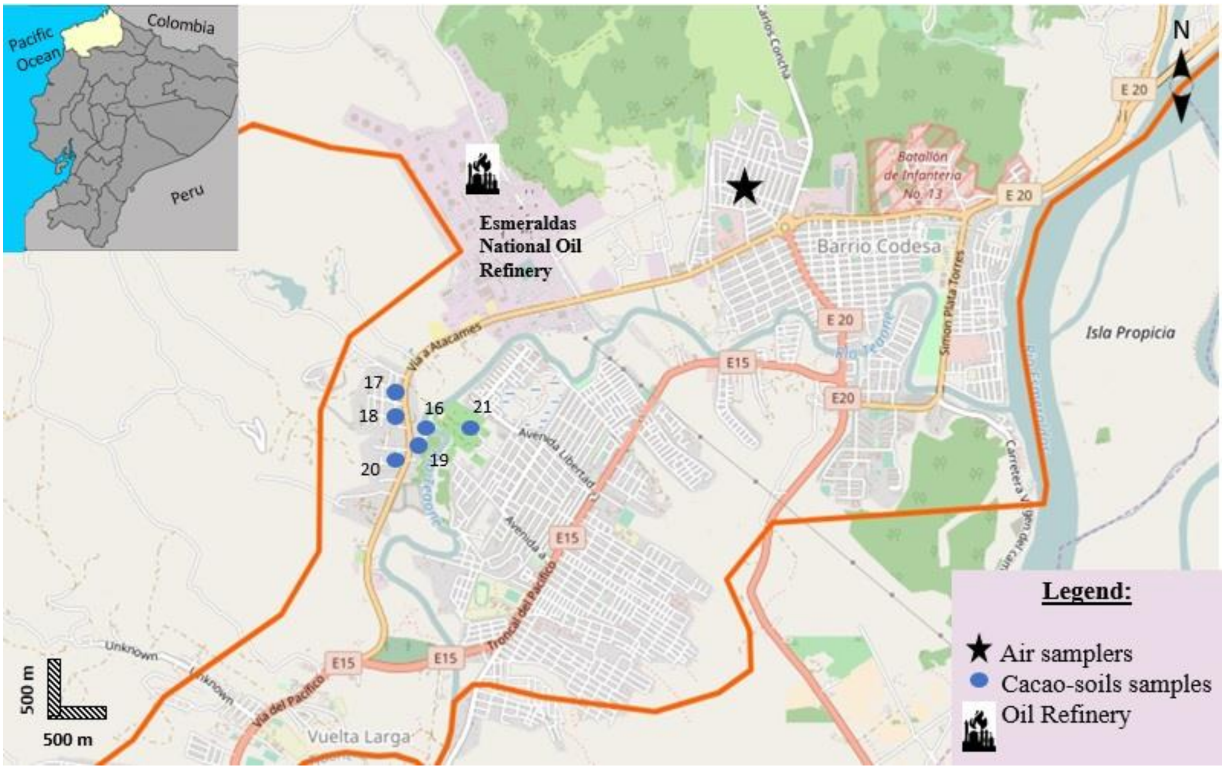

Figure IX. Sampling site locations in Esmeraldas (North Pacific Coast, NPC). The black star represents the location of two air samplers. Blue points represent the cacao and soil sampling points. The National Oil Refinery is depicted by the black pattern.

Crude oil, transported by a pipeline system, is stored in tanks before being processed (see Figure IV, in the **Introduction**, for more details). Once it enters the refinery, chloride salts are eliminated from the oil in order to reduce corrosion and fouling of the equipment (EP Petroecuador, 2013). In the next stage, oil, previously heated, enters into two atmospheric distillation units and passes through a fractionation tower where different quality products are obtained: LPG, naphtha, jet fuel and diesel 2. These products enter into a catalytic cracking unit to be transformed into heavier ones (gasoline, LPC, cyclic oils). Metals such as Mo, Ti, Rh and Pt are commonly used as catalyzers. In addition, residues like sulfur (S), water, halogens, olefins, arsenic (As) and other metals are reduced to improve the quality of the final products and to accomplish the environmental standards for S content in diesel. Residues produced during this step and industrial waters are sent to a treatment unit before being discharged in the Teaone River, a small tributary of the Esmeraldas River (EP Petroecuador, 2013).

Gases contained in the oil and those generated in the different units are used as fuel in the refinery. These gases require the elimination of sulfur and nitrogen compounds which otherwise would contaminate the environment when burned in furnaces and boilers (EP Petroecuador, 2013).

Sampling and methodology

A total of six small-scale farms were sampled between 2014 and 2016 within a radius of 1 km from the oil refinery. Moreover, the main difference between the fincas in the NPC and those from the NAR is the type of crops. Farms only cultivated cacao, except at sampling site 21 where there were also lemon trees.

We collected a total of 18 soil-composite samples from 0 to 20 cm depth. Air samplers were installed in an Elementary School in front of the refinery in La Florida district. PM_{10} samples were collected monthly on decontaminated quartz fiber filters. A total of 10 drinking water samples were collected in Teflon bottles and acidified in the field to prevent biogeochemical speciation processes.

Meta(loid) concentrations were also determined by ICP-MS after microwave (soils) or hotplate (filters) acid digestion.

Results and discussion

Metal(loid) concentrations in the topsoil (0-20 cm) are shown in Table I. Ba, V, Zn, Cr, Cu, Ni and Co levels were 1.6 to 2.8 times higher than the threshold established by the Ecuadorian Legislation. Chromium and Ni mean concentrations were twice the values found in the NAR. Arsenic, Ba and Cu concentrations were slightly higher than those reported in the Amazon region, whereas Mn concentrations were 3-fold lower (Figure X).

Table I. Total metal(loid) concentrations (mean ± standard deviation) in topsoils (0-20cm) in Esmeraldas (NPC). Mn, Ba, V, Zn, Cr, Cu, Ni, Co, Pb and As are expressed in mg kg^{-1} whereas Mo and Cd are expressed in µg kg^{-1} (denoted by the * sign).

Sampling code	Mn	Ba	V	Zn	Cr	Cu	Ni	Co	Pb	As	Mo*	Cd*
(TULSMA, 2015)[a]	--	200	76	60	54	25	19	10	19	12	5000	500
16	452±8	340±5	142±3	118.5±7.9	81.4±0.8	55.9±0.7	34.0±2.9	14.4±0.9	8.4±0.2	7.0±0.3	1794.3±186.4	449.8±54.0
17	375±7	273±4	143±3	86.3±0.9	94.3±0.9	53.8±0.6	36.2±3.1	14.5±1.0	8.1±0.2	8.5±0.4	1369.6±142.2	367.5±44.1
18	381±7	264±4	161±3	98.2±1.0	106.1±1.0	74.4±0.8	40.1±3.4	15.9±1.1	8.5±0.2	10.9±0.5	2073.7±215.5	385.7±46.3
19	416±8	382±6	148±3	90.4±1.2	126.2±1.2	51.3±0.6	138.9±11.7	18.9±1.3	7.5±0.2	9.9±0.4	3210.1±333.6	320.7±38.5
20	410±8	324±5	131±3	86.1±0.8	85.8±0.8	43.9±0.5	34.8±2.9	15.2±1.1	8.0±0.2	10.4±0.4	2720.8±282.7	453.1±54.4
21	465±9	438±6	136±3	81.8±0.7	73.9±0.7	34.7±0.4	30.8±2.6	15.5±1.1	6.4±0.1	4.9±0.2	740.0±76.9	325.1±39.0

[a] Unified Text of Secondary Environmental Legislation of Ecuador.

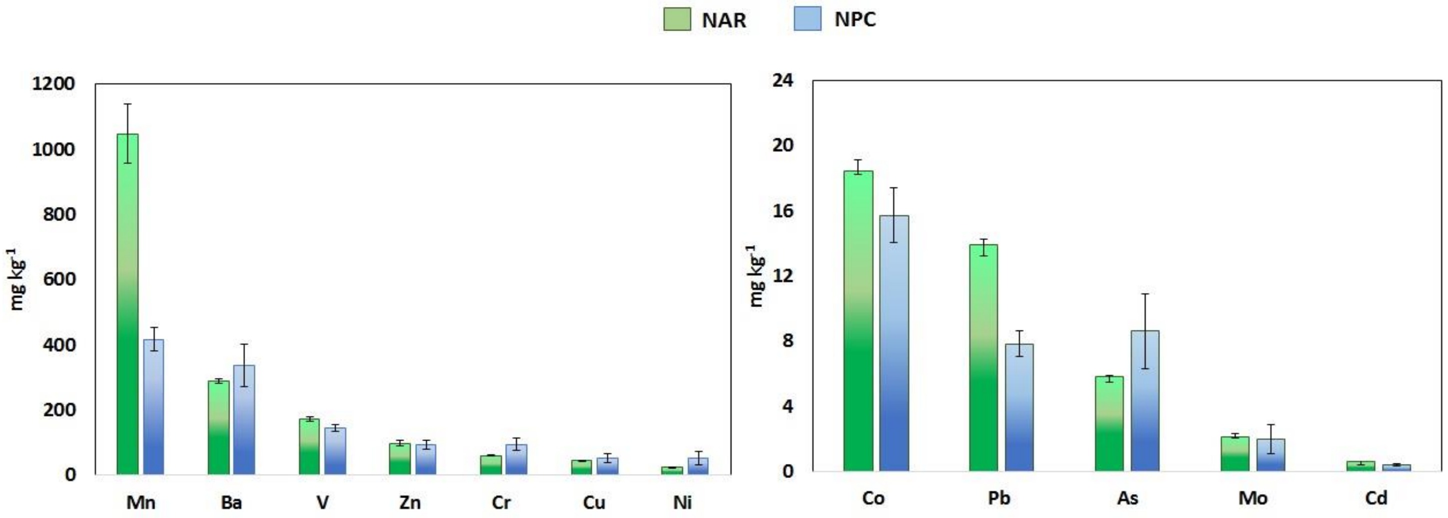

Figure X. Comparison between metal(oid) mean concentration in the North Amazon Region (NAR) and in the North Pacific Coast (NPC).

Table II. Metal(loid) annual mean concentrations* (mean ± standard deviation) in PM_{10} (ng m^{-3}) in Esmeraldas (North Pacific Coast, NPC).

ng m^{-3}	**Mo**	**Zn**	**Ba**	**Cr**	**Mn**	**V**	**Ni**
EU[a]	--	--	--	--	--	--	20
PM_{10}	226.67±24.79	56.50±39.50	61.04±42.47	61.39±5.73	30.25±15.98	22.10±6.87	16.59±3.93

ng m^{-3}	**Cu**	**Pb**	**As**	**Co**	**Cd**	**Sb**
EU[a]	--	200	6	--	5	--
PM_{10}	10.65±8.89	8.11±4.09	2.60±1.41	0.90±0.25	0.44±0.13	2.76±1.17

[a] European Union Directive 2004/107/EC

*sampling for 2015.

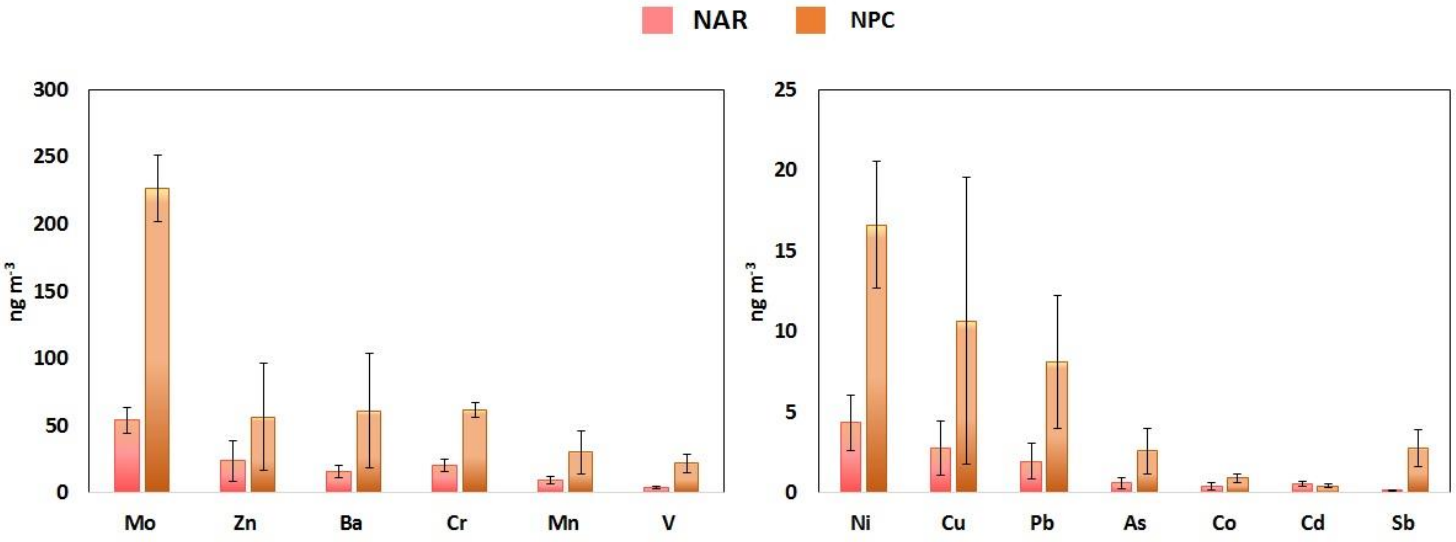

Figure XI. Comparison between metal(oid) annual mean concentration in the North Amazon Region (NAR) and in the North Pacific Coast (NPC).

However, it was not possible to calculate the EF (enrichment factor) because the control area selected for the NPC (in the province of Manabí, on the south Pacific Coast) was an area of intensive agriculture regarding the use of chemical products, potentially a high source of metal(loid)s, as discussed later in **Chapter 2 (Section 4.2).**

Aerosol (PM_{10}) annual mean concentrations in Esmeraldas (Table II) were also higher than in the NAR (Figure XI), especially for Mo and Ba. Arsenic, Cd, Ni and Pb did not exceed the limits established by the European Union.

Metal(loid)s in drinking water (Appendix C, Table C.8) remained below the WHO thresholds, except for Cd in one sampling point (16.34 $\mu g\ L^{-1}$). Concentrations of As, Ba, Cu, Cr, Mn, Mo, Pb, V and Zinc were higher in the NAR than in the NPC.

Health risk assessment calculations (HI, TCR) were also done, as in the previous research article manuscript, taking into account water and soil ingestion, inhalation, water and soil dermal contact. Crop ingestion won't be included because the diet in Esmeraldas is based on products purchased in local markets and not harvested from our study area farms. Risks from crop ingestion (concerning essentially cacao beans) will be focused on and discussed in **Chapter 2.**

Table III and Table IV show the values obtained for Hazard Index (HI) and Total Cancer Risk (TCR), respectively. Children and adults are equally exposed to metal(loid)s. HI values are greater than 1 suggesting a probable risk to develop non-carcinogenic adverse effects mainly by inhalation and water ingestion. This exposure pathway accounts for 95% in adults and more than 70% in children (Figure XII.A). Soil ingestion represented almost 29% of the exposure to these compounds in children whereas in adults it is less than 5%.

Table III. Hazard Index (HI) for each exposure pathway for adults and children from Esmeraldas (North Pacific Coast, NPC).

Exposure route/ Population	Water ingestion	Soil ingestion	Inhalation	Water dermal contact	Soil dermal contact	TOTAL
Adults	3.23×10^{-1}	1.29×10^{-1}	1.91	4.45x10-4	2.10×10^{-2}	2.39
Children	1.40	1.12	1.27	1.70x10-4	4.94×10^{-3}	3.80

TCR values are two to four times higher than the reference value proposed by the U.S. EPA. Inhalation was the main exposure pathways for carcinogenic effects in adults and children, accounting for 95% (Figure XII.B).

The elements with the highest and lowest HQ were Cr and Sb, respectively. The highest CR were obtained for As and Cr.

If we consider the same exposure routes in the Amazon region for adults and children, HI were 2.05 and 4.87, respectively, while TCR were 2.72x10^{-4} and 2.38x10^{-4}, respectively. Comparing these values to those of the NPC, we can conclude that population is equally exposed in both areas and that both population groups are vulnerable to the potential effects of these chemical compounds.

Table IV. Total cancer risk (TCR) for each exposure pathway for adults and children from Esmeraldas (North Pacific Coast, NPC).

Exposure route/ Population	Water ingestion	Soil ingestion	Inhalation	Water dermal contact	Soil dermal contact	TOTAL
Adults	1.28x10^{-5}	3.63x10-^{6}	3.52x10^{-4}	1.90x10^{-8}	1.88x10^{-6}	3.71x10^{-4}
Children	1.11x10^{-5}	6.29x10-^{6}	3.05x10^{-4}	1.70x10^{-8}	7.18x10^{-7}	3.23x10^{-4}

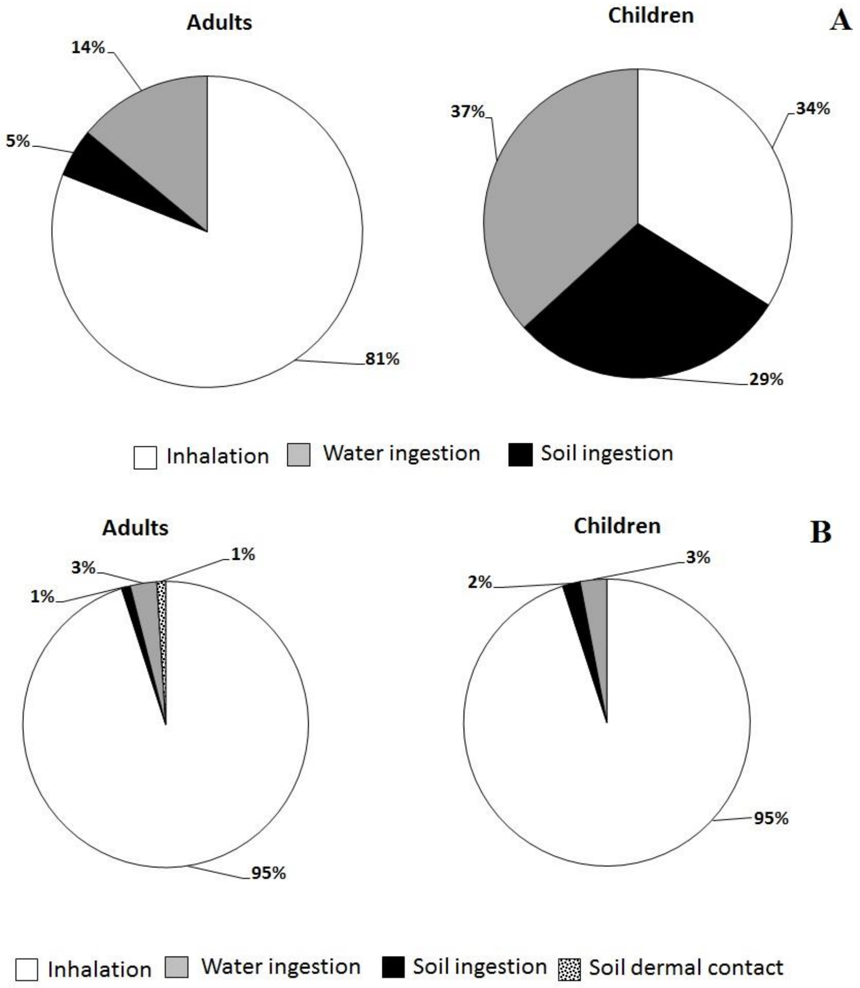

Figure XII. A. Hazard Index (HI) for non-carcinogenic health effects of metal(loid)s and **B.** Total Cancer Risk (TCR) considering inhalation, water and soil ingestion and soil dermal contact routes for adults and children living in Esmeraldas (North Pacific Coast, NPC).

To conclude:

High levels of metals were found in the small-scale farms of the NPC, close to the oil refinery in Esmeraldas. As this is considered an industrial site, it was expected that concentrations of metals would be higher than those reported for the NAR. However, only metal(loid)s in the air compartment showed a clear difference in concentrations, being four times higher than in the NAR. Thus, inhalation is one of the major exposure routes for non-carcinogenic and carcinogenic effects in children and adults from this area.

Chapter 2. Transfer of metal(loid)s in the air-soil-crops continuum

Foreword

As plants are immobile and therefore unable to escape stressful environments, they are constantly exposed to environmental pollutants such as metals and metalloids. Plants absorb, accumulate and integrate these inorganic pollutants into their systems. Depending on their sensitivity level to pollutants and on their ability to bioaccumulate trace elements, plants show visible changes including alteration of their biochemical and physiological processes (Rai, 2016). Consequently, metal accumulation in vegetables usually consumed by people may pose a direct threat to human health (Islam et al., 2015).

What are the metal transfer mechanisms involved in plant uptake?

Essential and non-essential elements from soils are taken up by plants as a response to concentration gradients generated by the uptake of specific ions by roots or by diffusion of elements in soil (Peralta-Videa et al., 2009). In adverse conditions (i.e., contaminated areas) and as a self-defense mechanism, some plants are able to tolerate high concentrations of metals like As, Cd, Ni and Pb. But “tolerate” doesn’t mean “accumulate.” Toxic metals are usually retained and detoxified in root tissues, reducing translocation to leaves. The opposite behavior, the distribution of toxic elements to the aerial organs, is called “accumulation” (Rascio and Navari-Izzo, 2011). Then, accumulation of toxic elements can vary between and within plant species and also depends on geographical conditions (Li et al., 2009). As an example, the Figure XII shows the mechanisms of transport and accumulation involved for Cd in the soil-plant continuum. Complex processes imply modifications, especially to the synthesis of proteins and enzymes. As a result, specialized transporters with the possible contribution of essential metal transporters are able to translocate Cd from roots to shoots (Clemens et al., 2013; Shao et al., 2017). Although root uptake is the major pathway for metal absorption by plants, foliar uptake has also been identified as a potentially significant route of Cd (or other metals) exposure (Shahid et al., 2016). Indeed, leaves can trap small airborne particles but the extent of this mechanism depends on morphological differences between plants such as cuticular layer with waxes, presence of trichomes, stomatal types, etc. (Gajbhiye et al., 2016).

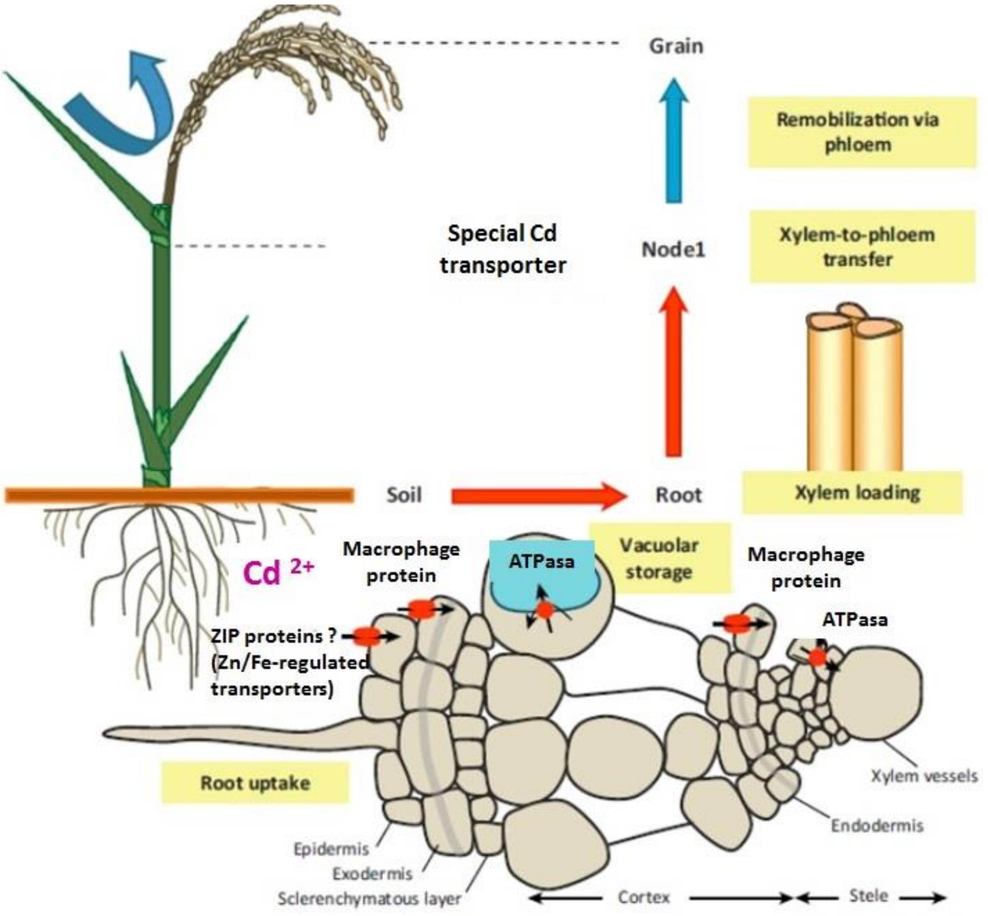

Figure XIII. Transfer and accumulation mechanisms involved for Cd in the soil-plant continuum for rice plant. Uptake into the root cell symplasm (transport through the cells), retention in the root through vacuolar storage, xylem loading (transport of dissolved nutrients from roots to shoots), xylem-to-phloem transfer (transport of metabolic products), accumulation in the grains and remobilization processes are exposed in this scheme, modified from Clemens et al. (2013).

What happens when people eat a fruit or a vegetable contaminated by a toxic metal?

It has been reported that nearly half of the mean ingestion of Pb, Cd and Hg through food is due to plant origin: fruits, vegetables and cereals (Islam et al., 2015).

Two main processes occur at the same time during the digestion phase:

- mechanical transformations that reduce the size of food particles—mainly occurring in the mouth and stomach—and
- enzymatic transformations to hydrolyze macromolecules into smaller constituents that are absorbed into the bloodstream—mainly in the small and large intestine (Guerra et al., 2012).

As we already discussed in Chapter 1, human health risk assessment is based on the total concentration of a contaminant measured in air, water or crops. However, only a percentage of the ingested toxic compound is released from the food matrix into the gastrointestinal tract and potentially available for absorption. This percentage is called the "bioaccessible fraction" (Oomen et al., 2002). The "bioavailable fraction" is the fraction of ingested component that is stored or can be available for physiological functions (Guerra et al., 2012). Then, the contaminant must be bioavailable (in its dissolved phase) to reach the blood stream (Figure XIV). In the case of metal(loid)s, the accessible fraction is produced mainly in the upper part of the digestive tract, particularly in the stomach compartment (Cave et al., 2011).

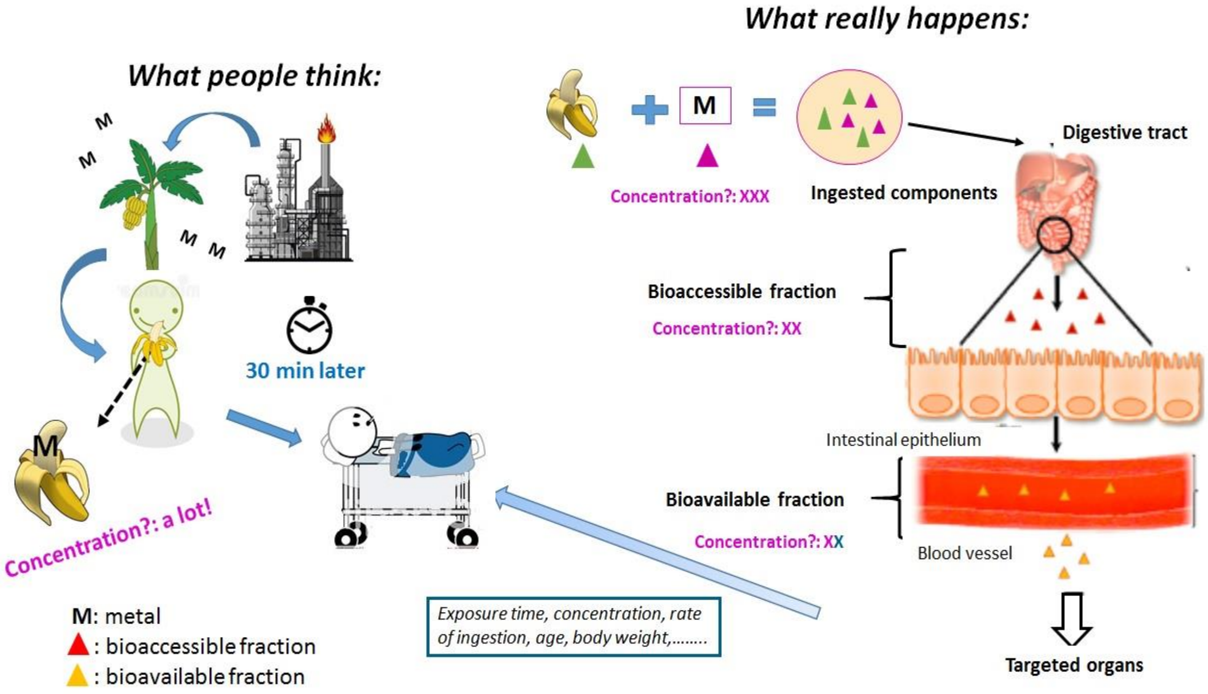

Figure XIV. Example of the ingestion of a contaminated banana (with a metal, M) showing the differences between bioaccessible and bioavailable fractions (Adapted from: Guerra et al., 2012)

Several models have been developed to determine the bioaccessible fraction of a contaminant using synthetic gastric fluids (Guerra et al., 2012). Among them, the Unified Bioaccessibility Method (UBM) developed by the Bioaccessibility Research Group of Europe (BARGE) has been widely used for contaminated soils (Pelfrêne et al., 2011) but also for vegetables (Pelfrêne et al., 2015; Xiong et al., 2014). The protocol simulates the human gastro-intestinal tract through three different compartments: mouth (5 minutes), stomach (1 hour) and small intestine (4 hours). The procedure of the *in vitro* test is presented in Figure XV. Table V shows the composition of the synthetic solutions, but only for the oral-gastric phase.

Among the collected crops, is there one that seems to accumulate a toxic metal(loid)?

Concentrations of non-essential metal(loid)s in crops from the NAR were usually below the limits of detection (as previously discussed in Chapter 1). However, Cd levels in some collected cacao beans were higher than in soils and exceeded the European recommended value, deserving more attention.

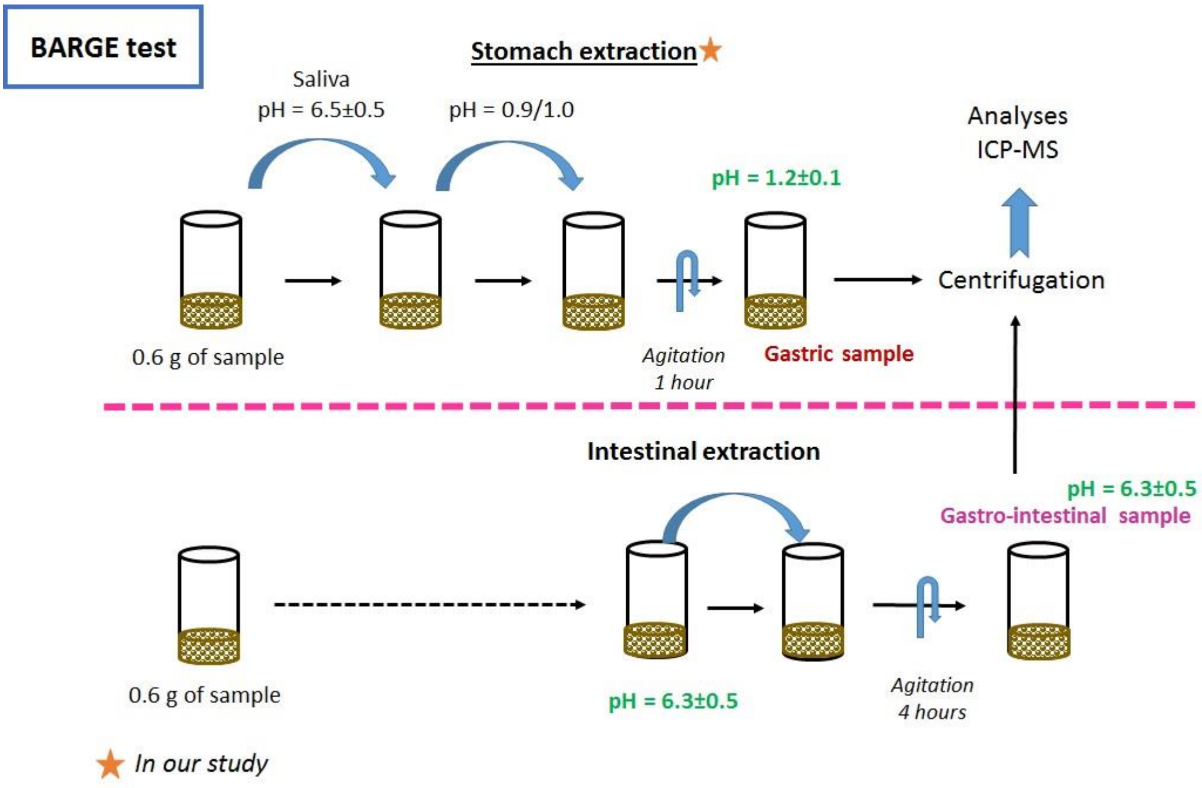

Figure XV. Description of the BARGE test**.** (Modified from: Caboche, 2009)

Table V. Composition of digestive solutions for the BARGE test, according to Denys et al. (2009).

	Saliva solution (pH= 6.5±0.5)	**Gastric solution (pH= 1.0±0.2)**
Inorganic solution*	448 mg KCl 44 mg NaH_2PO_4 100 mg KSCN 285 mg Na_2SO_4 149 mg NaCl 0.9 ml 1M NaOH	1376 mg NaCl 133 mg Na_2PO_4 412 mg KCl 200 mg $CaCl_2$ 153 mg NH_4Cl 4.15 ml HCl (37% w/w)
Organic solution*	100 mg Urea	325 mg glucose 10 mg glucuronic acid 42.5 mg urea 165 mg glucosamine hydrochloride
Reagents added to the inorganic and organic solutions	72.5 mg alpha-amylase 25 mg mucin 7.5 mg uric acid	500 mg bovine serum albumin 1500 mg mucin 500 mg pepsin

*masses indicated for 250 ml of solution.

As we have already mentioned in the Introduction, Cd is toxic for several biological tissues. Long-term exposure to Cd produces nephrotoxicity, osteotoxicity, immunotoxicity and it is also carcinogenic. Absorption from the gastrointestinal tract is the main route of Cd exposure in humans (Klaassen et al., 2009). Moreover, cacao is widely cultivated in Ecuador and represents a product of high economic concern.

Why is cacao important for Ecuador?

Between 5 and 6 million farmers in developing countries produce around 90% of cacao worldwide. For these farmers, cacao comprises about 70-100% of their annual household incomes (Kongor et al., 2016). In Ecuador, cacao bean production for 2015 reached 264 000 metric tons, up 10% compared to 2014 (Anecacao, 2015) and equivalent to 800 million USD. About 80% of the production is concentrated in the coastal provinces of Manabí, Los Ríos and Guayas, whereas cacao cultivation in the Amazon provinces accounts for 7% of the total planted area (Vega and Beillard, 2015).

Figure XVI shows the different cacao varieties collected during the second part of the MONOIL Program fieldwork in 2016. The "Nacional" and the CCN-51 are the most valuable varieties on the international market because of their special flavor, used to produce "fino de aroma" chocolate.

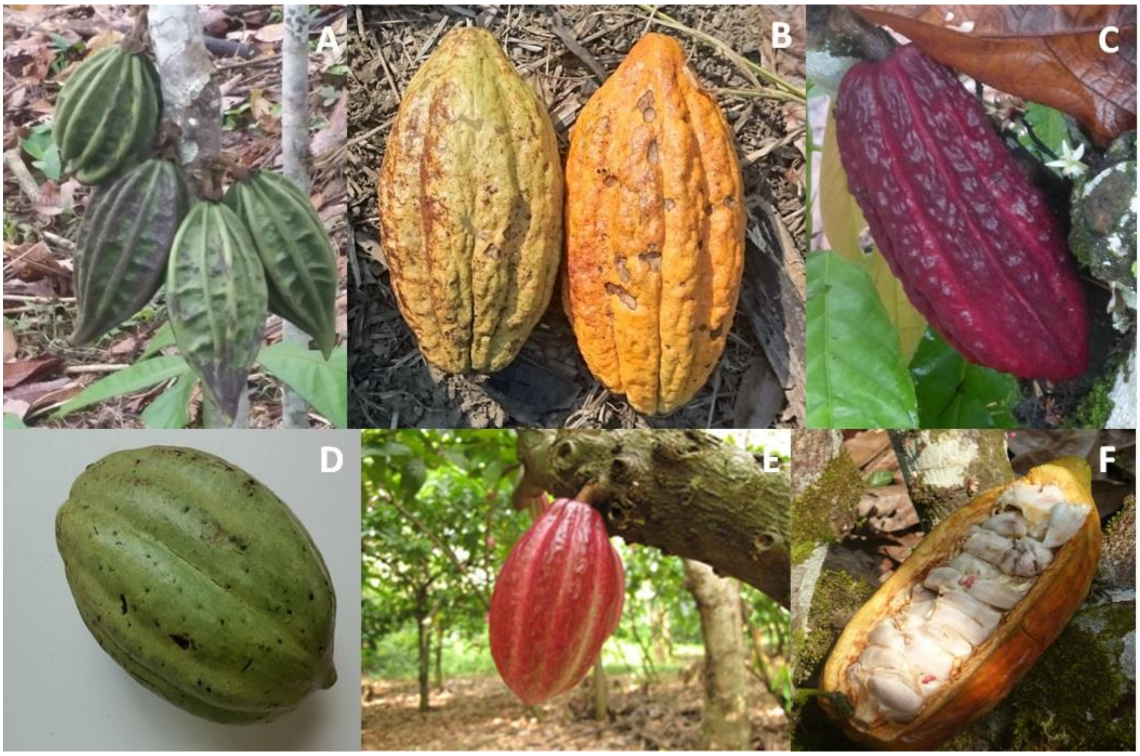

Figure XVI Cacao varieties collected in Ecuador. A. Nativo Amazonico. B. Nacional. C. CCN-51. D. Zamora. E. Forastero. F. Cacao pod with beans inside (from Nacional variety). (Pictures taken by: Fiorella Barraza).

Besides, value-added products like chocolate have specific international directives for contaminants and substances that have arrived in food during production, packaging or transport in order to ensure public health (European Union, 2015).

After the first findings about Cd in cacao tissues, four important questions came up:

- What is the distribution of Cd contamination in cacao cultivated in the NAR and NPC regions in Ecuador?
- Is Cd equally distributed within the cacao plant tissues?
- Where does Cd come from?
- Is there a risk associated with eating cacao-based products from these impacted and investigated areas?

The answers will be given in the following article *published* in the ***Environmental Pollution*** journal.

Note: *Full article is available on Appendix D (Page 223)*

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I.

Cadmium bioaccumulation and gastric bioaccessibility in cacao: A field study in areas impacted by oil activities in Ecuador☆

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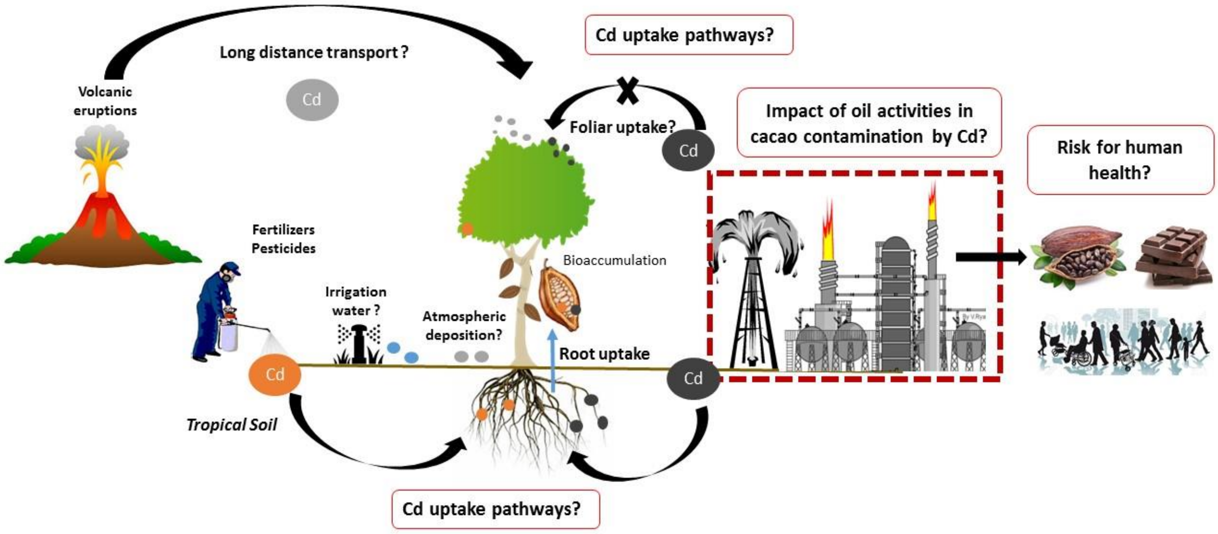

Abstract:

Cacao from South America is especially used to produce premium quality chocolate. Although the European Food Safety Authority has not established a limit for cadmium (Cd) in chocolate raw material, recent studies demonstrate that Cd concentrations in cacao beans can reach levels higher than the legal limits for dark chocolate (0.8 mg kg^{-1}, effective January 1st 2019). Despite the fact that the presence of Cd in agricultural soils is related to contamination by fertilizers, other potential sources must be considered in Ecuador. This field study was conducted to investigate Cd content in soils and cacao cultivated on Ecuadorian farms in areas impacted by oil activities. Soils, cacao leaves, and pod husks were collected from 31 farms in the northern Amazon and Pacific coastal regions exposed to oil production and refining and compared to two control areas. Human gastric bioaccessibility was determined in raw cacao beans and cacao liquor samples in order to assess potential health risks involved. Our results show that topsoils (0–20 cm) have higher Cd concentrations than deeper layers, exceeding the Ecuadorian legislation limit in 39% of the sampling sites. Cacao leaves accumulate more Cd than pod husks or beans but, nevertheless, 50% of the sampled beans have Cd contents above 0.8 mg kg^{-1}. Root-to-cacao transfer seems to be the main pathway of Cd uptake, which is not only regulated by physico-chemical soil properties but also agricultural practices. Additionally, natural Cd enrichment by volcanic inputs must not be neglected. Finally, Cd in cacao trees cannot be considered as a tracer of oil activities. Assuming that total Cd content and its bioaccessible fraction (up to 90%) in cacao beans and liquor is directly linked to those in chocolate, the health risk associated with Cd exposure varies from low to moderate.

1. Introduction

Ecuador is ranked as the sixth largest oil producing country in South America and 27th in the world (Ecuador Oil Production, 2016). Since the 1960s, most extraction activities have taken place in the northeastern Amazonian region, while refining occurs in Esmeraldas along the Pacific coast. During operations of the Texaco Oil company (1960s–1992), outdated practices and technologies used for oil production (Buccina et al., 2013) generated millions of gallons of untreated toxic waste, gas and oil, which were partially released into the environment (San Sebastián and Hurtig, 2005). Local populations were and continue to be exposed to a mixture of toxic compounds, such as trace metals and polycyclic aromatic hydrocarbons (PAHs), from water consumption, inhalation of airborne particles or ingestion of contaminated crops.

Although the economy of the country revolves around the oil industry, agriculture is the second most important activity in exportation. Ecuador is currently the fourth largest cacao producer in the world from which 87% is directly exported as cacao beans to Europe, mainly France, Germany and England, as well as to the United States (Anecacao, 2015; ProEcuador, 2013). *Theobroma cacao* is mostly cultivated in the western part of the country but the growing area now extends to the Amazon region, covering a total area of 433,978 ha. Cacao plantations utilize a wide variety of soils: from clayey highly eroded soils to volcanic sands and silty soils, with pH varying between 4 and 7. Nevertheless, cacao requires depth, well drained and clay loamy soils, with high organic matter content in order to grow in optimal conditions (Quiroz and Agama, 2006).

The cocoa tree, as a plant species, includes a wide range of vastly different varieties. In order to characterize its forms and cultivars, morphological (e.g. flowers), agronomic (e.g. resistance to diseases, fruit shape and grain size) and molecular (e.g. enzymes) properties are frequently used (Dostert et al., 2012). Of all the varieties cultivated in Ecuador, the 'Nacional fino de aroma' and CCN-51 are the most appreciated on the international market (Amores et al., 2009; Loor et al., 2009).

However, there is recent international concern regarding the presence of trace metals in cacao tissues. Recent studies have shown that As, Bi, Cr, Cd, and Pb can be accumulated in cacao beans, pod husks, and cacao-based products (Bertoldi et al., 2016; Chavez et al., 2015; Huamani et al., 2012). Among these elements, cadmium (Cd), a non-essential trace metal, seems to accumulate mainly in the edible parts of cacao, which entails potential risks for human health by ingestion of contaminated products. It has been also reported that cacao beans have different Cd concentrations depending not only on the variety but also on the geographical site, with mean concentrations reaching 1.4 mg kg^{-1} in South America, 0.5 mg kg^{-1} in East Africa and Central America, 0.3 mg kg^{-1} in Asia, and 0.09 mg kg^{-1} in West Africa (Bertoldi et al., 2016).

Indeed, Cd is considered to be one of the most toxic metals that exhibit adverse effects on all biological processes. It reveals very harmful impacts on the environment and food quality (Kabata-Pendias and Szteke, 2015). Based on its carcinogenic effects and, more precisely, the adverse effects observed in the brain, kidneys and bones, the European Union classified Cd and its chlorinated, oxygenated, sulfurated, and sulfate derivatives, into category 1B. Similarly, the International Agency of Research on Cancer (IARC) and the United States Environmental Protection Agency (US EPA) classified Cd into Group 1 and class B, respectively.

In a global context, the major sources of Cd seem to be atmospheric deposition from industries, sewage sludge, and P fertilizers (Kabata-Pendias, 2011). But in Ecuador, Cd can be present in the environment due to natural sources such as volcanic eruptions, which are particularly frequent in the Andes, or the leaching of volcanic rocks, as well as a result of anthropogenic activities including industrial and oil processing (FAO and WHO, 2015). Indeed, it is well known that crude oil can naturally contain metal(oid)s (e.g. As, Cr, Cd, Cu, Co, Ni, Pb, Ti, V, Zn) as a result of the mineral composition of the source rock, or these elements may be added during oil production (e.g. Ba and Mo), refining, transportation, or storage (Fu et al., 2014a; Khuhawar et al., 2012; Lienemann et al., 2007). As a consequence, soils near oil fields can be contaminated by trace metals that are easily transferred to crops (Fu et al., 2014b)

However, to our knowledge, no previous study has investigated the link between Cd bioaccumulation in cacao plants and the proximity of oil production or refinement infrastructures. The Ecuadorian Amazon region houses a vast network of roads, pipelines, and oil facilities (San Sebastián and Hurtig, 2005) that are located close to both large- and small-scale farms, called '*fincas*.' Along the Pacific coast, the city of Esmeraldas is also exposed to air contamination by organic and inorganic compounds due to emissions from the national oil refinery, while cacao crops are cultivated in the adjacent areas. Even though it is currently well known that cacao tissues can contain high concentrations of Cd, the sources and transfer mechanisms (e.g. by foliar and/or root uptake) and the bioaccumulation processes remain poorly described in the literature and several questions remain unsettled.

It should be noted that 90% of human exposure to Cd for the non-smoking population is related to food products (EFSA, 2012). Cadmium dietary exposure is estimated to about 2.04 µg kg^{-1} of body weight per week over a lifetime assuming an average life span of 77 years. Within the 20 main groups described in the Foodex (food classification system), vegetables and confectionary products account for 11 and 3% of food exposure to Cd, respectively, and more precisely, cacao beans and chocolate contribute from 4 to 26% and from 15 to 92%, respectively, depending on the consumer's age (EFSA, 2012).

Nevertheless, the determination of the fraction of total Cd concentration that may potentially affect human health after ingestion has not yet been studied in cacao-based products. Beyond the determination of total trace metal concentrations within a given food product, health risk assessments must also consider their gastric bioaccessibility (Caboche, 2009). This parameter indicates the maximum amount of a compound that, after being released from its matrix during digestion, can be absorbed by the human intestinal epithelium and then enter the blood stream (Hu et al., 2013; Intawongse and Dean, 2006; Peixoto et al., 2016).

A large-scale field study in two areas highly impacted by oil activities in Ecuador was therefore conducted in order to determine Cd concentrations in soils from small-scale farms in the northern Amazon region and near the national oil refinery along the Pacific coast. The aims of this study are to (i) investigate if oil activities constitute a real source of Cd contamination in cacao tissues, (ii) better understand the mechanisms of Cd transfer from soils to cacao plants and its potential bioaccumulation in edible parts, and finally (iii) assess the potential health risk involved after ingestion of cacao-based products, taking the gastric bioaccessibility of Cd in raw materials (cacao beans and liquor) into account.

2. Materials and Methods

2.1 Global localization and site descriptions

Cacao samples and associated soils were collected between 2014 and 2016 in the Ecuadorian provinces of Orellana and Sucumbíos in the northern Amazon region (NAR), close to oil production fields, and in Esmeraldas, along the north Pacific coast (NPC), in a two km radius from the national oil refinery.

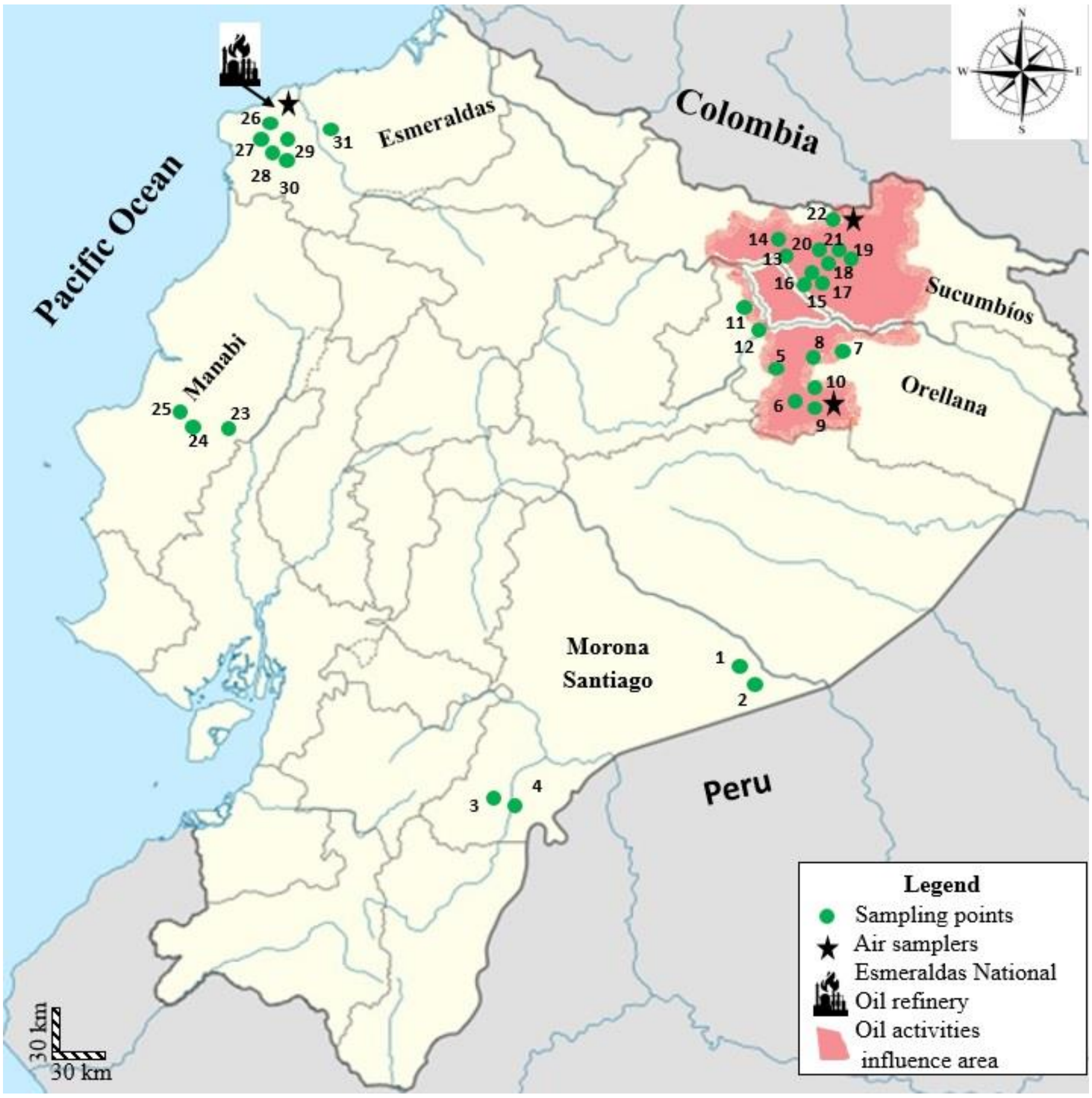

Fig 1. Sampling sites location in the Ecuadorian provinces of Sucumbíos, Orellana, Esmeraldas, Manabí and Morona-Santiago. Each sampling point is materialized by a green circle and its associated number. The pink area represents the large zone impacted by oil production activities (according to Pérez Naranjo et al. (2015)), whereas the Esmeraldas National Oil Refinery is described by a little pattern. Air samplers are depicted by black stars.

For comparison, two other provinces were studied: Manabí (MC) in the south of the Pacific coast and Morona-Santiago (MS) in the southern Ecuadorian Amazon. These additional sites were considered control areas in the absence of any oil production or mining extraction activities.

A total of 31 small-scale farms (Fig.1) were chosen within the large area of oil environmental liability, according to the Program of Social and Environmental Reparation and the Ministry of the Environment (PRAS and MAE, 2010), where oils spills, accumulation of drilling muds, formation of water in open pits, and emissions of waste gas into the air are the most common sources of contamination (Fig. SI-1, Supplementary Information). Sampling sites in Esmeraldas were chosen because of their proximity to the national oil refinery, that refines 110,000 barrels of oil per day (EP PetroEcuador, 2016), and the central thermoelectric power plant, which has 12 smokestacks (Ministry of Electricity and Renewable Energy of Ecuador, 2016).

The wet season in the Pacific coastal region usually occurs from mid-December until May, whereas the dry season begins in June and lasts until mid-November. By contrast, precipitation in the Amazon region is continuous over the year but more pronounced between June and September. The total annual precipitation can reach more than 3000 mm in both areas, ranging between 200 to 500 mm per month. The annual mean temperature is around 25 °C, and ranges from 13 °C to 38 °C (FAO, 2006; INAMHI, 2014; Pourrut, 1995). Farm crops are not artificially irrigated except for those located in Portoviejo (MC).

2.2 Soil, cacao, and aerosols sampling

For each sampling point, one mature and healthy cacao pod and ten leaves were selected from three different trees. Three soil samples around each cacao tree were also collected from the surface to 60 or 80 cm depth, according to the site, and to 1 m depth in the control area, namely between three and five horizons. Different cacao varieties (Nacional, CCN-51, Forastero) were collected whenever possible, depending on their availability in the selected *finca*. Three genotypes of Nacional cacao (EET103, EET116, EET576) were also provided by the National Institute of Agriculture and Livestock Research of Ecuador (INIAP-Portoviejo). Amazon Native, Zamora, and CCN-51 cacao varieties were also collected in the control areas of Gualaquiza and San Miguel (MS). Additionally, two cocoa liquor samples from Pacayacu (NAR) were collected to verify Cd gastric bioaccessibility in cacao beans and mass, and to assess health risks involved by their consumption. Detailed information of the cocoa liquor procedure is provided in Section 1 of the Supplementary Information.

Aerosol samples, and more precisely PM_{10} (particulate matters of 10 micrometers or less in diameter), were collected monthly on pre-calcined quartz fiber filters using a low volume air sampler (3 L min^{-1}, Microvol® devices) in three sites. Two air samplers were settled in the NAR on the roof of two houses located 300 m away from gas burning open flares (15 °E and 15 °S, downwind direction), and one was placed in the NPC in an elementary school, 1500 m east of the national oil refinery and downwind of the major wind directions, S-SE, S, and S-SW (EP PetroEcuador, 2013).

All samples were freeze-dried and transported to France for chemical analysis. Soil composite samples were then air-dried, sieved at 2 mm, and ground with agate ring mills. Cacao beans for the same site and variety were separated from their pod husks, mixed together, air-dried in Teflon trays for three days before being oven-dried at 40 °C for two days. Finally, cacao leaves were oven-dried at 40 °C and ground using an agate mortar and pestle and liquid nitrogen.

2.3 Soil physico-chemical properties

Soil morpho-pedological descriptions were performed in the field and soil classes were determined according to the United States Soil Taxonomy (USDA, 1999), and to the soil maps of the Ecuadorian Ministry of Agriculture, Livestock, Aquaculture and Fisheries and the General Coordination of the National Information System (MAGAP and CGSIN, 2012). Soil pH was measured in the laboratory using a high precision pH meter (744 Metrohm pH meter) using a 1:5 dilution. The cation exchange capacity (CEC) was measured only for the topsoil samples (i.e. top layers of 0–5 cm and 5-20 cm) by spectrocolorimetry using cobalt hexamine trichloride (Fallavier et al., 1985). The total organic carbon (TOC) was determined by combustion using a HORIBA EMIA-320V carbon/sulfur analyzer.

2.4 Cadmium content determination in soil, aerosols, and cacao samples

For each soil sample, 0.1 g was digested with a mixture of bi-distilled and suprapur acids (11 ml of aqua regia solution and 3 ml of HF) using a Mars 5-CEM microwave. Aerosol samples on quartz fiber filters were digested on a hotplate using 9 ml of aqua regia, 1 ml of H_2O_2 and 2 ml of HF. Mineralization solutions were then completely evaporated at 60 °C on a hotplate and the dry residue was resuspended by adding 0.5 ml of HNO_3.

For plant samples (leaves, pod husks, beans), 0.125 g were mineralized with a hotplate system (Digripep, SCP Science) using 5 ml of HNO_3 and 5 ml of H_2O_2. After digestion, samples were diluted with bi-distillated water and filtered through a 0.22 µm cellulose acetate membrane. The same protocol was applied for the two cocoa liquor samples.

All samples were diluted with HNO_3 0.37 N before ICP-MS (inductively coupled plasma mass spectrometry) analyses (Agilent 7500) in order to determine trace metal, and especially Cd, concentrations. Certified reference materials, SRM 2709 San Joaquin Soil, SRM1648a Urban Dust and SRM1515 apple leaves, were used to validate the mineralization methods for soil, aerosols, and plant samples, respectively. Recoveries of 90-110% were calculated for Cd in all sample types. Each composite sample was analyzed in triplicate and measured values are presented as the mean ± standard deviation (SD).

2.5 Cadmium transport and bioaccumulation in edible parts

The global enrichment factor (GEF) was calculated to determine the degree of metal accumulation in fruits of cacaos grown on a contaminated site with respect to control zones. The GEF method calculation normalizes the measured metal on contaminated sites to the metal content contribution from the biogeochemical background according to Schreck et al. (2014):

Equation (1):

$$\textbf{GEF} = \frac{[Cd]\ cacao\ beans\ grown\ on\ contaminated\ sites}{[Cd] cacao\ beans\ from\ control\ site}$$

where [Cd] is the Cd concentration of dry weight (d.w.) in mg kg^{-1}.

The Cd concentration in cacao beans samples from control sites was calculated as the mean value of Cd results in samples 3 and 4 (0.14 ± 0.01 mg kg^{-1}), corresponding to the CCN-51 variety, collected in the MS area of the Amazon region. These sites were chosen because there were not significant variations between Cd concentrations in topsoils and deep soils, and because they were located in the Amazon control area (MS). Even if CCN-51 can differ from the Nacional variety when unhybridized, we decided to use it for our estimations because it is more representative of our sampling (13 of 34 cacao samples) compared to the Native species or Zamora variety. Besides, CCN- 51 is often used as a parent in crosses on the breeding programs in Ecuador (Boza et al., 2014). It is also common to find Nacional fino de aroma and CCN-51 hybrids, which can favor natural hybridization, on local farms.

Cadmium transport from soil to cacao beans was also measured by the bioaccumulation factor (BF) (Mombo et al., 2016), which is defined as:

Equation (2):

$$\textbf{BF} = \frac{[Cd] cacao\ beans}{[Cd]\ soil(5-20\ cm)}$$

In essence, the BF represents the ratio between the total Cd concentration in the cacao beans compared to that in the surface soil (5–20 cm), as the root system in cacao trees is almost completely concentrated in the first 20 cm of the soil layer with most of the fine roots in the first 10 cm (Almeida and Valle, 2007). GEF and BF factors were calculated only for samples for which Cd concentrations in beans exceeded 0.8 mg kg^{-1}, according to the legal Cd limit established by the European Union for dark chocolate (i.e. contains more than 50% of total dry cocoa solids), beginning January 1st, 2019 (European Comission, 2014).

2.6 Human gastric bioaccessibility of Cd

Gastric bioaccessibility (GB) was only determined for cacao beans (i.e. edible part of cacao tree) and cocoa liquor samples with Cd concentrations up to 0.8 mg kg^{-1} in order to detect a potential risk to human health. GB was measured according to the BARGE (Bioaccessibility Research Group of Europe) unified method (Denys et al., 2012; Uzu et al., 2014; Mombo et al., 2016). Using synthetic digestive solutions, this two-step extraction procedure mimics pre-digestive chemical processes that occur in the mouth and stomach. Digestive fluids were prepared as described by Denys et al. (2009).

Dried and ground samples (0.6 g) were mixed with 9 mL of saliva (pH 6.5) and 13.5 mL of gastric solution (pH 1.1), as reported by Denys et al. (2012) and Wragg et al. (2011). This range of sample-to-fluid ratios is assumed to cover all physiological conditions *in vivo*, as described by Oomen et al. (2003) for soil samples. If the pH of the final solution was not in the range of 1.15 to 1.25, HCl was added to adjust it. After one hour of agitation at 37 °C using and end-over-end rotation agitator, the pH was checked again to ensure a value under 1.5 before centrifugation at 3000 rpm for 15 min. Finally, the supernatant was filtrated with cellulose acetate membranes (0.45 µm porosity) and acidified with HNO_3.

Cadmium concentrations in the gastric phase were also measured by ICP-MS (Agilent 7500). The Cd bioaccessible fraction was expressed as a percentage, dividing the gastric bioaccessible Cd concentration by total Cd concentration.

3. Results

3.1 Physico-chemical properties and total Cd contents in soils

As shown in Table 1, soils from the sampling areas are divided into three types according to the USDA classification typically used in Latin America: inceptisols in NAR and its control area (MS); entisols in NPC; and alfisols in the MC control area.

In the MS area, the soil pH varied between 4.15 ± 0.01 and 6.19 ± 0.06 and was more acidic in the first 20 cm. CEC values showed no difference between the first two horizons (0–5 cm and 5–20 cm), varying between 13.2 and 17.5 cmol kg^{-1}, except for the sampling point 2 at Yawi-Gualaquiza (CEC = 7.1 cmol kg^{-1} with a slightly less acidic pH). The TOC contents ranged between 1.71 and 20.33 g kg^{-1} of d.w. soil. In the NAR, the pH ranged from 4.44 ± 0.05 at the Manduro Yaku Kichwa community to 6.98 ± 0.18 at Pacayacu. The pH and CEC trends were the same as observed in the MS control area. TOC contents varied between 4.23 and 20.13 g kg^{-1} of DW soil with higher concentrations in the topsoils. In contrast to NAR, NPC and MC control areas exhibited slightly neutral to basic pH values (from 6.47 ± 0.01 to 8.00 ± 0.04) that increased slightly with depth. CEC and TOC values were also lower in these areas: between 1.0 and 7.3 cmol kg^{-1} and 1.45 and 10.19 g kg^{-1}, respectively.

In general, the total Cd concentrations decreased with depth, excluding Pacific coast soil sample 24 in MC and sample 26 in the NPC. In the Amazonian control area (MS), Cd remained below 0.27 mg kg^{-1} for points 1 and 3, and 0.5 mg kg^{-1} for points 2 and 4. Nevertheless, some points in the NAR exhibit lower Cd concentrations in all the horizons: about 0.18 and 0.24 mg kg^{-1} (0-60 cm depth) in Auca Sur and Nuevo Oriental, respectively.

Table 1. Physico-chemical properties and total Cd concentrations (in mg kg^{-1} d.w.) measured in soil samples collected in the North Amazon Region (NAR), the North Pacific Coast (NPC) and in the control areas of Morona-Santiago (MS) and in Manabí (MC), in Ecuador.

Sample Code	Site location (sampling area)	Description	Depth (cm)	Soil type (USDA classification)	pH water	CEC (cmol kg^{-1})	TOC (g kg^{-1})	Total Cd (mg kg^{-1} DW soil)
1	Yawi - Gualaquiza (MS)	Control area Amazon Region	0-5	Inceptisol	4.15±0.01	17.4	6.99	0.25±0.01
			5-20		4.32±0.01	17.5	15.21	0.24±0.01
			20-60		4.37±0.04		8.99	0.25±0.03
			60-80		4.69±0.02		6.42	0.23±0.02
			80-100		4.68±0.02		3.65	0.21±0.03
2	Yawi - Gualaquiza (MS)	Control area Amazon Region	0-5	Inceptisol	6.19±0.06	7.1	2.83	0.50±0.01
			5-20		5.33±0.01	15.1	12.66	0.27±0.01
			20-60		5.22±0.13		48.2	0.27±0.02
			60-80		4.82±0.01		10.35	0.24±0.02
			80-100		4.70±0.02		9.11	0.25±0.03
3	San Miguel (MS)	Control area Amazon Region	0-5	Inceptisol	5.09±0.02	16.4	5.23	0.27±0.02
			5-20		4.95±0.02	17.7	5.19	0.24±0.01
			20-60		5.31±0.13		11.52	0.25±0.01
			60-80		5.08±0.04		4.38	0.24±0.04
			80-100		5.10±0.01		3.10	0.28±0.03
4	San Miguel (MS)	Control area Amazon Region	0-5	Inceptisol	4.22±0.01	13.2	1.82	0.49±0.02
			5-20		4.22±0.02	13.9	1.70	0.50±0.01
			20-60		4.41±0.05		20.33	0.51±0.01
			60-80		4.40±0.00		8.15	0.39±0.04
			80-100		4.49±0.02		6.58	0.48±0.03
5	Dayuma (NAR)	Auca 9 Oil Well	0-5	Inceptisol	4.51±0.09	16.7	18.12	2.23±0.07
			5-20		4.61±0.01	11.7	7.62	0.79±0.03
			20-60		5.00±0.02		2.76	0.57±0.01
6	Com.Cristal (NAR)	Auca 22 Oil well	0-5	Inceptisol	5.10±0.03	14.3	7.43	0.29±0.03
			5-20		5.11±0.02	14.8	4.11	0.26±0.03
			20-60		5.01±0.03		1.64	0.25±0.01
			60-80		5.29±0.03		0.84	0.24±0.04
7	Com.Shuar Tzakim (NAR)	Hormiguero Oil block	0-5	Inceptisol	4.57±0.03	17.2	17.38	0.34±0.02
			5-20		4.72±0.01	18.7	11.79	0.25±0.01
			20-60		4.72±0.01		4.85	0.31±0.01
			60-80		4.79±0.02		3.09	0.28±0.04

Note. *The criteria of elaboration of this table was to separate the NAR from the NPC regions, starting with the control areas for both sites.*

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Table 1. (continued). Physico-chemical properties and total Cd concentrations (in mg kg^{-1} d.w.) measured in soil samples collected in the North Amazon Region (NAR), the North Pacific Coast (NPC) and in the control areas of Morona-Santiago (MS) and in Manabí (MC), in Ecuador.

Sample Code	Site location (sampling area)	Description	Depth (cm)	Soil type (USDA classification)	pH water	CEC (cmol kg^{-1})	TOC (g kg^{-1})	Total Cd (mg kg^{-1} DW soil)
8	Com.Shuar Rio Tiputini (NAR)	Hormiguero Oil block	0-5	Inceptisol	5.49±0.06	14.1	18.17	0.54±0.02
			5-20		5.60±0.02	16.4	7.81	0.40±0.01
			20-60		5.50±0.04		5.26	0.35±0.01
			60-80		5.50±0.06		2.25	0.28±0.04
9	Auca Sur (NAR)	Auca Sur Oil Platform	0-5	Inceptisol	4.86±0.01	17.0	6.67	0.18±0.03
			5-20		4.86±0.04	16.9	3.04	0.19±0.03
			20-60		5.03±0.01		1.44	0.16±0.01
			60-80		4.98±0.00		1.16	0.17±0.04
10	Auca (NAR)	Influence Area of oil activities	0-5	Inceptisol	5.35±0.04	13.3	13.78	0.32±0.02
			5-20		4.87±0.01	15.1	2.68	0.27±0.01
			20-60		5.04±0.01		2.21	0.28±0.01
11	Com.Kichwa M. Yaku (NAR)	Gacela Oil Platform	0-5	Inceptisol	4.44±0.01	17.8	10.4	0.33±0.01
			5-20		4.37±0.01	18.0	7.54	0.32±0.01
			20-60		4.71±0.01		7.25	0.31±0.01
12	Flor de Manduro (NAR)	Gacela Oil Platform	0-5	Inceptisol	5.44±0.02	16.2	14.16	0.60±0.02
			5-20		5.08±0.08	18.3	4.09	0.35±0.01
			20-60		5.11±0.00		3.82	0.36±0.01
13	Shushufindi (NAR)	Aguarico 3 Oil Platform	0-5	Inceptisol	5.99±0.05	14.0	15.75	0.54±0.03
			5-20		6.09±0.01	16.0	8.11	0.34±0.03
			20-60		5.95±0.00		6.54	0.42±0.04
14	Shushufindi (NAR)	Aguarico 3 Oil Platform	0-5	Inceptisol	6.00±0.02	15.5	10.41	0.51±0.01
			5-20		5.91±0.04	16.6	6.45	0.48±0.03
			20-60		5.54±0.04		3.33	0.28±0.01
15	Coop. Nueva Aurora (NAR)	Cobra 1 Oil Platform Well 43	0-5	Inceptisol	6.15±0.01	8.8	4.23	1.09±0.02
			5-20		5.48±0.04	16.9	25.47	0.39±0.01
			20-60		5.39±0.09		8.99	0.48±0.01
16	Nueva Quevedo (NAR)	Oil activities influence area	0-5	Inceptisol	5.12±0.01	16.7	10.55	0.40±0.03
			5-20		5.10±0.01	16.7	7.22	0.37±0.05
			20-60		5.28±0.01		4.13	0.31±0.01

Note. *The criteria of elaboration of this table was to separate the NAR from the NPC regions, starting with the control areas for both sites.*

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Table 1. (continued). Physico-chemical properties and total Cd concentrations (in mg kg^{-1} d.w.) measured in soil samples collected in the North Amazon Region (NAR), the North Pacific Coast (NPC) and in the control areas of Morona-Santiago (MS) and in Manabí (MC), in Ecuador.

Sample Code	Site location (sampling area)	Description	Depth (cm)	Soil type (USDA classification)	pH water	CEC (cmol kg^{-1})	TOC (g kg^{-1})	Total Cd (mg kg^{-1} DW soil)
16	Nueva Quevedo (NAR)	Oil activities influence area	0-5	Inceptisol	5.12±0.01	16.7	10.55	0.40±0.03
			5-20		5.10±0.01	16.7	7.22	0.37±0.05
			20-60		5.28±0.01		4.13	0.31±0.01
17	Nueva Quevedo (NAR)	Oil activities influence area	0-5	Inceptisol	5.86±0.04	15.77	20.13	0.51±0.02
			5-20		5.93±0.03	17.0	15.00	0.49±0.01
			20-60		6.06±0.06		7.35	0.44±0.01
18	Coop. Pacayacu (NAR)	Pichincha 2 Oil well	0-5	Inceptisol	6.38±0.01	14.9	14.66	0.65±0.03
			5-20		6.57±0.01	17.3	7.49	0.45±0.03
			20-60		6.65±0.01		7.55	0.44±0.01
			60-80		6.81±0.35		3.79	0.43±0.09
19	Coop. Pacayacu (NAR)	Pichincha 2 Oil well	0-5	Inceptisol	6.39±0.01	14.3	13.24	1.49±0.03
			5-20		6.23±0.04	15.4	14.22	1.02±0.03
			20-60		6.61±0.03		5.5	0.64±0.01
			60-80		6.98±0.18		2.53	0.39±0.04
20	Coop. Pacayacu (NAR)	Pichincha 2 Oil well	0-5	Inceptisol	6.61±0.07	17.9	8.58	0.48±0.03
			5-20		6.52±0.10	18.3	7.05	0.46±0.03
			20-60		6.47±0.11		4.16	0.33±0.01
			60-80		6.60±0.00		3.62	0.37±0.04
21	Coop. Pacayacu (NAR)	Pichincha 2 Oil well	0-5	Inceptisol	6.65±0.03	9.0	10.66	0.88±0.03
			5-20		6.32±0.02	18.0	28.56	0.42±0.03
			20-60		6.42±0.04		7.88	0.47±0.01
			60-80		6.15±0.21		10.95	0.32±0.04
22	Nuevo Oriental (NAR)	Shuara 9 Oil Platform	0-5	Inceptisol	4.65±0.21	18.1	5.86	0.23±0.02
			5-20		4.57±0.04	18.4	9.45	0.25±0.01
			20-60		4.60±0.01		6.43	0.25±0.01
23	Santa Ana (MC)	Control area NPC	0-5	Alfisol	7.24±0.01	5.3	4.59	0.46±0.02
			5-20		7.14±0.01	5.6	3.6	0.44±0.03
			20-60		7.17±0.01		1.09	0.40±0.01
			60-80		7.53±0.06		2.45	0.23±0.04

Note. *The criteria of elaboration of this table was to separate the NAR from the NPC regions, starting with the control areas for both sites.*

(continued on next page)

Table 1. (continued). Physico-chemical properties and total Cd concentrations (in mg kg^{-1} d.w.) measured in soil samples collected in the North Amazon Region (NAR), the North Pacific Coast (NPC) and in the control areas of Morona-Santiago (MS) and in Manabí (MC), in Ecuador.

Sample Code	Site location (sampling area)	Description	Depth (cm)	Soil type (USDA classification)	pH water	CEC (cmol kg^{-1})	TOC (g kg^{-1})	Total Cd (mg kg^{-1} DW soil)
24	Portoviejo (MC)	INIAP Control area NPC	0-5	Alfisol	6.94±0.01	2.5	6.43	1.00±0.05
			5-20		6.65±0.00	2.3	4.29	0.77±0.03
			20-60		6.47±0.01		4.12	1.02±0.01
			60-80		7.10±0.02		2.16	0.62±0.04
25	Portoviejo (MC)	INIAP Control area NPC	0-5	Alfisol	7.14±0.30	4.7	6.79	0.74±0.03
			5-20		7.14±0.16	4.8	5.36	0.72±0.03
			20-60		7.20±0.23		3.93	0.74±0.01
			60-80		7.17±0.17		3.08	0.73±0.01
26	Lucha de los Pobres (NPC)	Esmeraldas National Oil Refinery	0-5	Entisol	7.44±0.13	7.3	2.29	0.40±0.02
			5-20		7.55±0.01	3.8	9.51	0.50±0.01
			20-60		7.78±0.03		5.75	0.47±0.01
27	Lucha de los Pobres (NPC)	Esmeraldas National Oil Refinery	0-5	Entisol	7.23±0.01	1.3	10.19	0.34±0.02
			5-20		7.20±0.00	1.0	5.70	0.40±0.01
			20-60		7.48±0.05		5.34	0.45±0.02
28	Lucha de los Pobres (NPC)	Esmeraldas National Oil Refinery	0-5	Entisol	7.43±0.01	1.0	9.16	0.42±0.02
			5-20		6.87±0.01	1.7	6.20	0.35±0.01
			20-60		6.91±0.01		5.66	0.33±0.01
29	Lucha de los Pobres (NPC)	Esmeraldas National Oil Refinery	0-5	Entisol	8.00±0.04	2.7	1.45	0.37±0.03
			5-20		7.60±0.01	4.7	3.84	0.27±0.03
			20-60		7.64±0.01		3.35	0.25±0.01
30	Lucha de los Pobres (NPC)	Esmeraldas National Oil Refinery	0-5	Entisol	7.50±0.01	3.2	6.43	0.47±0.03
			5-20		7.66±0.02	3.2	4.35	0.43±0.03
			20-60		7.90±0.01		2.18	0.39±0.01
31	Tiwinza (NPC)	Esmeraldas National Oil Refinery	0-5	Entisol	7.27±0.04	8.3	4.07	0.32±0.03
			5-20		7.44±0.01	8.1	3.05	0.33±0.03
			20-60		7.35±0.04		2.85	0.34±0.01

Note. *The criteria of elaboration of this table was to separate the NAR from the NPC regions, starting with the control areas for both sites.*

The highest concentrations in the topsoil measured in the NAR were: 2.23 ± 0.07; 1.49 ± 0.03; 1.09 ± 0.02; and 0.88 ± 0.03 mg kg^{-1} corresponding to points 5, 19, 15 and 21, respectively.

Additionally, Cd concentrations were higher (between 0.46 ± 0.02 and 1.00 ± 0.05 mg kg^{-1}) in the Pacific coast control area, MC, than in NPC area, within the first 5 cm of soil. Finally, 12 of the 31 (39%) investigated sites exceeded the maximum Cd level in soils of 0.5 mg kg^{-1} as established by the Ecuadorian legislation (TULSMA, 2015). The highest concentrations were measured in topsoils sampled in Dayuma in the oil block Auca 09 and Pacayacu in the oil block Pichincha 2. Both sites are characterized by intense oil activity and widespread oil spills. In the coastal control area, both soils sampled in the INIAP-Portoviejo research station showed Cd concentrations two times higher than the recommended level by the Ecuadorian legislation.

3.2 Cadmium concentrations in cacao leaves, pod husks, and beans

Cadmium concentrations in cacao tissues are given in the Supplementary Information (Table SI-1). In general, Cd concentrations are higher in leaves than in pod husks or beans (Fig.2), except for the points 13, 18 and 22 in NAR, and 29 in NPC. In Dayuma (sample 5, CCN-51 cacao variety), Cd concentrations are the highest of the NAR, reaching values of 7.87 ± 0.05, 2.81 ± 0.02 and 3.50 ± 0.16 mg kg^{-1} in leaves, pod husks, and raw beans, respectively. In the Amazonian control area (MS), the native cacao specie 1a and the Zamora variety (sample 2) exhibited higher Cd concentrations in beans, pod husks, and leaves than the CCN-51 varieties (samples 3 and 4).

Cadmium concentrations in leaves from the NPC study area varied between 0.34 ± 0.05 and 1.96 ± 0.05 mg kg^{-1}, whereas values in the pod husks and beans ranged from 0.34 ± 0.05 to 1.96 ± 0.05 and 0.24 ± 0.19 to 1.30 ± 0.19 mg kg^{-1}, respectively. Additionally, the highest concentrations of Cd were found in the coastal area in Portoviejo at the INIAP station with 6.43 ± 0.05, 4.09 ± 0.02 and 1.66 ± 0.19 mg kg^{-1} for leaves, pod husks and beans, respectively, corresponding to the “EET 116” Nacional cacao genotype. Depending on the sampling site, cacao beans from oil production areas (extraction or refinement activities) reached mean Cd concentrations of 1.21 ± 0.87 and 0.89 ± 0.64 mg kg^{-1} for the CCN-51 and Nacional varieties, respectively, whereas Cd concentrations were 1.71 ± 1.26 for the Nacional variety in the MC control area and 0.14 ± 0.01 mg kg^{-1} for the CCN-51 variety in the MS control area.

Finally, even if the Cd in cacao beans could not be compared to a reference value set by worldwide legislation, 18 of the 34 (53%) cacao samples exhibited Cd concentrations above the threshold of 0.8 mg kg^{-1} set for dark chocolate (i.e. contains more than 50% of total dry cocoa solids) established by the European Union to be effective from 2019 (European Commission, 2014).

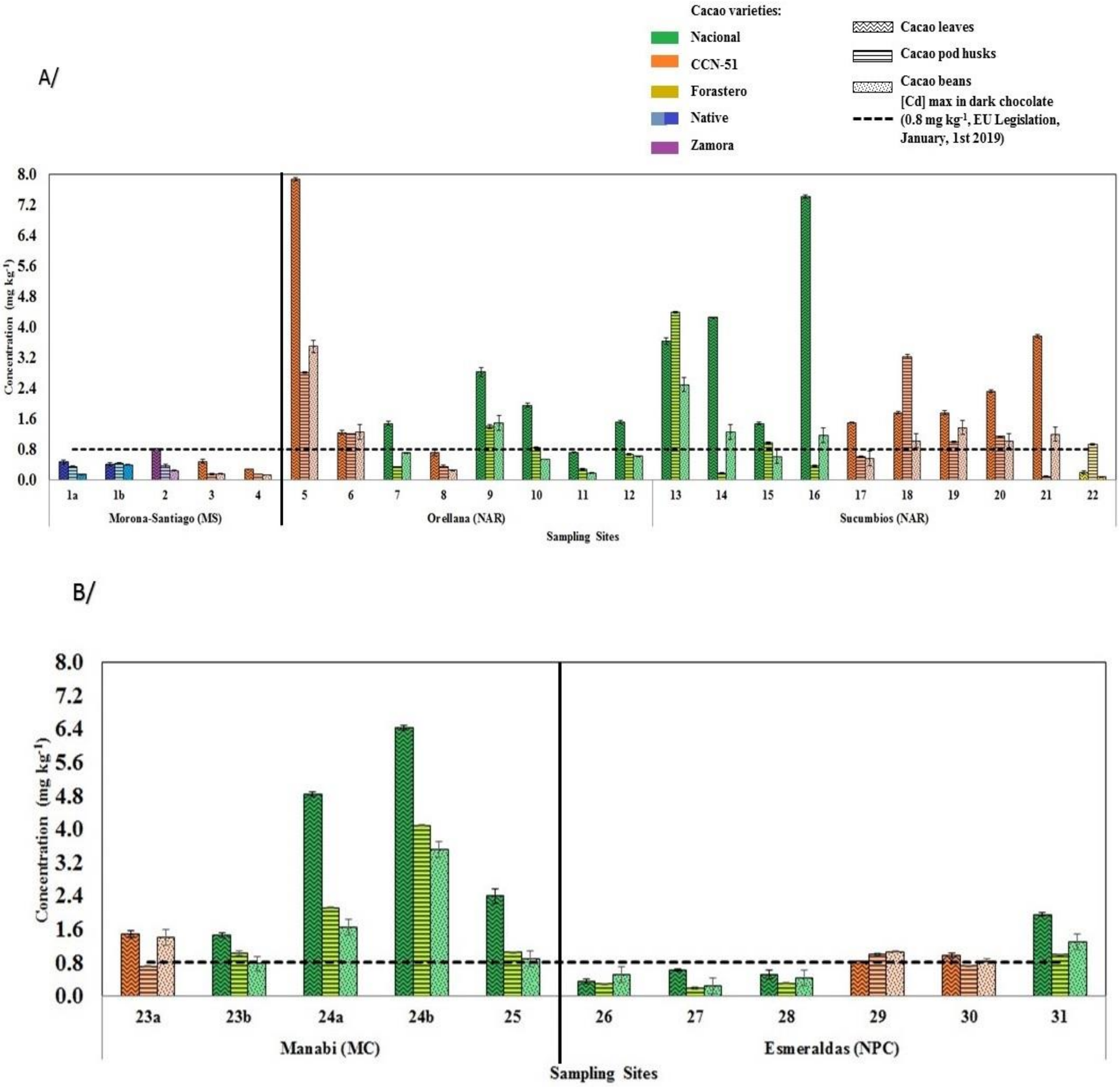


Fig.2. Total Cd concentrations (mg kg^{-1} d.w.) in cacao leaves, pod husks and beans from 31 small-scale farms in Ecuador. Sampling points are reported in the graphs according to their localization (A: MS and NAR; B: MC and NPC). The control areas (MS and MC) are presented on the left side of the graphs, separated by a black solid line. On the right side, sampling sites are located closed to oil infrastructures (A: wells, oil pools, platforms with open flares, B: refining facilities). Dotted black line represents the Cd threshold for dark chocolate (>50% cacao mass) according to European Legislation.

3.3 Cadmium concentrations in aerosols

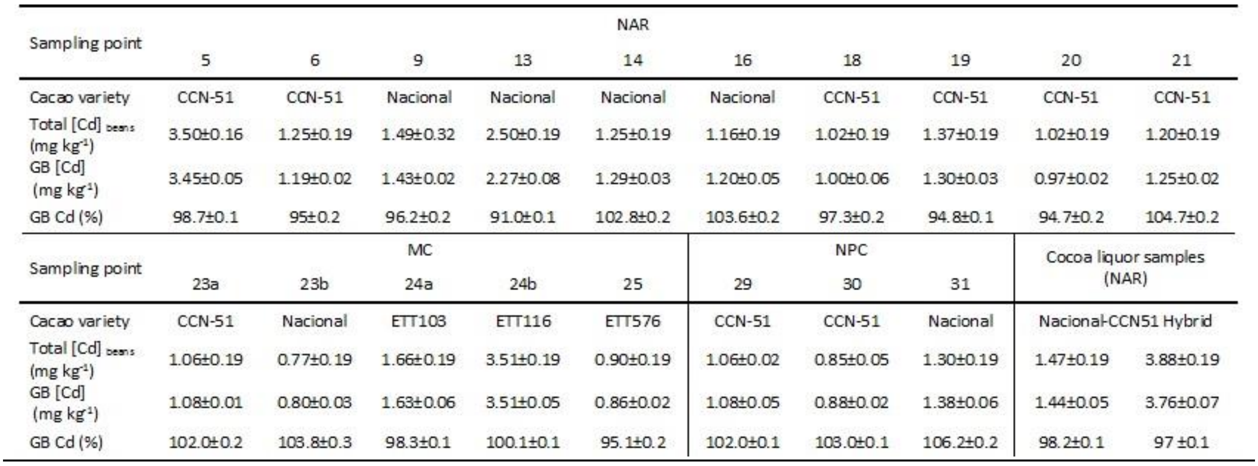
Mean annual Cd concentrations from the PM_{10} samples collected during the 2015 monitoring season (Table SI-2, Supplementary Information) ranged between 0.11 ± 0.03 and 0.17 ± 0.09 ng m^{-3} in the NAR (sampling sites 9 and 22, respectively), and averaged 0.44 ± 0.13 ng m^{-3} in the NPC in front of the most important refinery of Ecuador. These values were below the mean annual Cd concentration in PM_{10} of 0.5 ng m^{-3} according to the Ecuadorian Legislation (TULSMA, 2015). A maximum concentration of 0.57

ng m^{-3} was observed in the NPC, while a minimum concentration of 0.07 ng m^{-3} was observed at both of the NAR sampling sites. No marked short-term variations were observed for the three sites.

3.4 Gastric bioaccessibility of Cd

Total and bioaccessible Cd contents in cacao beans and cocoa liquor are extremely similar and even equal in some cases (Table 2). Between 90 and 100% of the total Cd is bioaccessible by human ingestion after digestion by gastric fluids. No differences were observed between the CCN-51 and Nacional fino de aroma cacao varieties.

Table 2. Gastric bioaccessibility (GB) of Cd in cacao beans and cocoa liquor after ingestion (% ± SD obtained in replicates)



Sampling point					NAR					
	5	6	9	13	14	16	18	19	20	21
Cacao variety	CCN-51	CCN-51	Nacional	Nacional	Nacional	Nacional	CCN-51	CCN-51	CCN-51	CCN-51
Total [Cd] $_{beans}$ (mg kg^{-1})	3.50±0.16	1.25±0.19	1.49±0.32	2.50±0.19	1.25±0.19	1.16±0.19	1.02±0.19	1.37±0.19	1.02±0.19	1.20±0.19
GB [Cd] (mg kg^{-1})	3.45±0.05	1.19±0.02	1.43±0.02	2.27±0.08	1.29±0.03	1.20±0.05	1.00±0.06	1.30±0.03	0.97±0.02	1.25±0.02
GB Cd (%)	98.7±0.1	95±0.2	96.2±0.2	91.0±0.1	102.8±0.2	103.6±0.2	97.3±0.2	94.8±0.1	94.7±0.2	104.7±0.2
Sampling point			MC				NPC		Cocoa liquor samples (NAR)	
	23a	23b	24a	24b	25	29	30	31		
Cacao variety	CCN-51	Nacional	ETT103	ETT116	ETT576	CCN-51	CCN-51	Nacional	Nacional-CCN51 Hybrid	
Total [Cd] $_{beans}$ (mg kg^{-1})	1.06±0.19	0.77±0.19	1.66±0.19	3.51±0.19	0.90±0.19	1.06±0.02	0.85±0.05	1.30±0.19	1.47±0.19	3.88±0.19
GB [Cd] (mg kg^{-1})	1.08±0.01	0.80±0.03	1.63±0.06	3.51±0.05	0.86±0.02	1.08±0.05	0.88±0.02	1.38±0.06	1.44±0.05	3.76±0.07
GB Cd (%)	102.0±0.2	103.8±0.3	98.3±0.1	100.1±0.1	95.1±0.2	102.0±0.1	103.0±0.1	106.2±0.2	98.2±0.1	97 ±0.1

4. Discussion

4.1 Cadmium bioaccumulation in cacao pods: uptake pathways and influence of external parameters

The mobility of trace metals, including Cd, in soils and their absorption by plants depends on many factors such as soil texture, pH, CEC, organic matter content, total concentrations in the soil, chemical speciation, plant species and varieties, and farming practices (Qasim et al., 2015; Song et al., 2015; Yang et al., 2016). Wang et al. (2012) reported that Cd uptake by plant roots and its transfer to fruits are influenced by soil properties, plant species and varieties, and also by crop management practices. In this study, soils displayed the same physico-chemical properties: acidic pH; low CEC; and variable TOC content, which fit with two categories of tropical soils: inceptisols and entisols. Due to the pedogenesis process that performs total hydrolysis of the bedrock in tropical environments, soils are naturally enriched in secondary minerals such as clays and Al and Fe oxy-hydroxides (e.g. gibbsite, goethite, kaolinite), which explains their low CEC and acidic pH (Juo and Franzluebbers, 2003). All of these factors are clearly in accordance with a high bioavailability of Cd in cacao plants. As reported by

He et al. (2015), there is an indirect linear relationship between soil pH and phytoavailability of Cd in soils; when pH decreases, Cd uptake by plant increases. Sekara et al. (2004) reported that Cd uptake by plants can be important even at low Cd concentrations in the soil, which explains the high Cd contents in plant tissues.

A correlation matrix was established to identify the potential relationships between Cd contents in cacao plants, soils, and soil physico-chemical parameters (Table SI-3, Supplementary Information). A significant correlation was found for the entire dataset between Cd concentrations in beans and those in pods or leaves, but not with Cd concentrations in soils, except for the NPC samples. Moreover, in the same NPC topsoil samples, Cd concentrations were negatively correlated with pH, indicating that acidic pH can favor the solubilization of this cation and its transfer by the roots to the cocoa plant. For the whole dataset, the Cd concentrations in topsoils did not depend on TOC or CEC.

In this field study, cacao leaves were found to have higher Cd concentrations than beans and pod husks, as reported in section 3.2. This trend has also already been observed in two previous studies. Gramlich et al. (2017) reported Cd average concentrations of 0.91, 0.54, and 0.21 mg kg^{-1} d.w. in leaves, pod husks, and beans, respectively, for two different cacao cultivars in Bolivia. Ramtahal et al. (2016) also found that Cd concentrations in leaves, pods, shells and nibs (beans without shell) were 2.9, 2.5, 2.4, and 2.1 mg kg^{-1}, respectively. Sekara et al. (2004) explained that Cd is transported via the transpiration stream of the plant and accumulates primarily in the leaves. As such, pods and seeds contain much less Cd than the leaves as they evaporate less water. But, in contrast, in a recent study conducted in southern Ecuador, Chavez et al. (2015) showed that Cd contents in leaves were below the detection limit while Cd was detected only in shells when its concentration in beans was above 1 mg kg^{-1}. All differences in the trends of Cd concentrations within cacao tissues might be explained, at least in part, as a function of crop species and cultivars. Hence, the genetic variation among cultivars means there is great potential for utilizing plant breeding as a way to select low-Cd characteristics in crops (Roberts, 2014). In our study, 17 of the 34 cacao samples belonged to the Nacional fino de aroma variety and 13 to the CCN-51 variety. Average Cd concentrations in leaves, pod husks, and beans for the two varieties are quite similar, however, they are slightly higher in the National variety, suggesting a potential impact of the cacao variety on Cd uptake and bioaccumulation in edible parts.

In Table 3, we report values of GEF and BF factors higher than 1, which suggests a bioaccumulation of Cd in the cacao plant and root transfer of Cd. The impact of cacao variety on Cd bioaccumulation in beans could not be clearly established because these factors are only based on the cacao varieties found in each farm, which makes it difficult to strictly compare the same field conditions.

Table 3. Global Enrichment Factor (GEF) and Bioaccumulation Factor (BF) values (mean ± SD) for sampling sites in which Cd concentrations in cacao beans exceeded 0.8 mg kg^{-1}

	NAR									
Sampling site	5	6	9	13	14	16	18	19	20	21
Cacao variety	CCN-51	CCN-51	Nacional	Nacional	Nacional	Nacional	CCN-51	CCN-51	CCN-51	CCN-51
GEF	24.38±1.86	8.73±2.30	10.38±1.47	17.41±1.70	8.75±1.42	8.11±1.41	7.15±1.39	9.57±1.44	7.14±1.39	8.34±1.42
BF	4.42±0.24	4.80±1.32	8.01±1.51	7.33±0.79	2.59±0.41	3.18±0.68	2.29±0.44	1.34±0.19	2.22±0.43	2.87±0.49

	MC					NPC		
Sampling site	**23a**	**23b**	24a	24b	25	29	30	31
Cacao variety	CCN-51	Nacional	ETT103	ETT116	ETT576	CCN-51	CCN-51	Nacional
GEF	9.78±1.45	5.37±1.36	11.58±1.50	24.47±2.01	6.27±1.37	7.42±0.48	5.93±0.49	9.07±1.43
BF	3.17±0.47	2.34±0.60	4.89±0.67	10.33±0.97	1.25±0.27	3.89±0.38	1.97±0.16	3.95±0.65

Cadmium can be released into the atmosphere through metal production activities, fossil fuel combustion, or waste incineration, and can also be enriched in soils by fertilizers or other phytosanitary products (EFSA, 2012; IARC, 2009; Kirkham, 2006). Elevated Cd levels in topsoils and water, even in remote locations, can sometimes result in the long-distance transport of contaminated aerosols (ATSDR, 2012) or by direct anthropogenic input. Since Cd is readily available from air sources, the contribution of airborne Cd to Cd in crops cannot be neglected (Kabata-Pendias, 2011). In order to consider atmospheric deposition as a possible source of trace elements, the natural concentration in the soil substrate must be low and its uptake and accumulation by plants cannot be related to soil borne elements (De Temmerman et al., 2015). It has been demonstrated that dust particles accumulated in the foliar surface can enter the plant as soluble ions through stomata, cuticular cracks, lenticels, ectodesmata, and aqueous pores (Shahid et al., 2017; Uzu et al., 2010). Once trace metals are accumulated inside the plant, they can be transported to the rest of the tissues contaminating edible parts and become bioaccumulated (Schreck et al., 2012; Xiong et al., 2014). In a review of three case studies of the contribution of airborne metal to plant Cd in agricultural crops, Smolders (2001) concluded that atmospheric Cd may be a significant source of Cd for crops grown in areas where its concentration is at least tenfold higher than 2 g Cd ha^{-1} y^{-1} , and where Cd content in soil is not high, or not available.

However, in this field study, as described in sections 2.2 and 3.3, aerosol samples were collected for two years but a complete dataset is still not yet currently available. Although air monitoring was not possible in the entire sampling area, these data provide insight into the Cd levels in air, highlighting that the atmosphere is not polluted by Cd. Furthermore, analyses were performed using SEM-EDX (Scanning electron microscopy with energy dispersive X-ray spectroscopy) and observations did not show any trace of Cd particles deposited on leaf surfaces (Fig. SI-2), suggesting that, in the present study, atmosphere-plant is not a pathway for Cd uptake by leaves or that Cd is quickly dissolved on the leaf surface and incorporated through the cuticle. Hence, we propose that soils remains the primary pathway of Cd uptake by plants cultivated on small-scale farms in areas impacted by oil activities and/or specific agricultural practices.

4.2 Potential sources of cadmium in soils of small-scale Ecuadorian farms

Cadmium is introduced into the soil through geogenic and anthropogenic processes (He et al., 2015). In uncontaminated soils, Cd contents are highly governed by soil texture and range generally from 0.01 to 0.3 and from 0.2 to 0.8 mg kg^{-1} in sandy and loamy soils, respectively (Kabata-Pendias, 2011). In a study on heavy metals in Brazilian soils in areas with minimal or no anthropogenic activity, Fadigas et al. (2006) recommended a Cd reference value, depending on the soil type, from 0.3 to 1.6 mg kg^{-1} (1 mg kg^{-1} for inceptisols). Even if higher Cd values in superficial horizons (compared to background soils) usually reflect anthropogenic input, it can also be derived from the parent material (Caridad-Cancela et al., 2005; Kabata-Pendias, 2011). However, the present study shows that average Cd concentrations in the deepest soil layers from the NAR and NPC (0.36 ± 0.09 mg kg^{-1}) remain low and comparable with the pedogenic background measured in the MC area (0.30 ± 0.12 mg kg^{-1}).

Nevertheless, a trend in the contaminated sampling points (i.e. where soil Cd concentrations exceed 0.5 mg kg^{-1}) is observed: Cd concentrations generally decrease from the top to deeper soil horizons, suggesting anthropogenic inputs instead of natural geological processes. It should be noted that in soils developed in humid climates, migration of Cd down the profile is more likely to occur than its accumulation in the surface horizon. Thus, the enrichment of Cd observed so commonly in topsoils should be related to contamination effects (Kabata-Pendias, 2011), instead of natural sources of heavy metals (i.e. from the parent rock material).

In order to confirm the initial hypothesis in which crude oils naturally contain Cd and other trace metals, four crude oil samples from the national oil pipeline, Auca 3 and Auca 8 oil camps, and from a natural outcrop of bituminous sandstones, all collected in the NAR, were analyzed (Table SI-4, in Supplementary Information). As these concentrations were found to be below the detection limit as well as below the concentrations in soils or air, the possibility to link the Cd content in cacao samples to oil spills or refining activities is quite limited, even if some sampling points of the NAR showed the highest Cd contents in topsoils. However, the existence of another anthropogenic source of Cd in soils is suggested. For example, the control area chosen along the Pacific coast showed high Cd levels in cacao tissues (mean concentrations of 1.65, 1.80, and 3.32 mg kg^{-1} in beans, pod husks, and leaves, respectively) in the absence of oil activities, suggesting a rather key role of agricultural practices in the Cd contamination of cacao plants compared to the impact of mining or oil activities. As highlighted by Zarcinas et al. (2004), the excessive application of inexpensive and contaminated phosphate fertilizers may have contributed to elevated levels of Cd in soil and cacao crops. In Ecuador, the total fertilizer consumption reached 368,370 tons in 2014 (FAO, 2017). The indiscriminate use of phosphate fertilizers and other chemical inputs, or crop irrigation with wastewater, can be considered as important Cd sources to topsoils that are partly responsible for cacao contamination in Ecuador (Mite et al., 2010). In fact, Cd concentrations in P fertilizers depend on the phosphate source rock (He et al., 2015; Jiao et al., 2012), varying from 2 to more than 100 mg kg^{-1} in triple super phosphate products (Roberts, 2014).

Most of the local farmers from the NAR and NPC did not indicate the use of phosphate fertilizers. Nevertheless, manuals of cacao crop management in Ecuador recommend the application of NPK (nitrogen, phosphorus and potassium) fertilizers in combination with micronutrient solutions or ammonia sulfate in controlled doses, and if not, they also promote the use of organic fertilizers (Amores et al., 2009; Quiroz and Agama, 2006; Rodriguez et al., 2010). Chicken manure, locally known as ‘gallinaza,’ fermented organic fertilizers known as ‘bocashi,’ and compost are some of the products named by Ecuadorian farmers but their Cd concentrations generally remain below 2.5 mg kg^{-1} (Carrillo, 2016). However, it is also well known that long-term application will result in Cd accumulation in soils (Nziguheba and Smolders, 2008; Salmanzadeh et al., 2016).

Additionally, in order to increase the organic matter content in soils and to contribute to the decomposition of plant litter, pod husks are left scattered or stacked around other trees after cacao harvest (Ardila and Carreño, 2011; Larrea, 2008). This procedure is not only considered inadequate because it becomes a source of inoculum of harmful pathogens, but it also becomes a pathway to reincorporate Cd already present in cacao pods into the topsoil. The same can occur with the accumulation of cacao leaves in the surface soil. The practice of not raking fallen leaves can favor Cd leaching into the soil solution or become absorbed by organic matter during litter decomposition (Scheid et al., 2009). As an example to illustrate this hypothesis, the highest Cd concentration in the topsoil was found in sampling point 5, and also in cacao beans and leaves. Later, the low soil pH increases Cd availability to the cacao plant roots.

In tropical lowlands with temperatures higher than 30 °C, annual precipitation above 2000 mm, wild winds and intense solar radiation, it is necessary to protect crops against fungal and pest diseases (Almeida and Valle, 2007; Norgrove, 2007; Pico et al., 2012). Since cacao crops are extremely vulnerable to black pod, frosty pod rot, and cocoa witches’ broom diseases (Norgrove, 2007; Pico et al., 2012), pesticides may be considered as another source of trace metal contamination (Castro et al., 2015; Khan et al., 2017; Kirkham, 2006). While these practices are not widespread on small-scale farms because farmers do not necessarily have sufficient income to afford such assorted chemical products, they are well developed on experimental farms managed by the Ministry of Agriculture, Livestock, Aquaculture and Fisheries (MAGAP) and research stations (INIAP) like those in Portoviejo (samples 24 and 25).

Finally, other Cd sources are mentioned in the literature. For example, Chavez et al. (2015) pointed out that elevated Cd levels have been reported in rivers near the study area of southern Ecuador, and consequently the irrigation of cacao fields with Cd-enriched water might have raised soil Cd to the current levels. But in the present study, cacao trees are not manually irrigated, except in Portoviejo (MC). In brief, the combination of many industrial and agricultural practices in Ecuador would confirm multiple sources of Cd in soils and cacao tissues, with agriculture practices probably being the main source of Cd bioaccumulated in cacao plants.

4.3 Human health risk assessment

Half of the investigated cacao samples exhibit Cd concentrations above the critical threshold of 0.8 mg kg^{-1} for dark chocolate (see section 3.2). Even if we assume that food quality standards for dark chocolate cannot be directly applied to raw cacao beans, Cd contents in cacao beans have often been compared to those in chocolate by several authors (Chavez et al., 2015; Gramlich et al., 2017; Pereira de Araújo et al., 2017; Takrama et al., 2015). In fact, cadmium content in chocolate appears to be directly related to the percentage of cocoa mass or cocoa liquor and cocoa powder (FAO and WHO, 2015). For example, in a premium chocolate with a cocoa mass of 70%, the Cd content would be more than 0.8 mg kg^{-1} if the level in the raw beans were higher than 1.15 mg kg^{-1}, a value found in 34% of our samples.

Moreover, the human risk assessment may now focus on the oral bioaccessible fraction, measured here on 18 raw cocoa beans and two cocoa liquor samples. Most studies on Cd gastric bioaccessibility have been performed using soil samples (Qin et al., 2016; Xia et al., 2016), with only a few studies on atmospheric particles (Goix et al., 2016), seafood (Ju et al., 2012), vegetables (Hu et al., 2013; Uzu et al., 2014), and mushrooms (Sun et al., 2012). Depending on the matrix, percentages can vary from 45 to 100, 35 to 99, 73 to 84, 14 to 71 and 30 to 70 in PM, soils, seafood, vegetables, and mushrooms, respectively.

In the present study, more than 90% of the Cd contained in cacao beans or cocoa liquor samples was bioaccessible after ingestion regardless of which cultivar was considered. According to Peixoto et al. (2016), the intestinal bioaccessibility of Cd ranged from 3 to 11% in five types of Brazilian chocolate drink powder, which contain cacao mixed with other ingredients like artificial sweeteners that could affect the chemistry of the whole process by the formation of soluble or insoluble complexes in the gastrointestinal tract. Moreover, the differences between gastric and intestinal bioaccessibility results are explained by the different analytical protocols used to estimate these percentages.

In our study, we have chosen to measure only the gastric bioaccessibility of Cd in order to maximize the health risks (Xiong et al., 2016). Even if the main part of Cd absorption occurs in the jejunum and ileum, the duodenum is a non-negligible absorption site (Denys et al., 2009). As described by Caboche (2009), at the exit of the stomach, a small amount of Cd contained in the gastric phase may be absorbed in the duodenum by passive paracellular diffusion or passive transcellular diffusion. For cationic elements like Cd, an increase of pH in the gastrointestinal phase induces precipitation and/or resorption of a part of the Cd solubilized in the gastric phase. This precipitated Cd will not be absorbed and is then eliminated via the feces (Denys et al., 2009). Cadmium absorption at these levels has been estimated to be 1–7% and is usually characterized by a high accumulation within the mucosa and low transfer rate into the organism (Vázquez et al., 2015).

Several authors have described a decrease of cationic element bioaccessibility in the gastrointestinal phase (Denys et al., 2009; Pascaud et al., 2014; Pelfrêne et al., 2015; Zhuang et al., 2016). As highlighted by Zhuang et al. (2016), a significant decrease of the gastrointestinal bioaccessibility of Cd in rice (63-72%) was observed compared to its gastric bioaccessibility (81-89%). This may be attributed to the pH increase and to the addition of organic components, such as bile extract and pancreatin, in the gastrointestinal phase. We therefore also suggest that gastric bioaccessibility is the most suitable method to assess the risks posed by ingestion of Cd-enriched cacaos.

Moreover, taking into account the Cd tolerable weekly intake (TWI) of 2.5 µg kg^{-1} of body weight (EFSA, 2012) and supposing that Cd content in cacao beans do not suffer any change during chocolate elaboration processes, human exposure to this trace element by chocolate consumption was calculated according to:

Equation (3):

$$\boldsymbol{Weekly\ Cd\ exposure\ level} = \frac{Cacao\ content\ in\ chocolate\ \times\ Total\ Cd\ in\ beans\ (and\ GB\ Cd\ in\ beans)}{Average\ adult\ body\ weight}$$

where the weekly Cd exposure level is expressed in µg of Cd per kg^{-1} of body weight per week. Chocolate consumption was considered on a weekly basis. Cacao content in chocolate was expressed in µg. Total Cd concentrations and GB of Cd in beans, expressed in mg kg^{-1}, were divided by 1000 to transform into units of µg kg^{-1} Average adult body weight was expressed in kg.

Considering a worldwide average chocolate consumption of 5.72 kg $year^{-1}$ $person^{-1}$ (Nieburg, 2014), equivalent to 110 g of chocolate per week, the weekly Cd intake for the cacao bean samples was calculated for two types of chocolate: a dark chocolate containing 70% cacao liquor; and a milk chocolate with only 40%. Results are presented in Table 4 as the maximum, minimum, and mean Cd concentration measured in cacao beans and liquor from our study. Even though the average concentration of Cd measured in the samples selected for GB, (described on Section 3.4, Table 2) is 1.63 mg kg^{-1}, it would not represent a risk for human health after ingestion of dark or milk chocolate made from Ecuadorian cacao beans. Conversely, if we consider the highest value reported in this field study (cocoa liquor samples), the weekly Cd intake from dark chocolate would raise to 4.48 µg kg^{-1} b.w., exceeding the TWI by a factor of 1.8.

Table 4. Weekly Cd intake for dark and milk chocolate consumption elaborated from Ecuadorian cacao beans and cocoa liquor

Type of cacao-based products	**Average chocolate consumption (g week^{-1})** ***≈ 1 chocolate bar week^{-1}***	**Average cacao consumption from chocolate (g week^{-1})**	**TWI-EFSA (µg kg^{-1} b.w)**	**Weekly Cd consumption from chocolate (µg)**	**Weekly Cd intake for an adult of 65 kg (µg kg^{-1} b.w)**	**Weekly bioaccesible Cd intake for an adult of 65 kg (µg kg^{-1} b.wt)***	**Risk for human health**
Dark chocolate (70% of cocoa liquor)	110	77.3	2.5	Mean: 125.9	1.94	1.88	Low
				Max: 299.7	4.61	4.48	High
				Min: 59.5	0.92	0.89	Very low
Milk chocolate (40% of cocoa liquor)	110	44.2	2.5	Mean: 71.9	1.11	1.07	Low
				Max: 171.3	2.63	2.56	Moderate
				Min: 34.0	0.52	0.51	Very low

Note. **A mean GB of 97% was considered for these calculations*

After human ingestion, Cd is accumulated in the kidneys and liver and has a biological half-life between 10 and 30 years (EFSA, 2012; FAO and WHO, 2015; MAK Value Documentation, 2012). In general, the digestive system of children and adolescents can more easily absorb Cd than adults (Yanus et al., 2014), so that the susceptibility of adverse effects in these groups is higher compared to adults. But, fortunately, the premium chocolate (i.e. containing a high percentage of cacao) manufactured from these Ecuadorian cacao beans is primarily consumed by adults and in small quantities due to its strong flavor and high price (FAO and WHO, 2015).

5. Conclusion

Cadmium distribution in Ecuadorian soils from the Amazon and Pacific coastal regions depends on natural (e.g. soil properties, geochemical background conditions) and anthropogenic (e.g. agricultural practices) factors. Cd concentrations in the first 0–20 cm of soil were typically higher than those in deeper layers, and 39% exceeded the Ecuadorian permissible limit, which suggests anthropogenic sources. Despite its low concentration in most of the study soils, Cd is easily uptaken by roots and accumulates in shoots and fruits. Leaf cadmium levels were always higher than pod husks or beans, however foliar uptake was not considered as the main pathway because of Cd low concentrations in PM_{10}.

Even if oil activities, which are widely spread across the study area, were initially considered a relevant source of Cd in soils and cacaos, our results suggest that Cd contents are more likely related to crop management practices including the extensive use of fertilizers and pesticides, and the natural decomposition of pods and leaves on the soil surface. A first approach to measure gastric bioaccessibility in chocolate raw material (cacao beans and liquors) revealed that almost 100% of the total Cd content is bioaccessible after ingestion. However, a risk assessment calculation shows that levels of Cd exposure by chocolate consumption (eventually processed from these samples) varies from low to high depending on the total Cd concentration, percentage of total cacao solids, and other factors concerning consumers (i.e. age, body weight, etc.).

Additional chemical analyses are currently being performed on raw and processed beans, as well as cocoa liquors and final chocolate products, in order to control each step of the fabrication process. We aim to determine if different steps of chocolate elaboration (e.g. fermentation, roasting, mucilage removal, etc.) can influence the Cd content of the final product and its gastric bioaccessibility. Further studies are also in progress to enhance the knowledge of Cd biological uptake mechanisms (e.g. sequestration and transport) for each commercial variety, as well as tracing its sources in Ecuadorian soils.

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Supplementary Information

Section 1. Cocoa liquor elaboration

Cocoa liquor samples collected in Pacayacu were produced in the ecological manufacturing center of Pacayacu District, following the next procedure:

- Cacao beans were separated from the pod husks, put into mesh bags and transported to the stockpile place
- Beans were fermented on clean wood trays (for 6 days), covered by banana’s leaves and removed every 48 hours. The fermentation is reduced to 2 days for Nacional cacao variety
- Then, they were sun dried on plastic sheets, usually in the roof of the stockpile place, for 3 days
- After that, beans were cleaned and selected manually and toasted for two hours
- A second selection was made manually, before being milled for 2 hours
- Beans coat was discarded using a peeling machine for 30 min
- Finally, cocoa liquor was put in plastic molds and chilled for 30 min

No agrochemical products were used in this cacao farm. The collected samples belong to a Nacional-CCN 51 hybrid harvest.

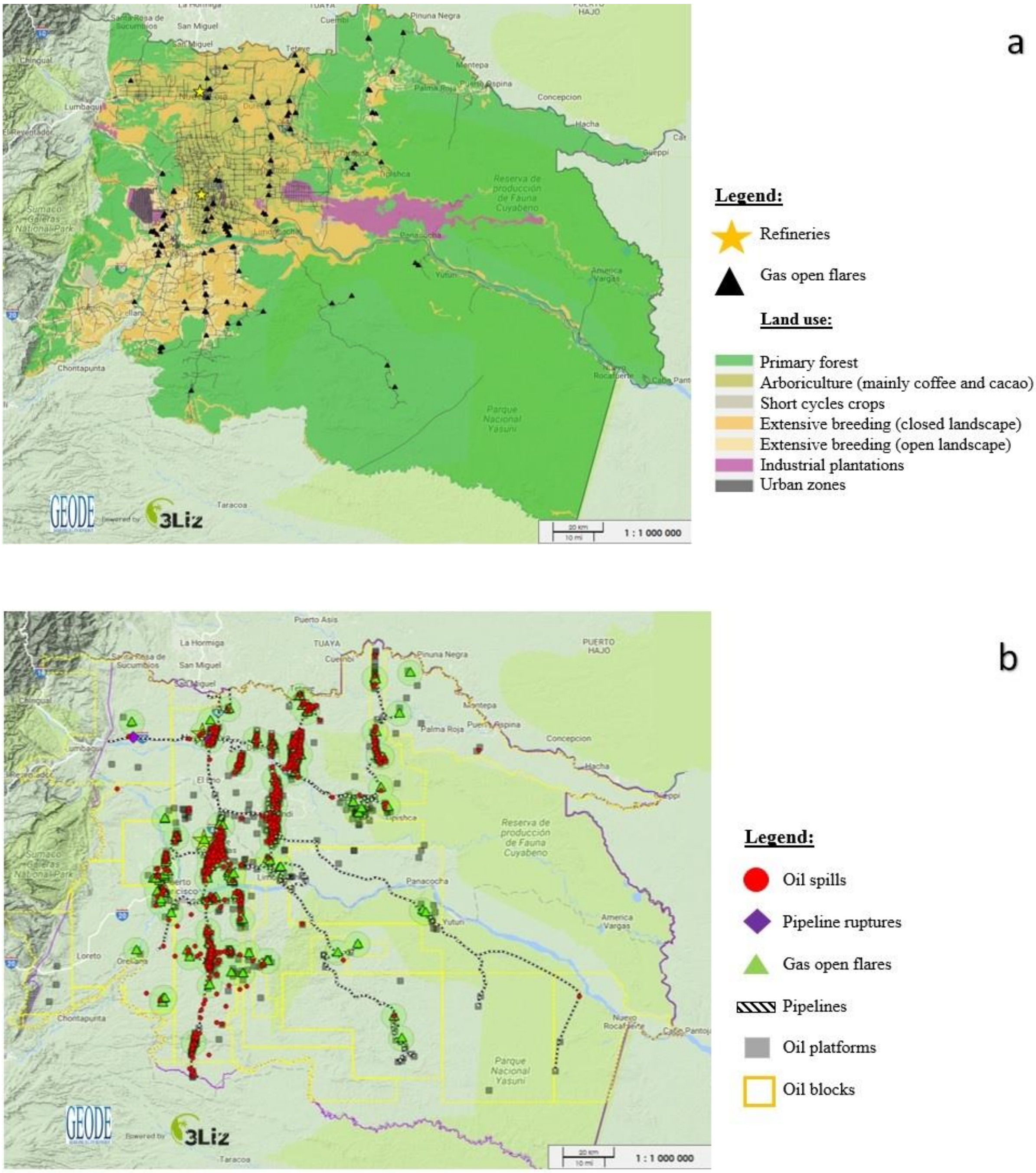

Fig. SI-1. Maps of land use (a) and oil contamination spots (b) in the Northern Amazon Region (NAR) of Ecuador.

**Elaborated by: Bosque S., Lerigoleur E., Maestripieri N., & M.Saqalli for Monoil Project, 2017.*

Table SI-1. Total Cd concentrations (mg kg^{-1} DW) in cacao leaves, pod husks and beans from 5 different varieties collected in 31 small-scale farms in NAR and NPC regions. Control areas (MS and MC) are represented by an asterisk next to the sample code.

Sample Code	Sampling site	Cacao variety	Leaves	Pod husks	Beans
1a*	Yawi-Gualaquiza (MS)	Native	0.47±0.05	0.35±0.02	0.14±0.01
1b*	Yawi-Gualaquiza (MS)	Native	0.41±0.05	0.43±0.02	0.40±0.01
2*	Yawi-Gualaquiza (MS)	Zamora	0.83±0.00	0.37±0.04	0.25±0.01
3*	San Miguel (MS)	CCN-51	0.48±0.05	0.14±0.02	0.16±0.01
4*	San Miguel (MS)	CCN-51	0.28±0.00	0.16±0.00	0.13±0.01
5	Dayuma (NAR)	CCN-51	7.87±0.05	2.81±0.02	3.50±0.16
6	Com. Cristal (NAR)	CCN-51	1.24±0.05	1.20±0.02	1.25±0.19
7	Com.Shuar Tzakim (NAR)	Nacional	1.47±0.05	0.34±0.02	0.71±0.01
8	Com.Shuar Rio Tiputini (NAR)	CCN-51	0.71±0.08	0.35±0.04	0.25±0.01
9	Auca Sur (NAR)	Nacional	2.83±0.12	1.39±0.05	1.49±0.19
10	Auca (NAR)	Nacional	1.95±0.05	0.85±0.02	0.53±0.00
11	Com. Kichwa Maduro Yaku (NAR)	Nacional	0.72±0.01	0.29±0.02	0.18±0.01
12	Flor de Manduro (NAR)	Nacional	1.51±0.03	0.68±0.02	0.62±0.01
13	Shushufindi (NAR)	Nacional	3.63±0.09	4.38±0.02	2.50±0.19
14	Shushufindi (NAR)	Nacional	4.25±0.00	0.17±0.02	1.25±0.19
15	Coop.Nueva Aurora (NAR)	Nacional	1.48±0.03	0.97±0.02	0.61±0.19
16	Nueva Quevedo (NAR)	Nacional	7.42±0.05	0.37±0.02	1.16±0.19
17	Nueva Quevedo (NAR)	CCN-51	1.50±0.01	0.61±0.02	0.56±0.19
18	Coop.Pacayacu (NAR)	CCN-51	1.76±0.03	3.23±0.05	1.02±0.19
19	Coop.Pacayacu (NAR)	CCN-51	1.76±0.05	0.99±0.02	1.37±0.19
20	Coop.Pacayacu (NAR)	CCN-51	2.32±0.04	1.14±0.02	1.02±0.19
21	Coop.Pacayacu (NAR)	CCN-51	3.76±0.05	0.08±0.02	1.20±0.19
22	Nuevo Oriental (NAR)	Forastero	0.19±0.05	0.93±0.02	0.09±0.01
23a*	Santa Ana (MC)	CCN-51	1.49±0.08	0.71±0.02	1.40±0.19
23b*	Santa Ana (MC)	Nacional	1.45±0.05	1.02±0.04	0.77±0.19
24a*	Portoviejo (MC)	Nacional ETT103	4.84±0.05	2.12±0.02	1.66±0.19
24b*	Portoviejo (MC)	Nacional ETT116	6.43±0.05	4.09±0.02	3.51±0.19
25*	Portoviejo (MC)	Nacional ETT576	2.39±0.17	1.04±0.02	0.90±0.19
26	Lucha de los Pobres (NPC)	Nacional	0.34±0.05	0.29±0.02	0.52±0.19
27	Lucha de los Pobres (NPC)	Nacional	0.62±0.03	0.19±0.02	0.24±0.19
28	Lucha de los Pobres (NPC)	Nacional	0.51±0.11	0.31±0.02	0.44±0.19
29	Lucha de los Pobres (NPC)	CCN-51	0.85±0.00	1.01±0.02	1.06±0.02
30	Lucha de los Pobres (NPC)	CCN-51	0.97±0.05	0.73±0.01	0.85±0.05
31	Tiwinza (NPC)	Nacional	1.96±0.05	0.99±0.02	1.30±0.19

Table SI-2. Monthly and annual mean Cd concentrations (ng m^{-3}) in aerosols (PM_{10}) in the Northern Amazon Region (Auca Sur and Shuara 9) and in the North Pacific Coast (Esmeraldas)

Date /Sampling site	Auca Sur (NAR)	Shuara 9 (NAR)	Esmeraldas (NPC)
Dec14-Jan15	0.10	0.13	--
Jan-Feb15	0.12	0.10	--
Feb-Mar15	0.17	0.20	--
Mar-Apr15	0.10	0.20	0.42
Apr-May15	0.11	0.13	0.37
May-June15	0.10	0.10	0.27
June-July15	0.09	0.16	--
July-Aug15	0.07	0.07	--
Aug-Sept15	0.10	0.08	--
Sept-Oct15	0.11	0.25	--
Oct-Nov15	--	0.39	0.57
Nov-Dec15	--	0.22	0.55
Annual mean concentration	0.11±0.03	0.17±0.09	0.44±0.13

Notes:
--: no available data due to technical issues
Analyzes in replicate were not possible, only one filter was collected each month.

Table SI-3. Correlation coefficients (r^2) between Cd content in cacao beans, pod husks and leaves and soil physico-chemical properties for Northern Amazon Region (NAR), North Pacific Coast (NPC), Morona Santiago (MS) and Manabi Control area (MC).

Variables	Cd beans	Cd pods	Cd leaves	Cd soil 0-5cm	Cd soil 5-20 cm	pH 0-5 cm	pH 5-20 cm	TOC 0-5 cm	TOC 5-20 cm	CEC 0-5 cm	CEC 5-20 cm
For all sites and all variables (N=34):											
Cd beans	1										
Cd pod husks	**0.79**	1									
Cd leaves	**0.81**	0.52	1								
Cd Soil 0-5cm	0.64	0.43	0.59	1							
Cd Soil 5-20cm	0.53	0.36	0.47	0.79	1						
pH 0-5 cm	0.17	0.12	-0.01	0.07	0.31	1					
pH 5-20 cm	0.19	0.15	0.00	0.04	0.33	0.97	1				
TOC 0-5 cm	0.20	0.21	0.28	0.30	0.16	-0.30	-0.25	1			
TOC 5-20 cm	-0.14	-0.19	-0.04	0.24	0.00	-0.13	-0.18	0.15	1		
CEC 0-5 cm	-0.12	-0.07	0.03	-0.07	-0.26	-0.83	-0.78	0.45	0.16	1	
CEC 5-20 cm	-0.23	-0.14	-0.06	-0.10	-0.36	-0.75	-0.75	0.40	0.41	0.90	1
For NAR and MS (N=23):											
Cd beans	1										
Cd pod husks	**0.71**	1									
Cd leaves	**0.79**	0.34	1								
Cd Soil 0-5cm	0.66	0.35	0.53	1							
Cd Soil 5-20cm	0.48	0.20	0.37	0.83	1						
pH 0-5 cm	0.18	0.22	0.14	0.24	0.34	1					
pH 5-20 cm	0.28	0.34	0.19	0.23	0.39	0.94	1				
TOC 0-5 cm	0.43	0.36	0.35	0.34	0.36	0.18	0.34	1			
TOC 5-20 cm	-0.06	-0.17	-0.03	0.28	0.12	0.34	0.27	-0.04	1		
CEC 0-5 cm	0.05	0.05	0.06	-0.23	-0.07	-0.54	-0.32	0.24	-0.49	1	
CEC 5-20 cm	-0.52	-0.30	-0.39	-0.58	-0.48	0.11	0.14	-0.05	0.26	0.28	1
For NPC and MC (N=11):											
Cd beans	1										
Cd pod husks	**0.96**	1									
Cd leaves	**0.90**	**0.96**	1								
Cd Soil 0-5cm	0.73	0.83	0.92	1							
Cd Soil 5-20cm	0.59	0.69	0.81	0.94	1						
pH 0-5 cm	-0.47	-0.52	-0.69	-0.68	-0.76	1					
pH 5-20 cm	-0.53	-0.61	-0.72	**-0.75**	-0.65	0.75	1				
TOC 0-5 cm	-0.11	-0.01	0.11	0.19	0.20	-0.48	-0.51	1			
TOC 5-20 cm	-0.39	-0.34	-0.33	-0.14	0.08	0.09	0.13	0.04	1		
CEC 0-5 cm	-0.06	-0.18	-0.16	-0.29	-0.11	-0.01	0.45	-0.64	0.04	1	
CEC 5-20 cm	-0.01	-0.14	-0.16	-0.33	-0.29	0.17	0.43	-0.66	-0.41	0.84	1

Note: *bold numbers indicate significant correlation between variables*

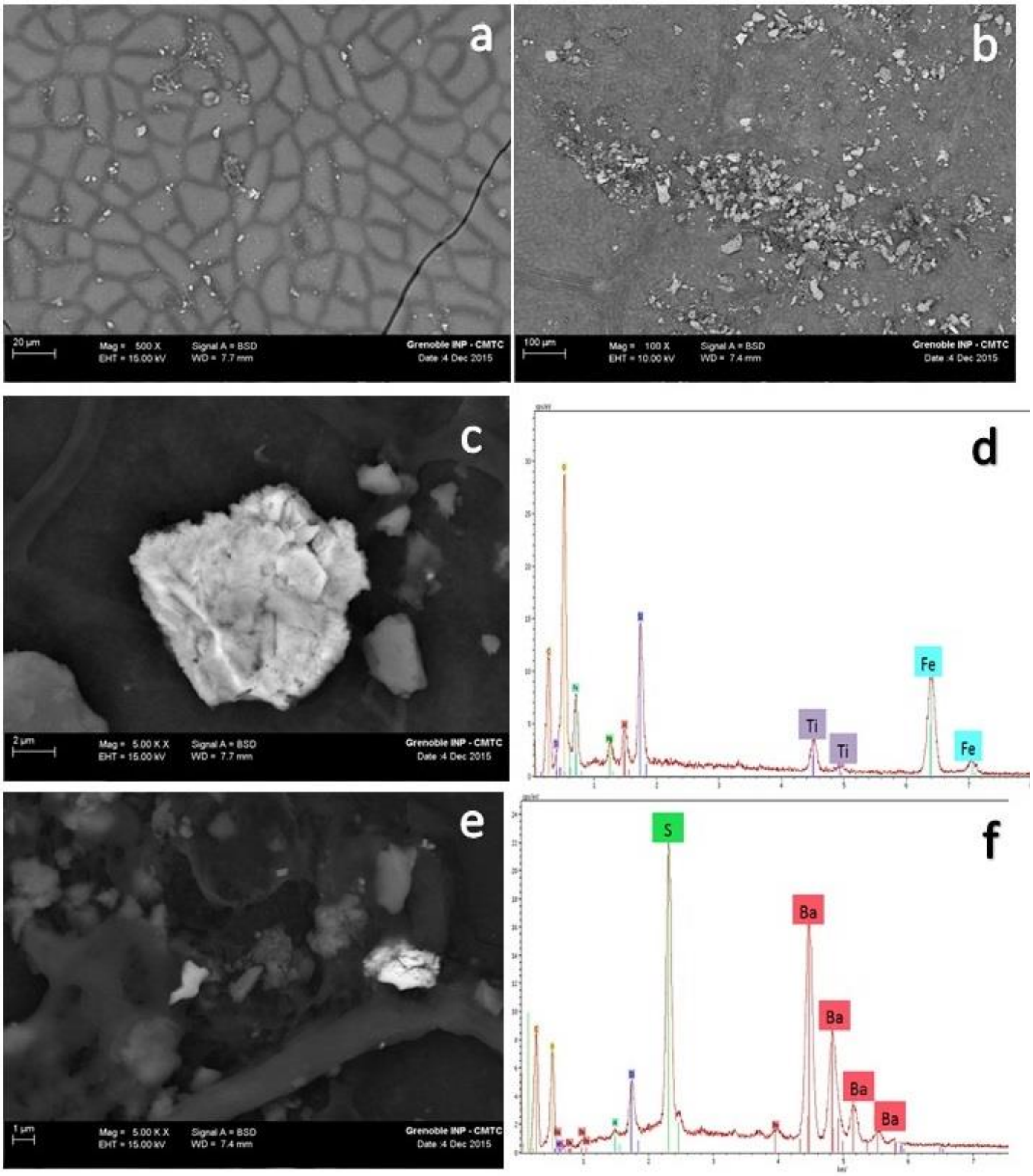

Fig. SI-2. SEM-EDX images of cacao leaves collected 300m far from an open flare in the NAR. **a.** Surface of a washed leaf. **b.** Surface of an unwashed leaf. **c.** Iron-Titanium (Fe-Ti) crystal. **d.** SEM spectra showing the chemical composition. **e.** Barium sulfate ($BaSO_4$) crystal. **f.** SEM spectra showing the chemical composition.

Table SI-4. Total Cd concentrations* (mg kg^{-1}) in 4 crude oil samples from the NAR.

Sample description	Sampling site	Cd concentration	LQ**
Crude oil	National Oil Pipeline LagoAgrio (Sucumbios)	<LQ	0.1
Crude oil	Auca 3 oil camp Dayuma (Orellana)	<LQ	0.1
Crude oil from a waste pool (in a soil matrix)	Auca 8 oil camp Dayuma (Orellana)	<LQ	0.1
Bituminous sandstones	Road from Coca to Tena (Orellana)	<LQ	0.1

*Determined by ICP-MS and certified by the Ultra traces Analyses Aquitaine (UT2A) Laboratory (Pau, France).
**LQ: Limit of quantification (mg kg^{-1})

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II. Other metals contents in cacao crops

Manganese, Ba, Zn, Cu and Ni mean concentrations (± SE, standard error) in cacao leaves, pod husks and beans of five varieties (Nativo Amazonico, Zamora, Nacional, CCN-51 and Forastero) collected in the Amazon and Pacific coast regions, as well as in control areas, are shown in Figure XVII. These metals followed the same tendency that was observed for Cd; concentrations were always highest in leaves, then in pod husks and finally in beans. Arsenic, Co, Cr, Pb, Mo and V concentrations were below the limits of detection.

In leaves

Manganese concentrations ranged from 12 (NPC, Nacional variety) to almost 1960 mg kg^{-1} (NAR, Nacional variety). The mean concentration of Ba (all sites, all varieties) was 114.99 ± 18.44 mg kg^{-1}, with the highest value reaching 354.44 mg kg^{-1} in the NAR for the Nacional cacao variety. Concentrations of Zn varied between 17 and 262.52 mg kg^{-1} in the NPC and in the NAR, respectively, corresponding also to the Nacional cacao variety. Copper and Ni concentrations were between 0.65 and 39.87 mg kg^{-1}, respectively. The highest concentrations for both elements were found in the CCN-51 variety in the NAR.

Concentrations of Zn and Mn in our study were 1.4 to 4.5 times higher than those reported by Arévalo-Gardini et al. (2017) for 8 different cacao varieties collected in Peru, which mainly include CCN-51 genotypes. In another study in Peru, Huamani et al. (2012) recorded Mn and Zn concentrations 2.7 to 5.4-fold lower than values found for cacao leaves in Ecuador. Arévalo-Gardini et al. (2017) reported Cu and Ni mean concentrations similar to those found in our study.

In pod husks

Manganese mean concentrations varied from 11.13 ± 2.25 to 135.67 ± 26.35 mg kg^{-1} in the NPC and MS, respectively. Mean concentrations of Zn ranged between 41.53 ± 5.96 in the NPC and 59.93 ± 9.95 mg kg^{-1} in MS. Barium concentrations varied from 2.46 (MC) to 98.10 mg kg^{-1} (NAR) in the CCN-51 cacao variety. The highest concentration of Cu (24.20 mg kg^{-1}) was found in the NAR, also in the CCN-51 variety. Nickel mean concentrations ranged from 3.10 to 6.27 mg kg^{-1}.

In beans

Mean Zn concentrations varied from 51 to 65 mg kg^{-1} in the NPC and in the MS, respectively. The highest concentration of Mn, corresponding to 176 mg kg^{-1}, was found in the NAR for the Nacional cacao variety in a specific fruit affected by a tropical disease. Barium concentrations ranged between 37.95 and 93.84 mg kg^{-1} in the MS control area (CCN-51 variety) and in the NAR (Nacional variety).

Copper and Ni concentrations were in the range of 1.01 to 93.84 mg kg^{-1} in CCN-51 and Nacional cacao varieties, respectively.

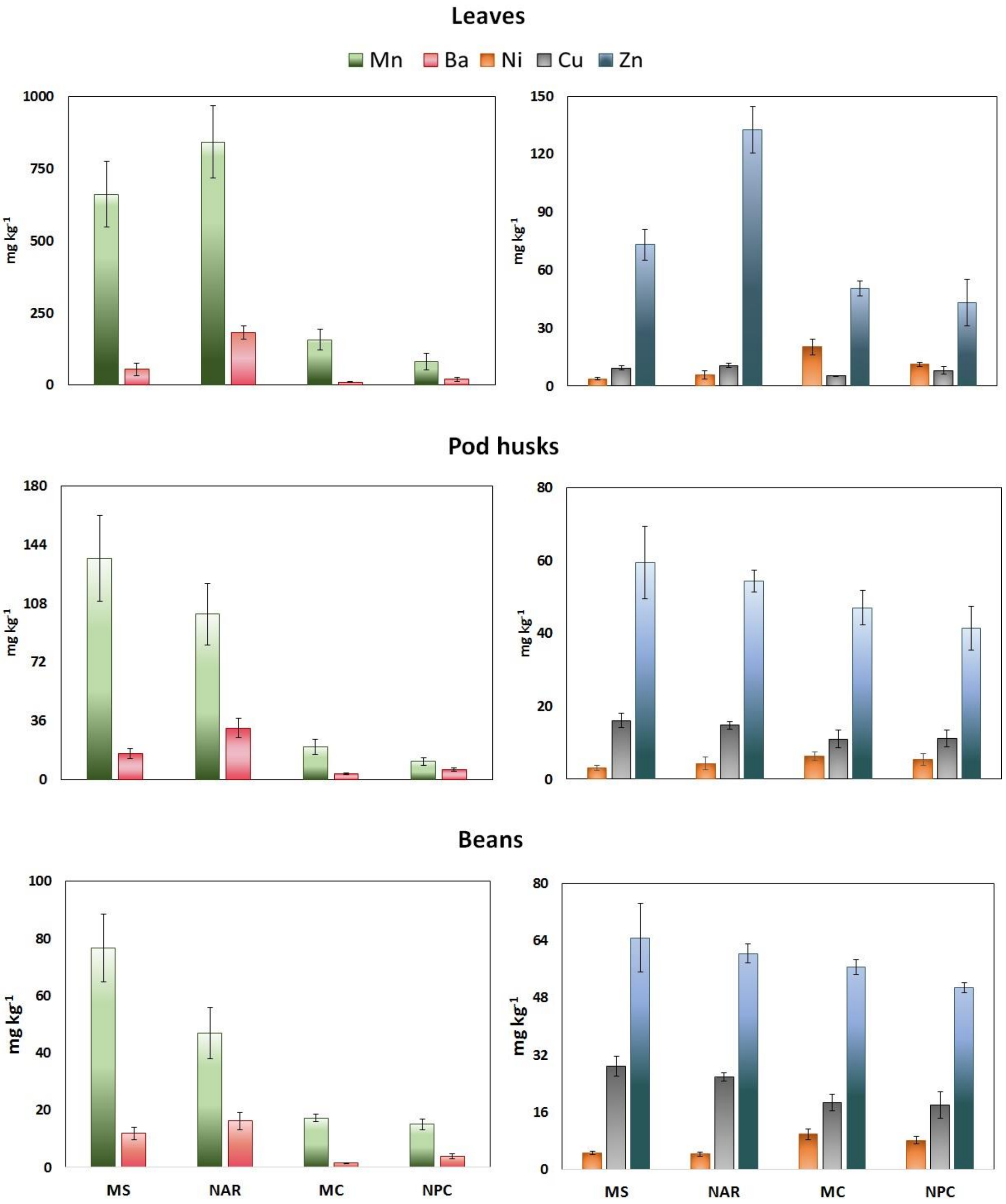

Figure XVII. Mean concentrations (± SE) of Mn, Ba, Zn, Cu and Ni in leaves, pod husks and cacao beans collected in: Morona Santiago control area (MS), North Amazon Region (NAR), Manabí control area (MC) and North Pacific Coast (NPC) of Ecuador.

In a recent study of the elementary composition of cacao beans (Forastero variety), Bertoldi et al. (2016) reported concentrations of Cu and Ni close to our values in samples collected in South America. Barium (22.2 ± 12.6 mg kg^{-1}), Mn (35.4 ± 11.6 mg kg^{-1}) and Zn (73.4 ± 60.8 mg kg^{-1}) concentrations in cacao beans for West Africa and Asia were also similar to our values reported in the NAR and in the MS.

In cocoa liquor:

Mean concentrations (± SD) for metals in two samples of cocoa liquor collected in the Pacayacu area are shown in Table VI and compared to the concentrations reported by Bertoldi et al. (2016).

Table VI. Metals concentrations (mg kg^{-1}) in cocoa liquor samples collected in Pacayacu (NAR).

Samples	Zn	Mn	Cu	Ba	Ni
Bertoldi et al. (2016)[a]	25.9±10.2	16.5 ± 2.8	13.3 ± 4.7	4.9 ± 1.84	4.67 ± 1.87
A*	59.16±2.99	18.67±0.18	24.67±0.06	6.95±0.06	4.85±0.02
B*	53.56±5.41	13.32±0.13	23.88±0.06	9.99±0.09	8.92±0.03

[a] *for 8 dark chocolate samples (>60% cacao solids = cocoa liquor, with beans from South America.*
** Hybrid of CCN-51 and Nacional varieties collected in the NAR (Pacayacu, Ecuador).*

Compared to our results, Mn and Ni concentrations were similar whereas Zn and Cu were 2-fold higher. Besides, Ba concentrations were slightly higher in our samples.

Differences between varieties and sampling sites

For all the metals already described, similar concentrations were found between the Nacional and the CCN-51 varieties. As we don't have enough samples of the three other varieties it's difficult to determine which species seems to concentrate the highest levels of metals within the cacao plant tissues.

According to the highest mean concentrations of metals, sampling areas can be ranked as follows: NAR/MS > MC > NPC.

Enrichment Factors between soils and cocoa tissues

Metal concentrations in soils associated with the cacao samples have been shown in **Chapter 1** (Table 2). Code equivalences between Chapter 1 and Chapter 2 (cacao-soil samples) are given in Appendix E, Table E.1.

Global enrichment factor (GEF) and Bioaccumulation factor (BF) are shown in Table VII. Both factors were calculated following the steps described in **Section 2.5** (accepted article). Based on these results, some cacao beans are enriched in Ba (62% of samples), Zn (59% of samples), Ni (55% of samples) and

Mn (28% of samples). Global enrichment factor of Cd was 4 to 18 times higher than GEF for the metals mentioned below. Bioaccumulation factor of Mn, Ba, Zn, Cu, Ni and Cu were 4 to 36 times lower than Cd-BF, suggesting that transfer from the soil to the rest of the plant is reduced (Kabata-Pendias, 2011).

Table VII. Global Enrichment Factor (GEF) and Bioaccumulation Factor (BF) values for cacao beans collected in the North Amazon Region (NAR), North Pacific Coast (NPC) and Manabí (MC).

	Mn		**Ba**		**Zn**	
	GEF	**BF**	**GEF**	**BF**	**GEF**	**BF**
Mean	0.77	0.08	2.81	0.09	1.21	0.66
Min	0.21	0.01	0.29	0.01	0.46	0.32
Max	3.61	0.52	11.77	0.36	1.93	1.31

	Ni		**Cu**	
	GEF	**BF**	**GEF**	**BF**
Mean	1.37	0.20	0.59	0.66
Min	0.24	0.04	0.15	0.15
Max	3.51	0.45	1.06	1.86

To conclude:

Even if some metals in cacao leaves, pods and beans in Ecuador have higher concentrations than those reported in the literature, it is not possible to affirm that cacao plants accumulate metals other than Cd. However, we observed a Mn enrichment in the cacao beans of fruits affected by diseases (black pod or frosty pod diseases). These data and the associated health risks will be presented in the next publication *In Prep*.

Chapter 3. Air quality and health exposure in the North Amazon Region and in the North Pacific Coast of Ecuador

Foreword

As previously reported in Chapter 1, inhalation is one of the main exposure pathways for metal(loid)s and their non-carcinogenic and carcinogenic effects in the NAR (North Amazon Region) and in the NPC (North Pacific Coast). Until this chapter, only one group of chemical compounds commonly found on airborne particles has been discussed: the inorganic compounds, especially metals and metalloids. But, aerosol composition is much more complex and unique at every sampling point, thanks to the influence of long-range transport and to changes in its size and sources of emission as well as chemical reactions occurring in the atmosphere. A better knowledge of the chemical composition and reactivity of aerosols is crucial to settle a number of control strategies to reduce aerosol emissions and minimize their impact on the environment and therefore on human health.

Chemical composition of aerosols

Figure XVIII displays an example of how Particulate Matter (PM) in the atmosphere was collected and which compounds currently contribute to the PM mass concentration. A criterion for PM classification is the type of emission. Primary aerosols are emitted directly from the source (natural or anthropogenic) into the atmosphere. This is the case for aerosols produced by a tearing effect (i.e., wind friction on the ocean or the earth's crust) and those emitted during incomplete combustion. Secondary aerosols are formed later by condensation of gaseous compounds which can undergo several chemical transformations (Boucher, 2011).

Particle size and human respiratory system

PM size is defined by the aerodynamic diameter, which is the diameter of a spherical particle with a density of 1 g cm^{-3} (Muhlfeld et al., 2008). The two most regulated classes are PM_{10} (with a diameter ≤ to 10 µm, coarse particles) and $PM_{2.5}$ (with a diameter ≤ 2.5 µm, fine particles). PM_{10} has been linked to serious cardiopulmonary diseases, acute respiratory infection and trachea, bronchus, and lung cancers (Loomis et al., 2013; Tecer et al., 2008; Vaduganathan et al., 2016), and $PM_{2.5}$ has been classified as carcinogenic since 2008. Moreover, epidemiological research indicates that the finer the particles are, the greater the health outcomes are, since fine and ultra-fine particles can enter deep into the lungs and partially into the bloodstream (Wolff and Perry, 2010).

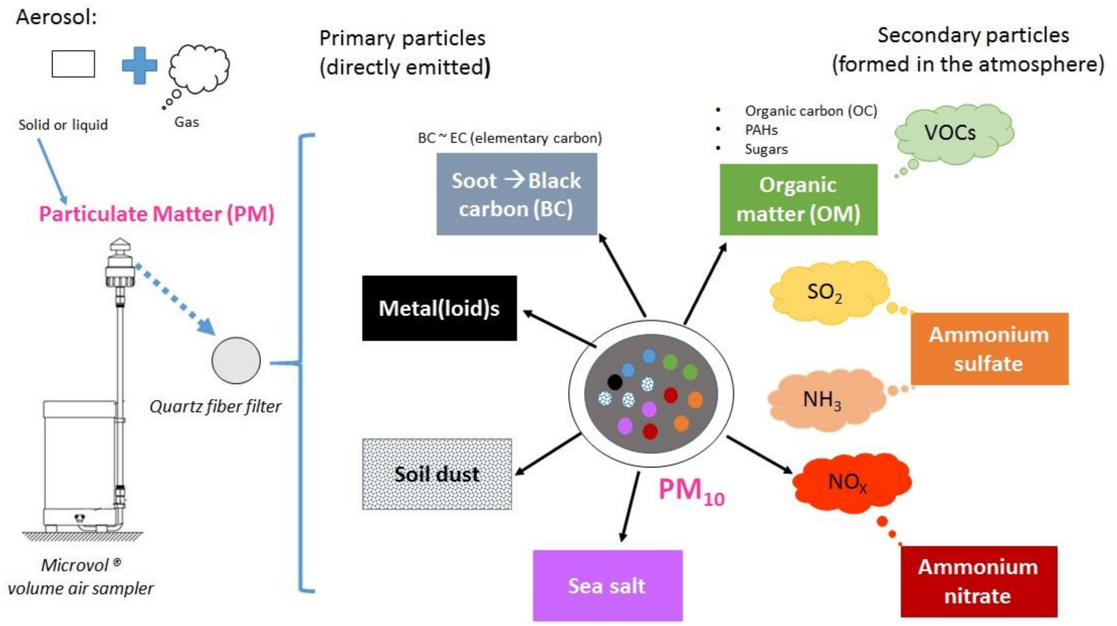

Figure XVIII Example of PM_{10} chemical composition, collected on quartz fiber filters using a low volume air sampler (Microvol ®). (Sources: Hassan et al., 2013; Pernelet-Joly, 2008; Pöschl, 2005; Wang et al., 2015).

A description of respiratory airways and the impact of PM inhalation according to its size is given in Figure XVI. Dust may be inhaled via either nose or mouth and, in addition with the pharynx and larynx, they form the nasopharyngeal region. Here, particles are equilibrated with body temperature and humidity, and large particles are restricted. Particles, then, enter the tracheobronchial region (airways connecting the larynx to the terminating bronchioles) and finally the alveolar region (bronchioles, alveolar ducts and sacs) (Kastury et al., 2017).

PM and Reactive Oxygen Species (ROS) formation

Results from toxicological research have shown that PM has several mechanisms of adverse cellular effects such as: cytotoxicity, oxygen-free radical-generating activity, DNA oxidative damage, mutagenicity and stimulation of pro-inflammatory factors (Valavanidis et al., 2008).

The key and unifying parameter of such biological effects is admitted to be the oxidative stress. Oxidative stress occurs when there is an imbalance between the level of reactive oxygen species (ROS), or free radicals, in the organism and the biological system's natural antioxidant defense. ROS can be generated directly on the PM surface or indirectly when cells interact with PM (Bates et al., 2015). The inherent capacity of PM to oxidize specific molecules, known as Oxidative Potential (OP), has been proposed as a promising and an additional air quality exposure metric alongside PM mass concentrations (Boogaard et al., 2011).

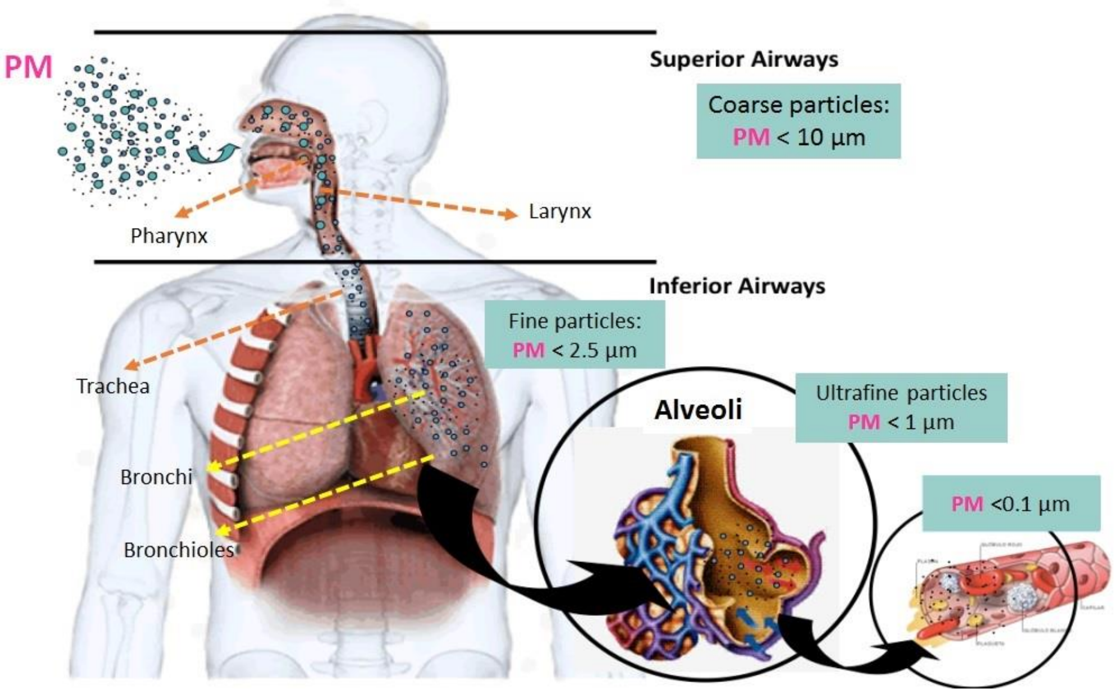

Figure XVI. Respiratory tract and particulate matter (PM) size classification. (Modified from: https://www.intechopen.com).

As already mentioned in the Introduction section, three different assays (DTT: Dithiothreitol, AA: Ascorbic acid and DCFH-DA: 2’,7’-dichlorodihydrofluorescein diacetate) were run to assess the OP from PM collected monthly in Ecuador. The principle of such reactions is to assess the depletion of a lung antioxidant or a surrogate when in contact with PM. Main reactions are given in Figure XVII and each methodology is described below, in Section 2.3 (following the *In Prep.* article).

Properties of each anti-oxidant or surrogate:

- ✓ Dithiothreitol is a strong reducing agent, responsive with the largest pool of chemical components present in PM (Xiong et al., 2017).
- ✓ Ascorbic acid, with antioxidant, anti-proliferation and anti-inflammatory properties, has a critical role in protecting the immune system, reducing allergic reactions and combating infections. It is also one of the main lung antioxidants (Jin et al., 2016).
- ✓ Dichlorodihydrofluorescein diacetate has been used for decades in biology to detect ROS and RNS (reactive nitrogen species). Non fluorescent fluorescein derivatives will emit fluorescence after being oxidized by hydrogen peroxide (Rastogi et al., 2010).

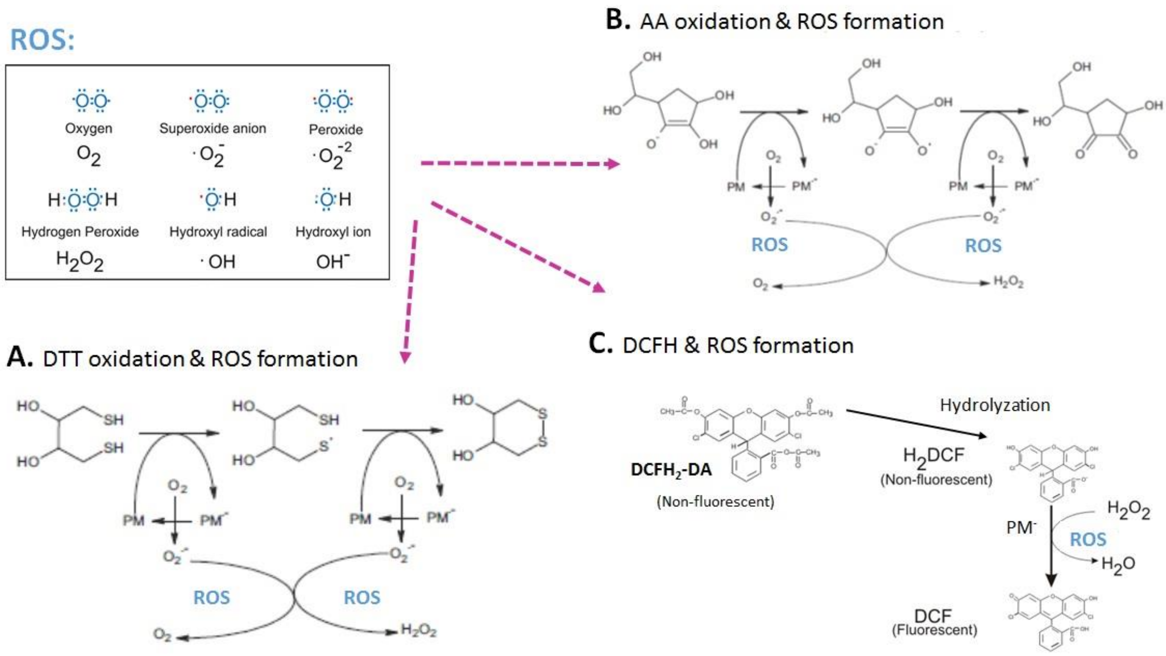

Figure XVII. Reactive oxygen species (ROS) and measurement of oxidative potential (OP) by three acellular assays. **A.** DTT: Dithiothreitol is oxidized to its disulphide form, which donates an electron to dissolved oxygen, forming a superoxide anion. **B.** AA: Ascorbic acid is oxidized to dehydroascorbic acid while ROS are reduced, then they transfer an electron to oxygen molecules promoting the formation of ROS. **C.** DCFH-DA: 2’,7’-dichlorodihydrofluorescein diacetate is hydrolyzed to H_2DCF (2’,7’-dichlorodihydrofluorescein) which undergoes a two-electron oxidation in the presence of ROS to yield DCF (2’,7’-dichlorofluorescein). (Visentin et al., 2016; Wang and Joseph, 1999).

In the following *In Prep* article, to be submitted to Atmospheric Environment, the chemical speciation and ROS generation ability of PM_{10} in areas impacted by oil extraction and refining in Ecuador is presented. Specific oil tracers, biomass burning tracers and industrial tracers were found in the sampling areas and were useful for assigning ROS generation to their source of emission.

I. Chemical composition of PM$_{10}$ and ROS generation in the vicinity of gas flares in oil extraction and refining areas in Ecuador

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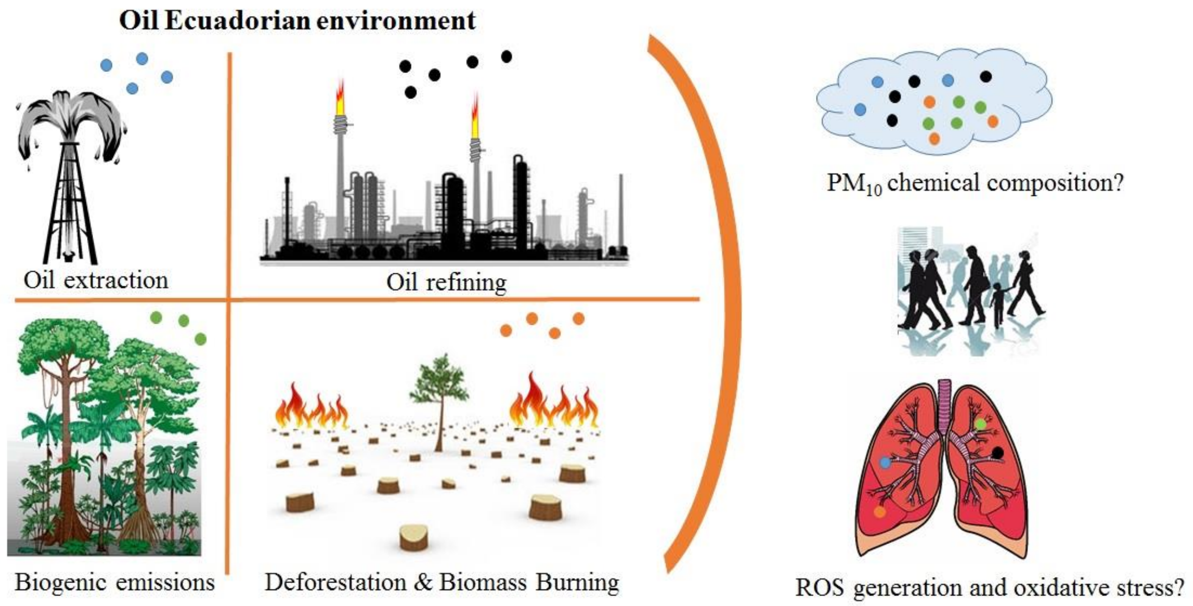
Graphical abstract:



Keywords: PM$_{10}$; oil extraction; oil refining; chemical speciation; PAHs; ROS formation; oxidative potential.

Abstract:

Oil extraction in rural sites in Ecuador, within the North Amazon Region (NAR), have generated for the last 40 years a mixture of atmospheric compounds, mainly polycyclic aromatic hydrocarbons (PAHs) and metal(loid)s, that could be toxic for local populations. Refinery activities, mainly located in Esmeraldas city, an industrial site in the North Pacific Coast (NPC) are also considered as an important source of air contamination for the country. Particulate matter (PM_{10}), emitted in both sites, could induce the formation of reactive oxygen species (ROS) associated with cell inflammation and respiratory diseases.

For the first time, PM_{10} mass composition was determined by a two-year monitoring at two sites. One is located in the NAR (close to oil platforms and open flares) and the second stands in NPC (direct vicinity of the Esmeraldas refinery). PM_{10} speciation was assessed thought metal(loid)s, organic and elementary carbon (OC, EC), monosaccharides (levoglucosan, manosan, galactosan), glucose, polyols (sorbitol, mannitol, arabitol), water soluble ions and PAHs (oxy and nitro-PAHs). Additionally, three complementary acellular tests (using reducing agents such as dithiothreitol (DTT), ascorbic acid (AA) and dichlorofluorescein DCFH) were performed to evaluate their oxidative potential (OP).

Results show that PM_{10} mass and toxic compounds concentrations were higher in the NPC than in the NAR. Barium and molybdène concentrations, commonly used in oil operations, were up to 1000 fold higher than values recorded in Quito. By contrast, OC/EC ratios were higher in the NAR than in the NPC, as well as polyols concentrations, suggesting larger biogenic contributions to the PM in this forestal region. Concentrations of PAHs, oxy- and nitro-PAHs remained low at the three sampling sites but they could have been underestimated for their volatility during sampling.

Chemical compounds related to ROS generation were specific to each sampling site and each one correlated to different sources. In the NAR, the main sources leading to the OP values are biogenic emissions and oil production, as highlighted by positive correlations between OP, sugars, polyols and Ba. By contrast, in the NPC, positive correlations between NH_4^+, Ba, As and Ni indicate that oil refining and industrial activities are the main sources of active compounds in the PM.

1. Introduction

In the last 50 years, atmospheric pollution has become a growing public health concern and accounts for almost 10% of all deaths worldwide, according to the Environmental Performance Index (EPI, 2016). Indeed, more than 3.5 billion people live in countries with unsafe levels of air pollution (Yang and Wang, 2017). Air quality and climate change from local to global scales are also influenced by airborne emissions from major urban and industrial areas (Baklanov et al., 2016).

Aerosols are composed of particulate matter (PM) and gaseous components. Particles (PM) are classified according to their aerodynamic diameter as coarse particles (≥ 2.5-10 µm), fine particles (≥ 0.1 -2.5 µm) and ultrafine particles or nanoparticles (< 0.1 µm) (Bourdrel et al., 2017)The smaller they are, the deeper they can travel into the respiratory system leading to many health outcomes, including cancer (Hoek et al., 2013; Raaschou-Nielsen et al., 2013). Depending on their emission sources, PM can exhibit many different compounds such as soot, metals and metalloids, salts, polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs) and even bacterial and fungi products (Santibáñez-Andrade et al., 2017). Agriculture, residential heating and cookingvehicular traffic, fossil fuel combustion, oil refining, mining, steel industries, marine transport, desert dust and biomass burning are the main anthropogenic outdoor pollution sources (Gurjar et al., 2016; Hadidi et al., 2016; Liang et al., 2016; Monks et al., 2009). International air quality standards focus on the quantification of six compounds: ozone, PM,SOx, NOx, Ox and Pb. Nevertheless humans are exposed to other harmful substances such as Cd, As, Ni, Hg, benzo(a)pyrene, or benzene (Baldasano et al., 2003; Boyd, 2006; Guerreiro et al., 2014). In this view, European Union air quality directives are amongst the most complete and restrictive in comparison to other countries.

In Latin America, emissions from industrial sectors have experienced a rapid growth. However, a lack of air monitoring, or with obsolete equipment and questionable methodology, have made difficult the collection of reliable data about air quality (Ossés de Eicker et al., 2010).

Among the well-known sources in the area, crude oil industry generates atmospheric emissions coming from different sources: gas flaring (Fawole et al., 2016), oil pits evaporation (Avdalović et al., 2016; Kirkhus et al., 2015) and high temperature combustion processes during refining (Yassaa and Cecinato, 2005).

Nowadays, Ecuador is the 5th largest oil producer country in South America and the 27th in the worldwide ranking (Ecuador Oil Production, 2016). Extraction activities have taken place in the North Amazon region (NAR), while refining occurs in Esmeraldas, in the North Pacific Coast (NPC). Texaco Oil Company has operated during 30 years (1960s–1992)and technologies used for oil production (Buccina et al., 2013) have generated millions of gallons of untreated toxic waste which were partially released into the environment (San Sebastián and Hurtig, 2005). For example, natural gas collected during oil exploration can be transported and re-used or recycled back, however, in developing countries, the excess of gas is flared or vented *in situ*, being a prominent source of black carbon (BC), the second highest contributor to global warming after CO_2 (Fawole et al., 2016). In the Ecuadorian Amazon, roughly 1.5 million cubic meters of wasted gas is burned daily without temperature or emissions controls (San Sebastián and Hurtig, 2004).

In addition, the development of oil industry, together with agriculture and pasture, has significantly contributed to the reduction of the tropical forest surface in Ecuador (Welford and Yarbrough, 2015).

For instance, approximately 34% of the prime Amazonian forest has been deforested (Mosandl et al. 2008; Rudel et al., 2002). Nevertheless, the remaining forest can still be considered as an important source of natural and anthropogenic aerosols emissions from the biosphere. Sources include high but intermittent biomass burning episodes and low but more consistent production of primary (i.e. pollen, spores, leaves and soil resuspension) and secondary bioaerosols (low volatile compounds formed in the atmosphere), as reported by Fröhlich-Nowoisky et al. (2016) and Martin et al. (2010). Biomass burning includes natural forest fires, being most intense in the Ecuadorian Amazon during El Niño events –characterized by severe drought and high temperatures (Boy et al., 2008)-, as well as human-initiated burning for land clearing or land-use change (Artaxo et al., 2013).

Oil production activities, biomass burning and biogenic emissions then release into the atmosphere contaminants such as PAHs, VOCs, metals, water soluble ions, making sources apportionment difficult. However, biomass burning tracers like levoglucosan and its isomers, mannosan and galactosan are exclusively generated from pyrolysis of cellulose and hemicellulose, the main constituent of plants cell walls (Zhu et al., 2015). The combustion of other materials, such as fossil fuels, does not produce levoglucosan (Cheng et al., 2013). Besides, polyols such as arabitol and mannitol are considered as good markers of biogenic emissions and more specifically linked to airborne microorganisms (fungal spores, bacteria) (Fu et al., 2016).

In this context, inhabitants living in the Amazon region affected by oil extraction activities or in refining areas may be exposed to a large cocktail of metal(loid)s and PAHs. But air quality in Ecuador remains poorly studied (Raysoni et al., 2017) and, to our knowledge, literature about aerosols' properties and their health impact after inhalation in Ecuador is scarce. Moreover, toxicity, carcinogenic and mutagenic potency of these atmospheric compounds differ considerably among them (European Communities, 2000; Liang et al., 2012). Actually, an epidemiological study conducted on a village surrounded by 30 oil wells and 4 powerful gas burners in the Ecuadorian Amazon evidenced that the apparent excess of cancer morbidity and mortality could be linked to population's exposure to toxic contaminants coming from oil production (San Sebastián et al., 2001). In addition, the origin of the health effects experienced from atmospheric PM is assigned to their ability to induce cellular oxidative stress by the generation of reactive oxygen species (ROS) in the lung (Idelchik et al., 2017; Secrest et al., 2016). ROS include superoxide radical (O_2•), hydrogen peroxide (H_2O_2), hydroxyl radical (•OH), alkoxyl radical (RO•), and singlet oxygen ($^1O_2$) (Hedayat et al., 2015). Quinones and transition metals have been identified as redox active species (Charrier and Anastasio, 2012; Crobeddu et al., 2017). This is why the inherent capacity of PM to oxidize a biological media is proposed as a unifying exposure metric to assess health risk from air pollution (Kelly et al., 2012; Sauvain et al., 2013). Several methods coexist for measuring the oxidative potential (OP) of PM and are based on the depletion of lung anti-oxidants or surrogate when in contact with PM.

This study was conducted in the Ecuadorian NAR and NPC areas, respectively the core of oil extraction and refining activities in the country, to evaluate their air quality and evolution over two sampling years. A detailed aerosol speciation was achieved and the potential link with oil activities (extraction and refining) was investigated by trying to isolate some specific tracers of industrial oil processes. Finally, we explore the ability of PM to induce ROS formation in the lung in order to assess its potential health impacts.

2. Materials and methods

2.1 Study areas and sampling strategy

Aerosols (PM_{10} for particulate matter with diameter under 10 µm) were collected every two weeks or monthly on pre-calcined quartz fiber filters, from January 2015 until December 2016, in three provinces of Ecuador impacted by oil activities (Fig.1): Orellana and Sucumbíos, in the Northeast Amazon region (NAR), and in Esmeraldas, in the North Pacific Coast (NPC).

A total of six one-stage low volume air samplers (MicroVol-1100) operated at a flow rate of 3 L m^{-1}, were placed on the roof of three buildings: in two houses 300 m away from gas-burning open flares, in the vicinity of the Auca (S 0° 42’ 16.49’’, W 76° 53’ 15.96’’) and Shuara (N 0° 3’ 33.26’’, W 76° 33’ 37.01’’) oil platforms, and in a primary school, and 1000 m away from the National Oil Refinery, in La Florida district of Esmeraldas city (N 0° 55’ 54.21’’, W 79° 40’ 40.91’’). Air samplers were powered with solar panels connected to a rechargeable battery pack in the NAR since no electricity was available, and plugged to the electric service in the NPC.

In the NAR, the sampling sites’ selection was done based on the socio-environmental indexes compiled by the Intelligence Subsystem of Environmental Statistics of Productive Activities in Ecuador (SIESAP, 2015) developed by the Ministry of the Environment and from the sociological surveys realized in the frame of the MONOIL “Monitoring of Oil activities in Ecuador: a cross-disciplinary approach between Environment, Health and People” Research Program. Such indexes categorize vulnerable areas as a function of the records of oil spills and dumping of formation waters. In the NPC air contamination hot spot’s maps elaborated by the National Oil Company (EP Petroecuador, 2013) provided the information to locate the samplers.

Predominant wind direction was NE and SW in Orellana and Sucumbíos respectively (INAMHI, 2015). In Esmeraldas, wind direction was S-SE and S-SW, following the Teaone and Esmeraldas River and ending in the Pacific Ocean in the morning, whereas in the afternoon wind comes from the ocean and the refinery (EP Petroecuador, 2013).

Wet season in the Pacific Coast region usually starts from mid-December until May and dry season from June until mid-November (GADPE, 2017). In contrast, in the Amazon region, precipitations are continuous over the year, but more pronounced between June and September (INAMHI, 2015).

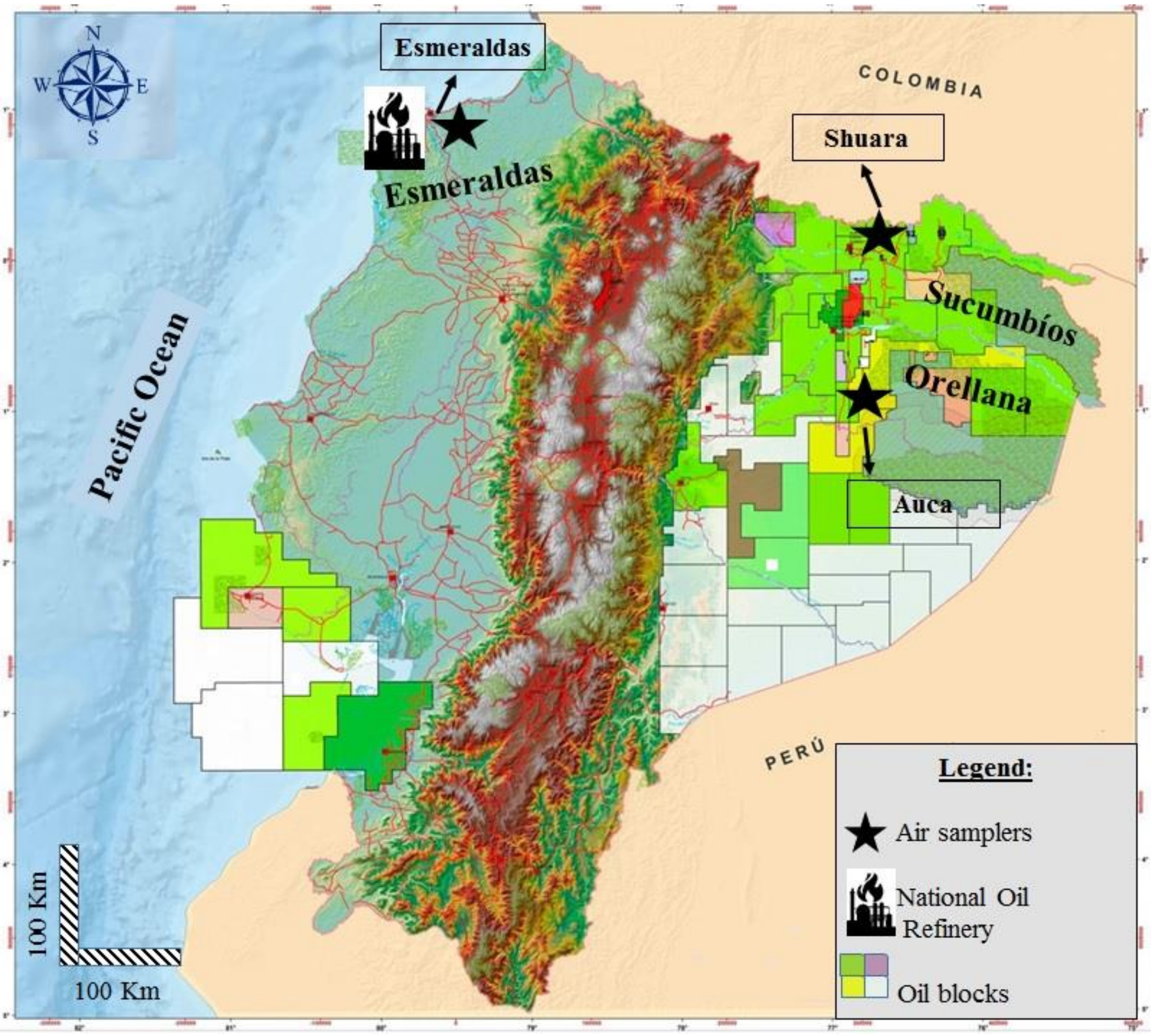


Fig 1. Sampling sites in Orellana (Auca) and Sucumbíos (Shuara) in the NAR (North Amazon Region) and in Esmeraldas (La Florida) in the North Pacific Coast (NPC). According to the Ministry of Non-renewable resources of Ecuador (SHE, 2015) the white and light blue blocks have not been assigned yet to oil exploitation, the others are currently being exploited.

Total annual precipitations in both areas can reach more than 3000 mm, meaning between 200 and 500 mm per month. Annual mean temperature is around 25°C, ranging from 13 up to 38°C (Pourrut, 1995).

Before analysis, PM_{10} samples were protected from the light with aluminum foils, frozen at -5°C, and then transported to France. Filters were weighted before and after sampling using an electronic microbalance (Mettler Toledo, ±10 μg), previously equilibrated during 24 h in conditioning chamber.

2.2 Aerosols chemical composition

- ***Major elements and trace metals concentrations***

Aerosol samples impacted on quartz fiber filters (punches of 5 cm^2) were digested on hotplate using 9 ml of aqua regia, 1 ml of H_2O_2 and 2 ml of HF (Trace metal grade analysis, Sigma-Aldrich). Mineralization solutions were evaporated at 60°C, the dry residue was then resuspended adding 0.5 ml of HNO_3 and diluted with ultra-pure water before ICP-MS (Agilent 7500) analyses in order to determine metals concentrations.

Certified reference material, SRM 1648a "Urban Particulate Matter" (from NIST) was used to validate the mineralization method. Recoveries of 80-105% were calculated for all trace metals. Field blank filters and blank acid samples were included on each batch of analyses in order to eliminate the quartz matrix composition.

- ***Soluble ions***

Water soluble cations, ammonium (NH_4^+), potassium (K^+), sodium (Na^+), magnesium (Mg^{2+}), calcium (Ca^{2+}) and anions, nitrate (NO_3^-), sulfate (SO_4^{2-}), chloride (Cl^-), oxalate $(COO)_2^{2-}$ and methanesulfonate ($MeSO_3^-$) were analyzed by ion chromatography (Dionex ICS-300) on a filter's punch of 4 cm^2 after extraction in 15ml of Milli-Q Water. This technique is based on the separation of different ionic species in aqueous solution on an ion exchange resin, using KOH and MSA (methanesulfonic acid) as eluents, followed by the quantification of each one by conductivity detection.

- ***Organic and elementary carbon***

Carbonaceous material was quantified by a thermal-optical analyzer (Sunset Laboratory, EUSAAR2 method) allowing to separate the Organic Carbon (OC) from the Elementary Carbon (EC) in the same sample. To separate the two fractions, a punch of 1 cm^2 of each filter was heated at different temperature ramps (up to 850°C) and more or less oxidizing atmospheres. Total Carbon (TC) was calculated as the sum of OC and EC.

- ***Organic compounds***

Monosaccharide anhydrides (levoglucosan, mannosan and galactosan), polyols (arabitol, mannitol, sorbitol), and sugars (glucose) were analyzed by LC-PAD (Dionex DX 500) in a filter's punch of 4 cm^2, using NaOH as eluent. Quantification of each compound was made by amperometric detection.

Quantification limits for each analysis are presented in Table SI-1, in Supplementary Information.

- ***Polycyclic aromatic hydrocarbons***

After extraction, purification and derivation, PAHs, oxy-PAHs and nitro-PAHs were analyzed by gas chromatography (GC) coupled with a mass spectrometer (MS).

List of quantified compounds is described in table SI-2, in Supplementary information.

2.3 Oxidative potential (OP) of PM_{10}

Oxidative potential of PM_{10} was assessed though three complementary acellular assays including DTT (dithiothreitol) assay, AA (ascorbic acid) assay and DCFH (dichlorofluorescin) assay. PM Extraction from the substrate (filters) for OP assays are usually performed in water, methanol or dichloromethane (Eiguren-Fernandez et al., 2010; Yang et al., 2014). However, to more closely mimic physiological processes when PM enters in contact with lung, it's suitable to use a medium close to human respiratory lining fluid, as well as an important interface between inspired air and respiratory tract cells (Schock et al., 2004; Visentin et al., 2016). For this purpose, a Gamble solution supplemented with surfactants such as dipalmitoylphosphatidylcholine (DPPC) is commonly used (Boisa et al., 2014)

Samples (punches of 0.5 cm^2) were extracted according to Calas et al. (2017) using the Gamble + DPPC solution and described in section 1 and table SI-3) with a constant concentration of 25 $\mu g\ ml^{-1}$ and vortexed for 1h15 at 37°C.

Blank filters were extracted in the same solution and included as triplicate in every assay.

- ***Dithiothreitol (DTT) assay* (OP^{DTT})**

DTT consumption by formation of DTT-disulfide form in presence of ROS (Yang et al., 2014) was measured using a plate-reader TECAN spectrophotometer Infinite® M 200 pro and 96 well CELLSTAR® plates.

For each sample, 40 µL of PM suspension (in triplicate) with 205 µL of phosphate buffer (pH=7.4) were incubated at 37°C in 12.5 nmol of DTT (DTT solution in phosphate buffer). Reaction was stopped at 0, 15 and 30 minutes adding 50 nmol of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB in phosphate buffer). A solution of 40 µL of 1,4 naphtoquinone (1,4-NQ 24.7 µM) was used as positive control. DTT loss after DTNB titration was read at 412 nm. OP was obtained from the slope of the linear regression of the consumed DTT (corrected from blank measurements and from matrix absorbance from particles) normalized by the PM_{10} volume (nmol $DTT.min^{-1}.m^{-3}$).

- ***Acid ascorbic (AA) assay* (OP^{AA})**

AA depletion by ROS production when in contact with PM, was monitored at 265 nm. 80 µL of PM extraction and 120 µL of water were injected manually on each well of Greiner UV-Star® (UV transparent) 96 wells plate before an automatic injection of 80µL of AA (80µM in the well). Absorbance was read at 265 nm every 4 minutes for 32 minutes after injection of 24 nmol of AA (100 µL of AA solution in Milli-Q water). 1,4 naphtoquinone (NQ) was used as a positive control.

The AA loss rate (nmol.min^{-1}) was determined from the slope of the linear regression of calculated nmol of consumed AA *vs* time (corrected from blank and matrix) . Units of OP^{AA} were calculated in the same manner as for OP^{DTT}.

- ***Dichlorofluoresceine (DCFH) assay*** **(OP^{DCFH})**

The oxidation of DCFH with horseradish peroxidase (HPR) by ROS and the formation of its fluorescent product 2′,7′-dichlorofluorescein (DCF) was measured by fluorescence (470 nm excitation/ 530 nm emission) in ROTH® 96 black multiwall plates (Foucaud et al., 2007).

In brief, 40 µL of sample extraction in Gamble + DPPC solution were injected on each well. Absorbance was read at 485 nm every minute for 15 minutes after injection of 220 µL of HRP/DCFH (5 U ml^{-1} and 40 µM respectively, in phosphate buffer). A solution of H_2O_2 at different concentrations (20, 40, 60, 100, 200 and 300 nM) and 2 blanks of Milli-Q water were including on each batch in order to calibrate/determine the formation rate of superoxide anions.

50 nM of H_2O_2 was used as a positive control. Units are expressed as nmol[H_2O_2]equiv m^{-3}

Detection limits (DL) for each analysis are presented in table SI-4, in Supplementary information.

2.4 Mass reconstruction

Reconstructed mass of PM is usually expressed as the sum of its representative chemical components: organic matter (OM), elementary carbon (EC), inorganic ions, soil minerals, salts (sea salts near oceans), trace elements and the remaining mass (Chow et al., 2015).

General equation used in this study was adapted from the methodology developed by Guinot et al. (2007) and Amato et al. (2016), in agreement with the environments of the sites:

Eq.1:

$$PM_{10}\,\mu g.m^{-3} = OM + EC + (\sum ions) + Soil\ elements + Trace\ metals$$

where:

OM = **f** x OC, with f = 1.7

OC = Organic Carbon

∑ions = anions + cations

Soil elements = 1.15 x (3.79 Al + 2.14 Si + 1.67 Ti) + Fe

The conversion factor **(f)** to transform OC in organic matter (OM) depends on the extent of OM oxidation and secondary organic aerosol (SOA) formation, values for **f** vary from 1.2 for fresh aerosol in urban areas to 2.6 for aged aerosol (Chow et al., 2015). The highest **f** values (up to 1.6) are usually used for oxygenated and/or functionalized organic species that can be expected in emissions from oil activities. For the oil Ecuadorian environment, we assumed to use **f = 1.7**.

As the NPC sampling station was located at 5 km from the seashore, sea salts (ss) were also included in the equation as follows, following the equation proposed by Amato et al. (2016):

$$\sum \text{Sea salt (ss)} = \text{ssNa} + \text{ssCa} + \text{ssK} + \text{ssMg} + \text{ssSO}_4^{2-}$$

where:

ssNa = Na – nssNa (=0.348Al); ssCa = 0.038 ssNa; ssK = 0.037 ssNa; ssMg = 0.119 ssNa and ssSO_4^{2} = 0.253 ssNa

**nss (non-sea salt)*

2.5 Statistical analyses

The software R version 3.1.1 was used for all statistical analyses. As our data did not follow a normal distribution, Spearman's correlation (r_s) was used to measure the strength and relationship between OP and the chemical composition of PM_{10}, for each sampling sites.

Correlation degree was interpreted as follows:

- Less than 0.40: weak
- 0.41-0.60: moderate
- 0.61-0.79: strong
- 0.79-1: very strong.

3. Results

3.1 PM_{10} mass in oil production and refining areas

PM_{10} contents over two years of sampling in the NAR and in the NPC are presented in table 1, as annual mean concentrations ± Standard Deviation (SD).

In 2015, PM mass concentration in the NAR (oil production area) was slightly higher in Shuara than in Auca, reaching values of 27.2 ± 8.3 and 23.7 ± 8.6 µg m^{-3}, respectively. In Esmeraldas (NPC, oil refining

Table 1. Annual chemical composition of PM_{10} in the 3 sampled areas of Ecuador: Auca and Shuara in the NAR (North Amazon Region) and Esmeraldas in the NPC (North Pacific Coast).

Sampling site	**Auca Sur (NAR)**		**Shuara 9 (NAR)**		**Esmeraldas (NPC)**	
Samples	**N=11**	**N=9**	**N=12**	**N=10**	**N=9**	**N=23**
Year	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**[a]
PM_{10}	23.7±8.6	23.3±8.2	27.2±8.3	25.3±9.6	51.6±23.4	107.2±40.4
Na	7785±1601	1451±3068	10260±1887	247±145	41150±5263	10549±17954
Al	2144±400	1089±686	2525±605	868±567	10347±1139	2620±3231
Ca	3575±664	859±1341	4266±526	219±175	17144±1249	3972±6639
Si	1455±266	1423±266	1816±736	1783±749	8354±2824	8393±2423
Fe	436±133	310±235	472±236	387±247	1308±131	577±174
Ti	41.92±9.25	35.67±24.01	60.91±34.50	46.15±27.85	196.44±194.03	51.83±14.37
Mo	49.00±11.10	38.80±8.21	59.37±8.07	43.13±7.51	226.67±24.79	225.97±60.40
Ba	16.92±3.76	22.09±8.13	14.75±5.37	29.36±6.80	61.04±42.47	94.60±42.05
Zn	14.72±5.58	17.32±7.77	32.53±24.40	24.22±5.65	56.50±39.50	75.95±36.95
Cr	16.91±6.90	4.19±4.48	24.06±2.18	2.48±1.06	61.39±5.73	21.75±20.71
Mn	9.75±2.29	6.09±4.01	9.98±3.24	6.89±3.95	30.25±15.98	11.56±5.45
V	4.13±1.75	5.60±5.37	4.53±1.65	6.75±3.93	22.10±6.87	31.83±10.09
Ni	3.5±1.3	3.8±2.5	4.4±1.3	4.4±2.1	16.6±3.9	20.0±3.8
Sn	3.21±0.71	1.23±1.97	4.49±0.81	0.37±0.18	16.76±2.63	5.97±8.16
Cu	2.19±0.85	1.05±0.72	3.39±2.52	1.41±0.82	10.65±8.89	3.81±1.86
Pb	1.26±0.38	0.52±0.11	2.62±1.86	0.61±0.37	8.11±4.09	2.16±0.82
As	0.60±0.33	0.24±0.17	0.63±0.34	0.37±0.16	2.60±1.41	1.81±1.03
Sb	0.54±0.15	0.29±0.23	0.58±0.14	0.31±0.22	2.76±1.17	1.74±1.12
U	0.45±0.08	0.21±0.18	0.57±0.07	0.14±0.06	2.10±0.24	1.00±0.81
Co	0.26±0.06	0.17±0.11	0.52±0.35	0.18±0.10	0.90±0.25	0.40±0.21
Cd	0.11±0.02	0.09±0.06	0.17±0.09	0.11±0.07	0.44±0.13	0.41±0.26
SO_4^{2-}	1109±524	997±705	731±555	804±557	2556. ±737	3119±909
NO_3^-	72±38	32±31	198±165	24±17	764±570	774±369
$(COO)_2^{2-}$	59±38	35±18	42±25	33±22	201±178	432. ±240
Cl^-	13±14	46±68	413±616	15±13	732±473	697±306
$MeSO_3^-$	3±2	4±3	4. ±2	4±2	18±14	43±30
Na^+	312±102	104±104	686±605	42±43	1486±1150	1159±460
K^+	224±84	220±185	258±168	192±123	190±70	201±42
NH_4^+	81±69	168±118	166±150	135±121	232±152	381±181
Ca^{2+}	156±56	61±45	140±173	50±43	473±284.	315±139
Mg^{2+}	24±8.4	23±19	17±8	13±8	77±44	107±37
EC	1.07±0.44	1.12±0.89	0.94±0.21	0.74±0.20	0.97±0.32	0.99±0.21
OC	4.58±1.57	5.05±3.67	4.89±1.30	4.44±1.34	3.37±0.72	2.79±0.72
OC/EC	4.56±1.15	4.56±1.58	5.48±1.98	5.99±1.06	3.59±0.49	2.91±0.80
Levoglucosan	4.67±2.25	31.48±10.49	8.35±4.87	56.58±33.80	19.33±12.89	50.83±27.09
Galactosan	<DL	2.55±0.85	0.80±0.53	1.60±0.51	0.59±0.54	1.88±1.16
Manosan	4.04±2.81	45.66±15.22	0.63±0.38	5.83±3.21	3.81±1.38	5.82±3.47
Glucose	54.86±36.19	54.58±60.71	35.53±32.63	56.79±40.80	33.30±18.84	30.25±20.49
Manitol	151.56±63.47	175.12±144.64	59.20±86.24	134.49±46.30	63.62±31.02	46.77±33.76
Arabitol	50.70±23.43	48.27±44.98	43.40±82.46	34.26±13.59	45.71±24.10	29.93±21.03
Sorbitol	5.46±3.19	2.08±1.48	2.15±1.74	3.63±2.16	6.85±3.80	4.29±2.87
∑PAHs[b]	123.12±43.91	316.44±330.05	44.55±35.46	190.59±402.28	789.98±566.48	1192±885.33
∑oxy-PAHs	640.66±897.93	333.16±270.19	64.31±87.35	139.54±221.49	1529.19±1213.52	844.13±1045.58
∑nitro-PAHs	2.4±0.9	2.4 ±1.4	1.4±2.0	0.8±1.1	2.9±0.5	5.3±39

Units: mean concentrations of elements (± SD) are expressed in ng m^{-3}. PM_{10} mass, EC, OC and TC are expressed in µg m^{-3} and PAHs in pg m^{-3}.

[a] National Oil refinery operated with 9 smokestacks in January 2015 and 18 (full capacity) since December 2015.

[b] ∑PAHs refers to 15 compounds classified as carcinogenic by the US-EPA (NTP, 2016). Chrysene and triphenylene were also included in this sum.

area), these concentrations were 2 to 4 times higher than in the NAR, exceeding the European and Ecuadorian legislation limits of 40 and 50 µg m^{-3} respectively (table SI-5).

3.2 Elemental and ionic species concentration in Ecuadorian NAR and NPC areas

Major elements mean concentration in the NAR (Al, Ca, Fe, Na, Si) ranged between 300 and up to 10 000 ng m^{-3}. In the NPC, same elements were 2 to 4 times higher than the NAR.

Trace elements have been classified into 4 categories according to their concentrations. The first one is represented by Ti and Mo, with annual mean concentrations varying between 36 to 60 and 56 to 227 ng m^{-3} in the NAR and the NPC, respectively. Barium, Zn and Cr are making up of the second group, with annual mean concentrations ranging from 3 to 33 ng m^{-3} in the NAR and from 22 to 95 ng m^{-3} in the NPC. The third group, ranging between 1.7 to 29 ng m^{-3}, correspond to the Mn, V, Ni, Sn and Cu elements. Finally, Pb, As, Sb, U, Co and Cd are in the fourth group, with concentrations below 3 and 8 ng m^{-3} in the NAR and NPC, respectively. For trace metals regulated by the Ecuadorian (Cd) and European (As, Cd, Ni and Pb) legislations, concentrations remained below the thresholds (table SI-5) during the two years sampling for all sites.

Cations concentrations in Esmeraldas are ranked in the following order: $Na^+ > Ca^{2+} > NH_4^+ > K^+ > Mg^{2+}$, whereas in Auca and Shuara the order is $K^+ > Na^+ > NH_4^+ > Ca^{2+} > Mg^{2+}$ and $Na^+ > K^+ > NH_4^+ > Ca^{2+} > Mg^{2+}$, respectively.

Among the water soluble anions, SO_4^{2-} and NO_3^- show the highest mean concentrations for NAR and NPC, ranging from 24 to 3119 ng m^{-3}. Sulfates, Cl^- and $(COO)_2^{2-}$ concentrations in Esmeraldas are between 2 and 16 times higher than in the NAR.

3.3 Sugars and polyols composition of PM_{10} in Ecuadorian NAR and NPC areas

OC/EC ratios in Auca and Shuara (NAR) were similar, ranging from 3 to 10, but higher than those calculated for Esmeraldas (NPC), as observed in Fig.2. The highest contents of OC were 11.4, 7.0 and 4.1 µg m^{-3} for Auca, Shuara and Esmeraldas, respectively. In Esmeraldas (NPC), OC/EC varied between 0.6 and 4.2, half of ratio values found in the NAR.

Marked differences were observed for some polyols and sugars between sites and sampling years (table 1).

Levoglucosan reached a maximum monthly concentration in the NAR of 107 ng.m^{-3}(Shuara, august 2016), and 111 ng m^{-3} in the NPC (Esmeraldas, Mars 2016). Mannosan and galactosan total mean concentrations ranged from 0.9 to 10 ng m^{-3}. It is important to note that low volume air sampler and the one-month sampling may have led to the degradation of these semi-volatil compounds towards secondary species and could have led to their underestimation.

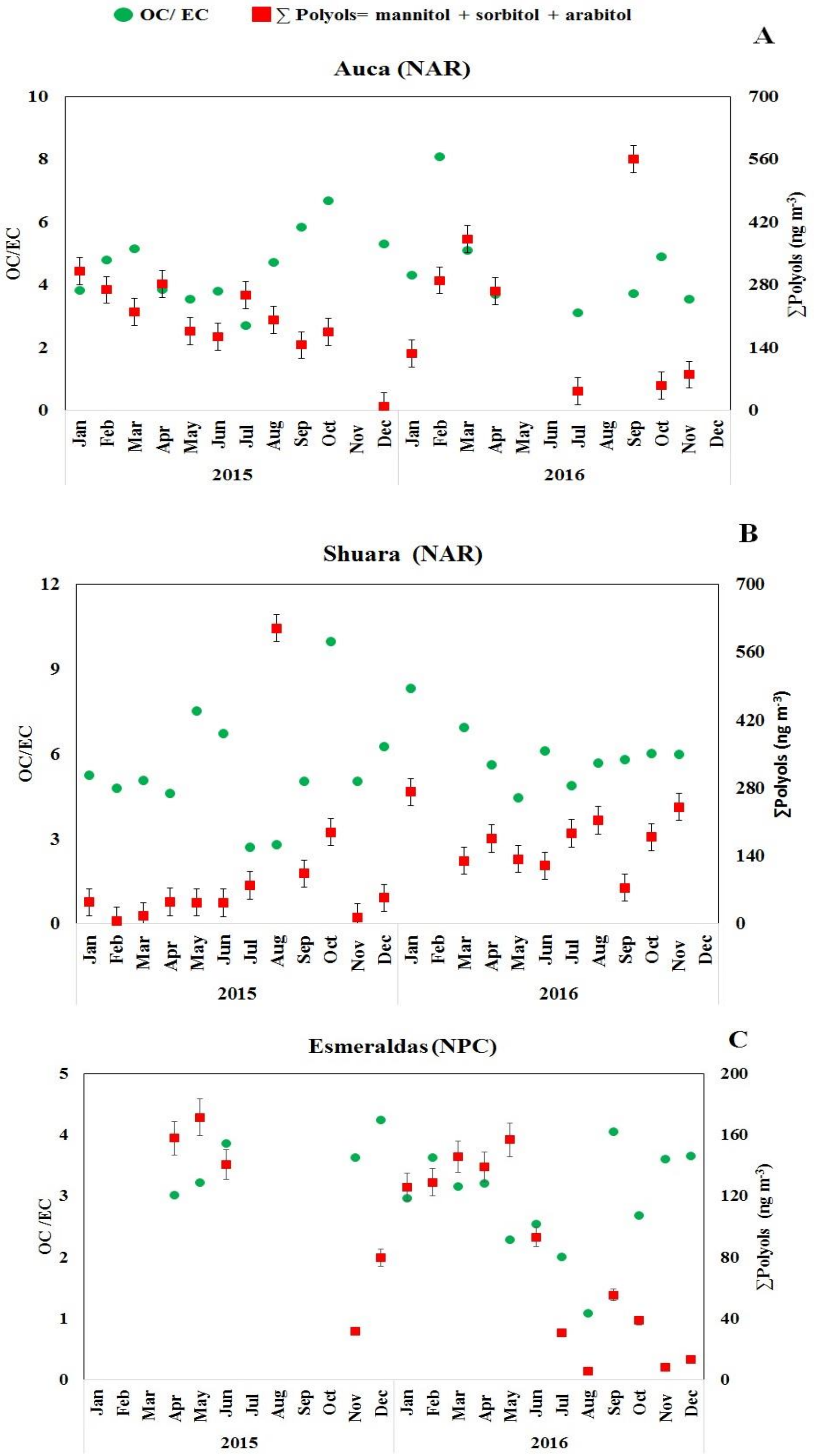


Fig 2. OC/EC ratios and ∑(Polyols) content (ng m^{-3}) in PM_{10} measured from January 2015 to December 2016 for the 3 sampling areas: **A.** Auca and **B.** Shuara, in NAR, close to oil burning open flares, and **C.** Esmeraldas in NPC, close to the Oil National Refinery.

**Blank spaces referred to missing data when sampling was not possible (due to technical issues)*

Monthly concentrations of the sum of polyols, also displayed in fig. 2, varied from 9 to 609 and from 6 to 171 ng m^{-3} in the NAR and in the NPC respectively. Polyols concentrations were classified as mannitol> arabitol> sorbitol over the three sampling sites, with the highest concentrations recorded in the NAR.

3.4 PAHs contents of PM_{10} in Ecuadorian NAR and NPC areas

The sum of oxy-PAHs, nitro-PAHs and PAHs was 4 to 6 times higher in Esmeraldas than in the NAR. Nitro-PAHs concentrations remain not significant (under 5 pg m^{-3}) for all the sites. Concentrations of these compounds in the NAR, were as well higher in Auca than in Shuara.

Among the PAHs, naphtalene, benzo(b)fluoranthene + benzo(j)fluoranthene + benzo(k)fluoranthene, indeno(1,2,3-c,d)pyrene and benzo(g,h,i)perylene (Table SI-6) reached the highest annual mean concentrations in Esmeraldas, varying from 180 to 373 pg m^{-3}.

Finally, in all Ecuadorian sampling sites, benzo(a)pyrene concentrations in PM_{10} remained below the maximum permissible levels of 1 ng m^{-3} according to the European Union (Table SI-5). As for levoglucosan, sampling design (one-month) may have led to the degradation of these compounds towards secondary species and induced an underestimation.

3.5 OP assays and ROS generation

Annual means of oxidative potential (OP) results from monthly PM_{10} filters (between 10 to 12 filters per year for the NAR stations and up to 23 for the Esmeraldas station) measured by three different assays are showed in Fig. 3. Among all sampling sites, Esmeraldas (NPC) presents the highest ROS generation potential for all OP assays, followed by Shuara and Auca (NAR). All the 3 OP assays present similar time evolution in Auca (NAR) and Esmeraldas (NPC) for the two-years sampling. For Shuara, OP^{DCFH} and OP^{DTT} provided similar information, whereas OP^{AA} displayed a significant difference between 2015 and 2016.

Annual OP^{DTT} in the NAR was below 1.2 nmol DTT min^{-1} m^{-3} for both locations whereas it was 3 fold higher in the NPC. Besides, OP^{DCFH} exhibited similar trends in the NAR (< 1 nmol[H_2O_2] eq. m^{-3} in Auca), being 3 fold higher in Esmeraldas. The lowest values were measured in Auca (NAR), with OP^{AA} below 0.5 nmol AA min^{-1} m^{-3}.

4. Discussion

4.1 PM_{10} mass reconstruction

Species contribution of PM_{10} mass for each site is shown in Fig.4. Even if Auca and Shuara are located

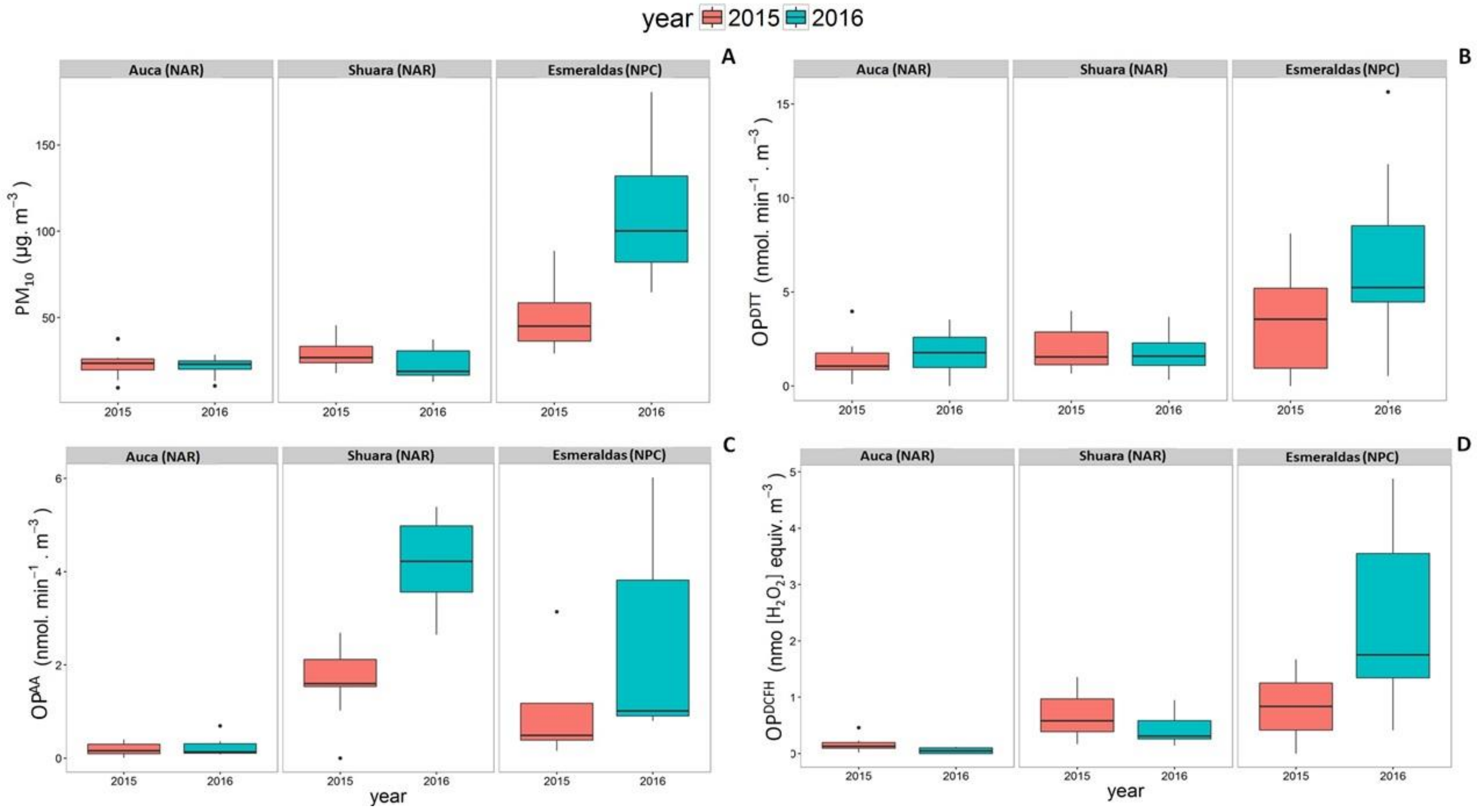


Fig. 3. A. PM$_{10}$ mass concentration (µg m^{-3}) and Oxidative Potential (OP.m^{-3}) measured by B. Dithiothreitol (DTT), C. Ascorbic acid (AA) and D. Dichlorofluoresceine (DCFH) assays in Auca, Shuara and Esmeraldas, for the 2015 and 2016 years.

in two different provinces, Orellana and Sucumbíos respectively, both sampling sites display similar mass contributions. Actually, small villages in the NAR appeared as a consequence of the roads opening in the rainforest to ensure the establishment and operating of oil activities (Lessmann et al., 2016). Hence, in these rural sites, OM rates in aerosol was dominated by biogenic emissions from the remaining forest (soils and plants) and by biomass burning episodes, related to agricultural practices or deforestation (Medeiros et al., 2006). The contribution of OM to the PM_{10} mass in the NAR was 32 to 37%. Indeed, in the Amazon basin, fires are usually caused by humans, as a clearing tool in preparation for agricultural fields after a forested patch has been slashed down, or to convert crops to pasture for cattle grazing (Artaxo et al., 2013; Mosandl et al., 2008). Besides, major elements accounting for 47% in PM_{10} in the NAR, have their origin from soil dust resuspension.

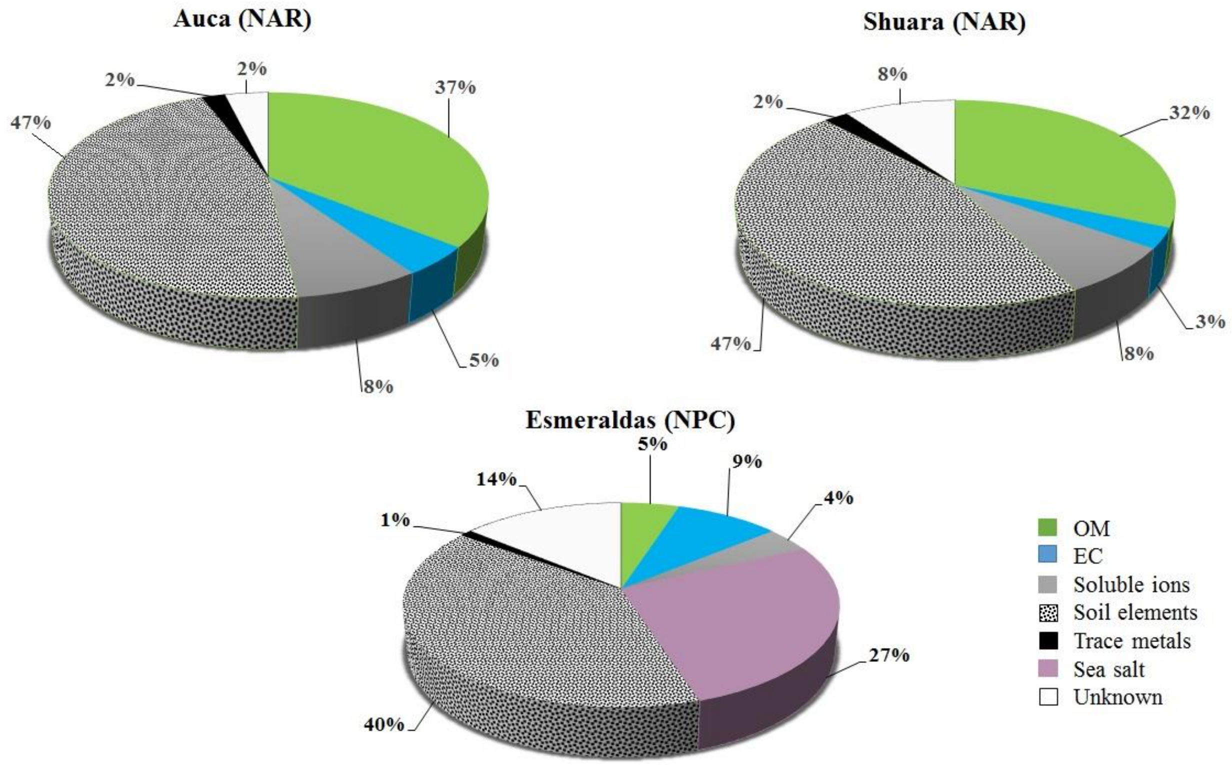


Fig 4. PM_{10} mass balance for Auca and Shuara in the Ecuadorian North Amazon Region (NAR) and for Esmeraldas in the North Pacific Coast (NPC).
OM stands for Organic Matter and EC for Elementary Carbon.

By contrast, the NPC is characterized by an urban and industrial profile. Esmeraldas is a coastal city with scattered patches of tropical rain and dry forest. Oil refining, thermoelectric stations, vehicular traffic and sea salts are the main sources of ambient particles in this area. OM contribution was 5%, about 6 times lower than in the NAR. Soil elements and sea salts accounted for 40 and 27%, respectively. Elementary carbon (EC) also exhibits higher percentage in the NPC than in the NAR: 9% against 3-5% of the total PM_{10} mass.

Soluble ions accounted for 4.5% in the NPC and for 8% in the NAR. Trace metals in both areas represented less than 2%. Chemical balance closure was satisfactory with not identified compounds within the range of 2 to 14%.

To compare with other PM_{10} mass balances, Waked et al. (2014) showed that, in Lens, France, in a region with many petrochemical, metallurgic, and non-metallurgic industries, depending on the season, OM, EC, SO_4^{2-}, NH_4^+, NO_3^- and other compounds (Cl^-, major elements and trace metals) accounted for almost 30, 5, 12, 8, 21 and 9%, respectively. The non-identified fraction was about 14%, whereas above 5% of the compounds in the OM was identified: 56% corresponding to levoglucosan, 24% to ∑polyols, 9% to mannosan, 3% to galactosan and 8% to glucose.

In the NPC, total contributions of SO_4^{2-} (sea salt and no sea salt) for PM_{10} mass balance were around 8%. Regarding the OM fraction, 4% of the compounds were identified: ∑ (monosaccharide anhydrides, polyols+glucose) and ∑(PAHs + oxy-PAHs + nitro-PAHs) accounted for 90 and 10%, respectively.

However, in the NAR, between 2 and 3% of the OM compounds were identified. PAHs contributed to less than 1%, whereas the other 99% come from monosaccharides, polyols and sugars.

In both areas, polyols (from biogenic emissions) contributions to OM were higher than biomass burning tracers.

Several organic compounds including carboxylic acids, naturally present in plant waxes (Santos et al., 2014), humic-like substances (HULIS) (Graber and Rudich, 2006; Zhao et al., 2016), volatile organic compounds, VOCs, such as BTEX (benzene, toluene, ethylbenze and xylene), originated from biogenic and anthropogenic sources (Baltrenas et al., 2011; Zhang et al., 2017) and PAHs in the vapor phase (Barrado et al., 2013; Ravindra et al., 2008; Szulejko et al., 2014) may also contribute to the OM fraction. However these compounds were not quantified in our study.

Finally, the remaining mass (called the "unknown" fraction in our study, Fig. 3), despite being acceptable, could also be explained by measurement errors (i.e. filters masses before and after sampling), water bounded to particles, volatilization of species due to the sampling time, or by the difficult choice among all possible mass balance equations displayed in the literature, as already suggested by Chow et al. (2015) and Malm et al. (2011).

4.2 PM_{10} composition and potential source identification

Very recent literature displayed data about PM_{10} mass concentration for Quito and Cuenca cities, but both are located in the Andean cordillera, at 2500 m above sea level. Raysoni et al. (2016) reported, for the 2004-2007 period, concentrations of 24.4, 34.1 and 77.0 µg m^{-3} in PM_{10} for 3 different sampling sites in Quito; they were influenced by textile industry, quarries, and vehicle exhausts and heavy traffic respectively. In the same area, mean concentrations measured indoor and outdoor environments a few years later, in 2010, varied from 8.2 to 45.3 µg m^{-3}. In a study conducted in Cuenca, Palacios and Espinoza (2014) reported that PM_{10} annual concentrations (2008-2013) ranged between 32.4 and 46.0 µg m^{-3}. Emissions in this area were associated to traffic, thermoelectric stations, and other industries. Even if these results are not comparable to the Amazonian or to the Pacific Coast regions of Ecuador, mainly due to different climate conditions and source's nature, PM_{10} concentrations were within such ranges mentioned above. In addition, the significant increase of all compounds concentrations at Esmeraldas between 2015 and 2016 was mainly explained by the downtime, renovation and restart of the refinery with a twice its initial capacity between the two periods.

PM_{10} concentrations in the NAR were higher than in the Brazilian Western Amazon, within the Cuieiras forest reserve in Manaus (pristine conditions), where a 4-years monitoring displayed an annual mean of 5 µg m^{-3} (Artaxo et al., 2013). However, values were in the same range than in Porto Velho (Brazil), an Amazonian area highly impacted by land use change and biomass burning (De Oliveira Alves et al., (2015). During the wet season (November–March), the mean concentration of PM_{10} was 13.38 ± 3.15 µg m^{-3} , whereas in the dry season (August-November) this value reached 30.20 ± 12.34 µg m^{-3}. However, NAR does not display well defined seasons, and as well, PM mass concentrations ranging from 9.39 to 45 µg.m^{-3} did not show any seasonality. In Manaus (Brazilian pristine Amazonian area), concentration of soil dust associated elements (Al, Ca, Fe, Si, Ti) were less than 300 ng m^{-3}, supported by the fact that soil is covered by plant debris, reducing natural emissions and resuspension. By contrast, in Porto Velho (impacted Brazilian Amazon), these concentrations were about 8 times higher (Artaxo et al., 2013), but still lower than those recorded in the Ecuadorian NAR. Regarding cations, total mean concentrations in the NAR were doubled comparing those reported by Gilardoni et al. (2011) in a pristine rain forest in the north of Manaus, where concentrations mainly associated to biogenic emissions varied from 20 to 80 ng m^{-3} in the coarse mode. In addition, in Porto Velho, trace metals (Cr, Cu, Ni, Pb, V, Ti, Sb and Zn) in the coarse fraction were twice higher than those measured in Manaus but between 8 to 28 times lower than the concentrations reported in our study (except for Pb and Sb that are in the same range). All these point suggests that anthropogenic activities in the NAR highly affect the air quality in comparison to pristine Amazonian areas.

Thereby, considering major elements as anions, Yamasoe et al. (2000),found SO_4^{2-} to be the most important inorganic ion in biomass burning aerosols in Amazonia. In their study, smoke and aerosols samples were collected over freshly biomass burning plumes in an Ecological Reserve (Cerrado, south of Brazilia) and in a tropical rain forest at Jamari (Porto Velho). Results showed that the percentage of sulphates varied in function of the burning phase, smoldering *vs* flaming, reaching mean values of 0.35-0.72 and 0.39-0.90% of the mass in the Cerrado Reserve and Jamari, respectively. In the NAR, sulfates concentrations represents between 3 and 5% of the total PM_{10} mass in Shuara and Auca respectively, suggesting that other sources must contribute to SO_4^{2-} concentrations in PM_{10} in this area. Likewise, chemical analyses of rain and fog water samples collected in the mountainous rain forest of south Ecuador showed frequent episodes of high sulfate and nitrate emissions, assigned to biomass burning from upwind areas of the Amazon basin (Fabian et al., 2005). Nevertheless, sulphates can also be secondary products from oil and gas extraction procedures (Sarnela et al., 2015), contributing to almost 40% to the $PM_{2.5}$ mass, as reported by Tuccella et al. (2017) in a modelling study conducted in the Norwegian Sea.

Sulphate is also a prevalent tracer in sea salt aerosol which could not be a significant source in the NAR but must be considered in the NPC. In Esmeraldas, SO_4^{2-} reached an annual concentration of 2556 and 3120 ng m^{-3} in 2015 and 2016 respectively, evidencing both industrial emissions in NPC and sea proximity. These values were within the range of concentrations found in coastal industrial cities like Barcelona, Porto and Athens, which also exhibit petrochemical plants (Amato et al., 2016).

For the other anions, Cl^- and NO_3^- concentrations found in the NAR were similar to those recorded by Yamasoe et al. (2000) in the Cerrado site (savannah-like vegetation) while $(COO)_2^{2-}$ was close to the values of the tropical forest site.

Within organic matter (OC) compounds, levoglucosan accounts for the main pyrolysis product from cellulose, leading to a unique tracer for biomass burning episodes and its concentrations varies depending on region and seasons (Chen et al., 2017; Cheng et al., 2013). Besides, galactosan and mannosan are derived compounds (by thermal decomposition) of hemicellulose, acting also as typical biomass burning tracers (Li et al., 2017). The mean concentrations of these three anydrosugars in Porto Velho reached 8400, 74 and 40 ng m^{-3}, respectively (De Oliveira Alves et al., 2015), whereas in the NAR (for both sampling sites) these values were 15, 2 and 6 ng m^{-3}, suggesting that NAR is less impacted by land use change and biomass burning than Porto Velho. This is in agreement with OC/EC ratios: De Oliveira Alves et al. (2015) reported OC/EC ratios of 11 and 18 in Porto Velho, 2 to 3 times higher than the ratios calculated for the NAR, pointing out the least biomass burning impact and more rural area in the NAR.

Total mean concentrations of levoglucosan, mannosan and galactosan in Esmeraldas (NPC) were 42, 6 and 2 ng m^{-3} respectively. The same compounds recorded in Barcelona (Spain) by Reche et al. (2012) in $PM_{2.5}$ showed concentrations of 60, 6 and 5 ng m^{-3}, respectively, related to agricultural waste burning and aerosols long-distance transport from forest fires. Oliveira et al. (2007) reported levoglucosan concentrations of 120 ± 80 ng m^{-3} in PM_{10} in Oporto (Portugal) from wood burning ovens and fireplaces. Thus, Esmeraldas can be impacted by such mentioned sources. Finally, concentrations for levoglucosan, mannosan and galactosan in all the sampling sites, were quite low in comparison with values from Porto Velho and for the literature about wood burning and deforestation in Amazonian region. Most probably, these low concentrations can mainly assigned to the sampling duration (up to one month because of samplers supplemented by solar panels since no electricity was avalaible in Amazonian forest and which have imposed a low air flow rate filtration) which can led to levoglucosan degradation, volatilization or transformations in the warm sampling conditions (up to 40°C in the NAR).

To follow with organic compounds, PAHs concentrations (section 3.4) were different at the two sampling sites located in the NAR. Since air samplers in both sites were located to equal distance from open flares, and similar PM_{10} concentrations were observed, such differences on PAH contents could be due to open flares dimensions, bigger in Auca, to their number (just one in Shuara and four in Auca) and to the close environment (wooded area in Shuara).

In Alenquer (Lower Amazon mesoregion of the Para state, Brazil), PAHs concentrations in total suspended particles (TSP) varied from 11 to 59 and from 54 to 393 pg m^{-3} during the rainy and dry season, respectively. Most abundant PAHs were benzo[g,h,i]perylene (25−113 pg·m^{-3}), indeno[1,2,3-cd]pyrene (23-153 pg·m^{-3}), chrysene (18-85 pg·m^{-3}) and benzo[b + j]fluoranthene (9.5-96 pg·m^{-3}), typical of remote locations (Gonçalves et al., 2016). These values concur with those found in the NAR in Ecuador (Table SI-6) but far less than the concentrations recorded in the NPC.

Choi et al. (2012) reported concentrations of 1030, 1860 and 2180 pg m^{-3} for benzo(g,h,i)perylene, indeno(1,2,3-c,d)pyrene and benzo(b)fluoranthene in PM_{10} samples collected in the city of Ulsan, Korea. These concentrations were 3, 6 and 7 times higher than those found in the Ecuadorian NPC area, mainly because Ulsan is the largest industrial city of Korea, with over one million inhabitants surrounded by petroleum refining, petrochemical, non-ferrous metal, automobile and shipbuilding industries. By contrast, in our study Esmeraldas (NPC), with over 500 000 inhabitants, displayed an industrial site on oil refining and thermoelectric centrals and it was in agreement with OC/EC ratios. They were close to urban and industrialized sites in Spain and Portugal, where OC/EC ratios in PM_{10} ranged between 1.8 ± 0.8 to 2.8 ± 1.3.

Finally, as for levoglucosan, a part of the PAHs could have been volatilized due to high temperatures occurring in the NAR and NPC during the sampling time (up to 40 °C) and could explain these differences towards other oil refining sites.

Regarding trace elements, barium and molybdenum are reported as air pollutants commonly emitted during oil refining operations (Islam et al., 2010). Barium and Mo in the NAR and in the NPC reached concentrations from 2 to 7 and from 200 to 1000 times higher, respectively, than those reported by Raysoni et al. (2017) in the Quito metropolitan district.

Molybdenum is used as a catalyzer during refining of heavy crude oil (Mironenko et al., 2017; Pradhan et al., 2013) and also as a weighting agent (McDaniel and Jamison, 2014; Oskarsson, 2015).

Molybdenum mean concentration in airborne and total dust generated during solid catalyst operations at one of the largest petroleum refineries in the world, in U.S. Virgin Islands, was 0.034 mg m^{-3} as reported by Lewis et al. (2012). Bozlaker et al. (2013) reported that Mo, in association with Ni, V and Sc were related to the combustion of crude oil in PM_{10} collected from a site located rear the heavily industrialized Houston Ship Channel.

Barium in PM_{10} usually originates from crustal dust. However, inorganic compounds in drilling muds used as weighting agents contain between 15-35% $BaSO_4$ (Pragst et al., 2017). In addition, PM_{10} collected between the Mediterranean and Atlantic coasts showed daily concentrations varying from 0.8 to 49.1 ng m^{-3} associated with crustal and anthropogenic sources such as shipping fuel oil combustion (Moreno et al., 2010).

Therefore, such Ba and Mo concentrations evidenced the impact of the extraction and refining processes of the oil industry on the local atmospheric composition and both elements can be considered as specific tracers of oil activities in the Ecuadorian environment.

4.3 Identification of PM_{10} chemical components generating ROS

Saffari et al. (2014) reported OP^{DTT} between 1.4 nmol min^{-1} m^{-3} for urban background up *to* 3.3 nmol min^{-1} m^{-3} for a highway traffic place. Total mean values for OP^{DTT} were 2 fold in Esmeraldas displaying an important oxidative burden exposure. Conversely, in the NAR, total mean OP^{DTT} were between 1.63 to 1.83 nmol min^{-1} m^{-3} displaying urban background values.

Hedayat et al. (2015) reported DCFH oxidation rates up to 3.81 nmol$[H_2O_2]$eq m^{-3} for urban sites also in agreement with Esmeraldas values.

In spite of almost equivalent PM_{10} mass concentrations for the two-years sampling in Auca and Shuara OP^{AA} is significantly different between 2015 and 2016, and trends different in the second NAR site of Auca. OP^{AA} is known to be highly sensitive to metals (Hedayat et al., 2015; Szigeti et al., 2015). Trace metals concentrations being in Shuara higher than in Auca, it is not surprising that OP values reached higher values at the former site.

Several studies have already indicated that ROS formation measured by OP assays is strongly driven by PM reactivity and redox compounds (Calas et al., 2017; Charrier et al., 2015; Eiguren-Fernandez et al., 2010; Yang et al., 2014). To determine which particulate compounds were responsible of ROS formation in the NAR and in the NPC, a correlation matrix according to Spearman test (statistical test chosen because of a non-normal data distribution) was calculated and results are shown in Fig.5. In Auca (NAR, oil extraction area), OP^{DTT} was correlated with Ba, Ni, NH_4^+, Si and Zn (r~0.7), moderately with OC, TC and polyols, and had a negative correlation with benzanthrone, fluoranthene and perylene (r_s>-0.8). OP^{AA} was strongly correlated to EC, mannosan and galactosan (r_s 0.7-0.8) and inversely correlated with Al, Co and Zn (r_s>-0.7). OP^{DCFH} was low or not positively correlated to any of the investigated chemical compounds.

In Shuara, OP^{DTT} was not correlated with the quantified chemical compounds in PM_{10}. However, OP^{AA} was strongly correlated ($r_s > 0.8$) with Ba, moderately correlated with some oxy-PAHs (i.e. napthtoquinone) and PAHs (i.e. BaP) and polyols and inversely correlated with Al, Cr, NO_3^- and Sn. OP^{DCFH} was not correlated with any compound.

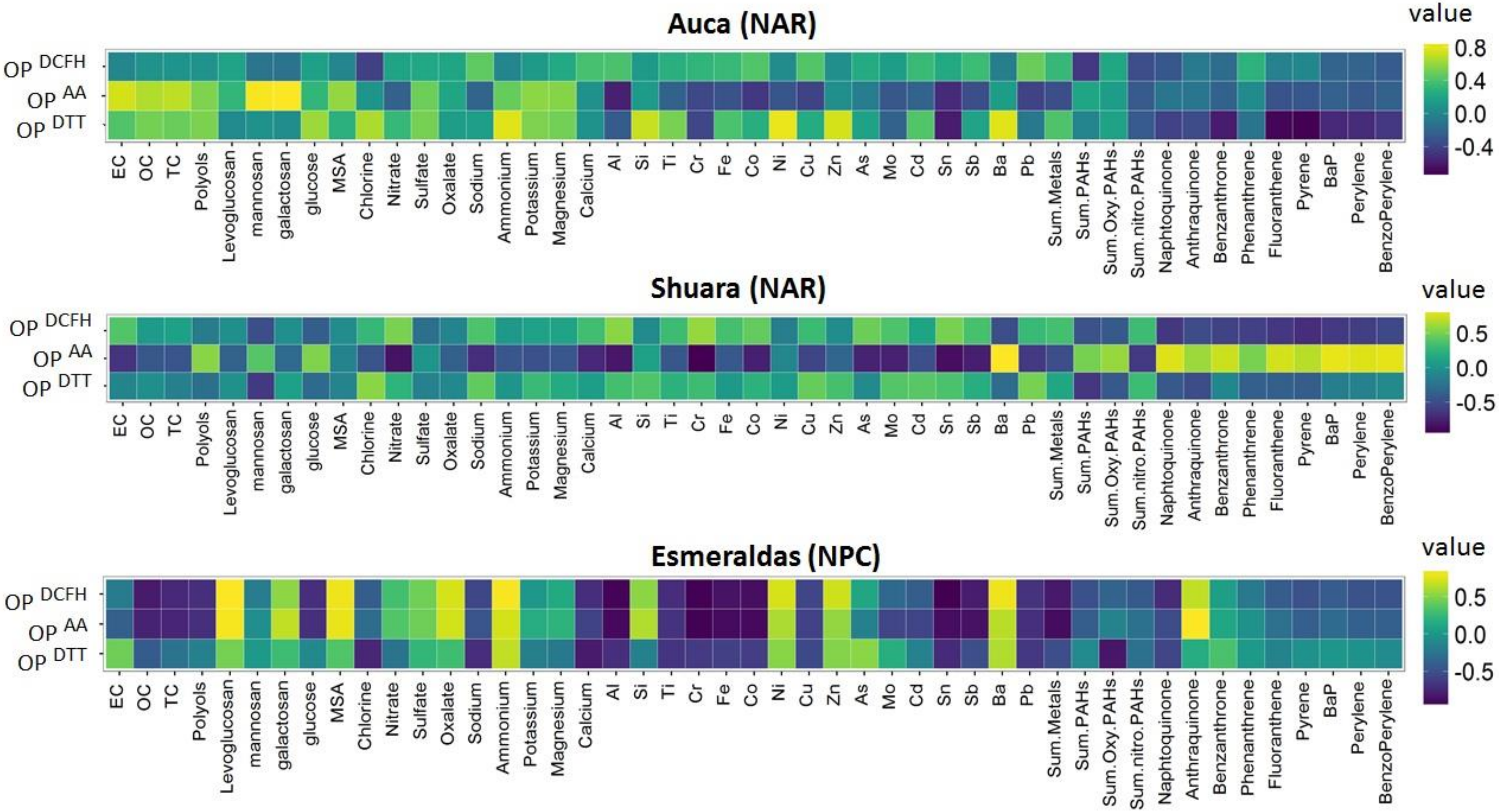
In the oil refining area (NPC), OP^{DTT} was moderately correlated As, Ba, Ni, Zn and NH_4^+ and inversely correlated with Cl^-, Ca^{2+} and ∑oxy-PAHs. OP^{AA} was strongly correlated with Ba and NH_4^+ ($r_s > 0.7$) but inversely to metals (Co, Cr, Fe, Sn). Finally, OP^{DCFH} was moderately correlated to MSA, NH_4^+, Ba and Ni and strongly correlated to levoglucosan and MSA.

From these results, correlations found between OP and chemical compounds in PM_{10} partially evidenced some sources responsible of ROS generation. For example, PAHs can induce indirectly oxidative stress if they are oxidized to polar compounds including quinones and possible nitro-PAHs that are redox active compounds (Cho et al., 2005; Li et al., 2002; Squadrito et al., 2001).

Assays were poorly correlated with the sum of oxy-PAHs or nitro-PAHs but some individual compounds were strongly correlated (e. g. 1,4 napthoquinone; 9, 10 antraquinone, benzenthrone with r > 0.7 in Shuara; 9, 10 antraquinone with r > 0.8 in Esmeraldas).

Polyols were moderately correlated with ROS generation in both sites of the NAR were biogenic emissions are prevalent while they are known to contribute to OP values (Samake et al., 2017). It has been observed that V and Ni are able to generate hydroxyl radicals once coming in contact with the biological cells (Verma et al., 2009). Ni was strongly correlated with OP assays in Esmeraldas and it's a specific tracer for shipping traffic (Pey et al., 2013) which could be an issue in Esmeraldas.

Our results showed that OP^{AA} and OP^{DCFH} were also correlated with the oil tracer Ba for all sites. Actually, Perrone et al. (2013) reported that Ba in $PM_{2.5}$ and PM_1 may induce cytotoxicity of human alveolar cells. It has been reported that exposure to Ba can produce oxidative stress in mammals and impair cellular antioxidant defense systems like glutathione (Elwej et al., 2017)


Auca (NAR)

Shuara (NAR)

Esmeraldas (NPC)

Fig.5. Spearman's coefficient for a subset of chemical species determined in PM_{10} and the three OP assays for Auca (NAR), Shuara (NAR) and Esmeraldas (NPC) sampling stations.

Nevertheless there is not conclusive information about Ba redox activity in lung cells but it is probable that Ba is emitted bound to other compounds that are redox active during oil activities.

Finally, such correlations provided interesting information about emission sources probably involved in the ROS exposure from PM: (1) In Auca, OP^{DTT} was mainly correlated to oil extraction tracers and to anthropogenic emissions from biomass burning, (2) In Shuara, OP^{AA} is associated to oil extraction and finally (3) In Esmeraldas, OP^{AA} et OP^{DCFH} assays agree in pointing oil refining industrial emissions, and possibly shipping. Thus, in all sampling sites, the oxidative burden from PM was related to specific tracers for oil activities and also from local additional sources. Finally, these results indicate that population living in the vicinity of the Esmeraldas National Oil Refinery and Thermoelectric plants present an acute ROS exposure and are potentially more impacted by oxidative stress provided by PM inhalation than those settled in the oil extraction area of the Amazonian region.

5. Conclusions

Chemical composition of PM_{10} in oil producing and refining areas in Ecuador not only depends on the type of oil activity but also on other anthropogenic sources and on their natural environment. Even if there is no seasonality in both study areas, variations with time between sampling years have been observed for some compounds, especially for sugars and PAHs, and mainly evidenced in Esmeraldas, in the vicinity of the national main refinery.

In the Ecuadorian NAR, OM and soil elements were the dominant fractions of PM_{10} composition, indicating that biogenic and soil dust emissions are the main sources of atmospheric particles. On the contrary, in the NPC, sea salts and soil elements were the major compounds found in the aerosols. EC contribution to the PM_{10} mass balance was significant, evidencing industrial emissions due to oil refining processes. PAHs also contribute for a higher percentage of OM in the NPC (10%) than in the NAR (less than 2%). Trace metals like Ba and Mo widely used in oil production, and not usually reported in studies about air quality, showed higher concentrations in the investigated sites than those generally found in urban-industrialized sites in Quito. These two elements can be considered as relevant tracers of oil activities in Ecuador.

ROS formation depends on the airborne chemical composition and was specific to each region. PM from Esmeraldas exhibited a very high ROS generation capacity, representative of industrials sites, whereas PM from Shuara and Auca presented an overall OP assigned to rural sites. Additionally, OP assays crossed with detailed composition evidenced that oil activities in combination with biogenic emissions in the NAR and with other industrial emissions in the NPC are responsible of the PM capacity for ROS generation. Finally, this study reported that oil activities can probably impact human health by inhalation in the Ecuadorian NAR and mainly in the vicinity of the main national refinery and thermo-electric plant. It opens the way for further investigations in epidemiology and risk assessment in these contrasted areas, but also in other Latin America or African countries where oil activities are widely developed without relevant environmental policies.

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Supplementary Information

Table SI-1. Quantifications limits (QL) of chemical compounds analyzed in field blank filters* in 3 areas of Ecuador: Auca and Shuara in the North Amazon Region (NAR) and in Esmeraldas (North Pacific Coast, NPC). Concentrations are expressed in ng m^{-3}. EC, OC and TC are expressed in µg m^{-3}

Sampling site	Auca Sur (NAR)		Shuara 9 (NAR)		Esmeraldas (NPC)	
	2015	2016	2015	2016	2015	2016
Na	302.5	70.15	318.96	50.33	401.65	224.46
Al	96.17	82.76	101.09	68.85	432.79	314.06
Ca	119.4	51.12	124.89	51.5	258	242.69
Si	120.28	120.28	118.09	15.75	421.61	421.61
Fe	5.74	19.14	5.71	16.17	25.26	80.11
Ti	9.22	2.05	9.35	1.77	42.54	9
Mo	3.52	3.92	3.77	3.56	16.39	17.33
Ba	0.72	0.16	0.5	0.09	2.3	0.49
Cr	0.86	0.17	0.91	0.15	1.96	0.33
Zn	0.43	0.1	0.25	0.05	1.17	0.25
Mn	0.26	0.07	0.28	0.06	0.59	0.15
V	0.065	0.015	0.061	0.011	0.355	0.075
Ni	0.54	0.12	0.56	0.11	2.42	0.51
Sn	0.13	0.01	0.13	0.01	0.11	0.01
Cu	0.18	0.09	0.18	0.08	0.83	0.34
Pb	0.034	0.016	0.036	0.013	0.228	0.042
As	0.052	0.023	0.057	0.02	0.21	0.059
Sb	0.026	0.011	0.026	0.01	0.105	0.035
U	0.032	0.011	0.034	0.01	0.157	0.038
Co	0.04	0.008	0.038	0.009	0.155	0.081
Cd	0.003	0.002	0.004	0.002	0.017	0.006
SO_4^{2-}	1.46	0.95	1.39	1.11	7.49	9.77
NO_3^-	3.44	1.81	4.25	2.41	14.52	8.44
$(COO)^{2-}$	1.39	0.29	0.74	0.15	1.64	1.88
Cl^-	0.84	3.31	1.05	2.95	5.75	13.52
$MeSO_3^-$	0	0	0	0	0	0.7
K^+	1.91	0.39	1.31	0.44	5.84	1.94
Na^+	3.92	1.31	5.08	0.89	18.08	3.2
Ca^{2+}	23.63	2.23	20.42	2.37	2.46	7.89
NH_4^+	4.3	5.01	2.96	5.86	15.19	30.74
Mg^{2+}	0.05	0.27	6.06	0.49	26.08	1.21
EC	0	0	0	0	0	0
OC	0.08	0.07	0.14	0.09	0.4	0.21
TC	0.08	0.07	0.14	0.09	0.4	0.21
Levoglucosan	2.83	0.62	2.83	0.6	3.83	2.41
Galactosan	0.47	0.31	0.47	0.1	0.47	0.21
Manosan	1.17	0.77	0.17	0.15	1.17	1.01
Glucose	1.17	0.77	1.17	0.75	1.17	3.01
Manitol	1.17	0.77	1.17	0.75	1.17	1.01
Arabitol	1.17	0.77	0.17	0.35	1.17	0.9
Sorbitol	0.47	0.31	0.47	0.2	0.47	0.21

*Two filters per year and per site

Table SI-2. Chemical compounds analyzed by GC-MS

∑PAHs	∑Oxy-PAHs	∑Nitro-PAHs
Naphthalene	Phthalic anhydride	1-Nitropyrene
Acenaphthylene	1,4 Naphtoquinone	7-Nitrobenz(a)anthracene
Acénaphthene	1-Naphthaldehyde	6-Nitrochrysene
Fluorene	1,4 Chrysenequinone	1,3-Dinitropyrene
Phenanthrene	9-Fluorenone	1,6-Dinitropyrene
Anthracene	9,10 Anthraquinone	1,8-Dinitropyrene
Fluoranthene	1,4 Anthraquinone	6-Nitrobenz(a)pyrene
Pyrene	Benzo(a)fluorenone	1-Nitronaphthalene
Benzo(a)Anthracene	Benzanthrone	2-Nitronaphthalene
Chrysene + Triphenylene	Benz(a)anthracen-7,12-dione	2 Nitrobiphenyl
Benzo(b)Fluoranthene +		
Benzo(j)Fluoranthene +		2-Methyl-4-nitronaphtahalene
Benzo(k)Fluoranthene		
Benzo(e)Pyrene		3 nitrobiphenyl
Benzo(a)Pyrene		1,5 dinitronaphthalene
Perylene		2-Nitrofluorene
Indéno(1,2,3 -c,d)Pyrene		9-Nitroanthracene
Dibenzo(a,h)Anthracene +		9-Nitrophénanthrene
Dibenzo(a,c)Anthracene		
Benzo(g,h,i)Perylene		3-Nitrophénanthrene
		2 nitroanthracene
		3-Nitrofluoranthene
		4-Nitropyrene

Section 1. Gamble solution composition

The Gamble's solution represents the interstitial fluid deep within lungs and is mixture of salts (Marques et al., 2011) at pH 7.4. Lung macrophages are one of the main producers of ROS in the early inflammation phase. To compensate ROS production, and possibly to modulate ROS release, macrophages use antioxidant defenses. Gamble's solution supplemented with dipalmitoylphosphatidylcholine (DPPC) (pH: 7.4). This major phospholipid of lung surfactant helps reducing surface tension at the air-water interface of the terminal airways and improves lungs defensive function. The citrate in the solutions replaces proteins that can be found in lung lining fluid and acetate was used instead of organic acids. (Calas et al., 2017)

Table SI-3: Detailed composition of the simulated lung fluids solutions. The chemical compounds are listed in the order of their introduction to the solution to avoid salt precipitation.

Compounds	**Gamble solution**	**Gamble + DPPC solution**
	mg L^{-1}	
$MgCl_2$	95	95
NaCl	6 019	6 019
KCl	298	298
Na_2HPO_4	126	126
Na_2SO_4	63	63
$CaCl_2$, $2H_2O$	368	368
CH_3COONa	574	574
$NaHCO_3$	2 604	2 604
Na_3 citrate. $2H_2O$	97	97
Glycine	190	190
DPPC		0.02% (200mg.L-1)
pH	7.4±0.1	7.4±0.1

Table SI-4. Detections Limits (DL) of Oxidative Potential (OP) assays* for 3 sampling areas: Auca and Shuara in the North Amazon Region (NAR) and in Esmeraldas (North Pacific Coast, NPC), in Ecuador.

OP assay	**Auca (NAR)**	**Shuara (NAR)**	**Esmeraldas (NPC)**
DTT (nmol min^{-1} µg)	0.02	0.02	0.02
AA (nmol min^{-1} µg)	0.02	0.06	0.01
DCFH (nmol H_2O_2 L^{-1})	2.16	6.69	62.21

*Three blank filters per site

Table SI-5. International legislation about PM_{10}, $PM_{2.5}$ mean annual mass, trace metals and PAHs concentrations.

PM_{10} (µg m^{-3})	**Annual mean**			
• Ecuador [a]	50			
• European Union [b]	40			
Trace metals (ng m^{-3})	**Ni**	**Pb**	**Cd**	**As**
• Ecuador [a]	--	--	5	--
• European Union [c]	20	500*	5	6
PAHs (pg m^{-3})	**Benzo(a)pyrene**			
• European Union [c]		1000		

[a] Air Quality Standard. Ministry of the Environment of Ecuador. Ministerial agreement No 050. Official Register No 464 (2011).

[b] European Air Quality Standards: http://ec.europa.eu/environment/air/quality/standards.htm

[c] European Union Directive 2004/107/EC relating to As, Cd, Hg, Ni and PAHs in ambient air, OJ L 23, 26.1.2005, pp. 3-16.

*Limit value entered into force 01/01/2005

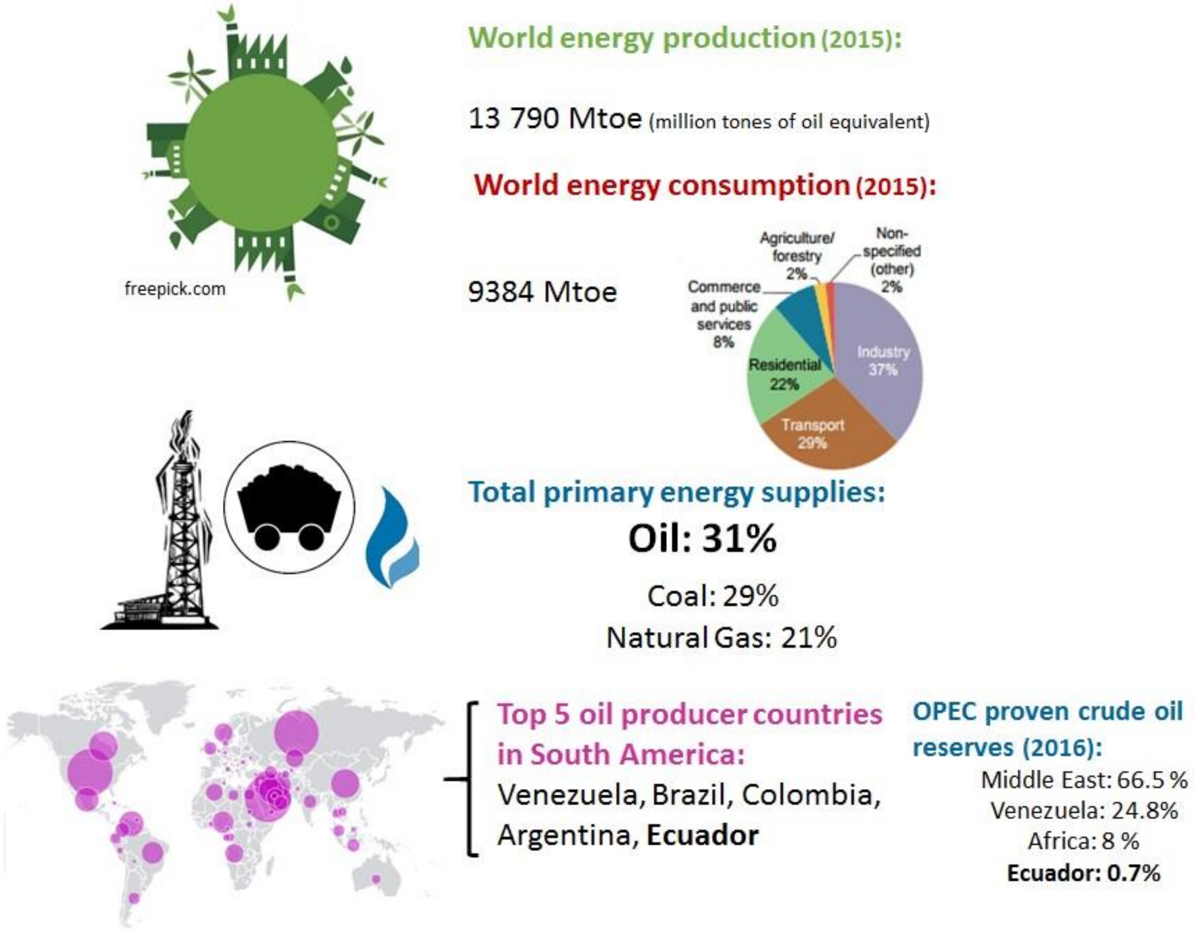
Table SI-6. Annual mean PAHs concentration (pg m^{-3}) in PM_{10} in the 3 sampling areas of Ecuador: Auca and Shuara in the North Amazon Region (NAR) and Esmeraldas in the North Pacific Coast (NPC).

Sampling site	**Auca (NAR)**		**Shuara (NAR)**		**Esmeraldas (NPC)**	
Number of samples	**N=11**	**N=10**	**N=12**	**N=10**	**N=11**	**N=23**
Year	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
Naphthalene	8.65±5.04	13.50±7.80	9.16±5.35	12.42±7.97	372.71±624.91	97.15±77.66
Acenaphthylene	0.86±0.34	2.04±1.27	1.22±0.52	1.59±1.13	8.45±8.28	28.38±35.19
Acenaphthene	<QL	1.14±0.48	1.70±0.49	1.92±0.83	4.48±2.80	4.68±1.75
Fluorene	2.07±1.21	1.80±0.96	<QL	1.97±1.08	3.96±1.60	11.51±5.64
Phenanthrene	8.63±3.87	14.95±10.34	12.96±7.87	9.35±2.96	23.12±9.41	89.57±61.01
Anthracene	1.07±0.60	2.70±1.68	2.51±0.72	1.77±1.00	6.58±3.84	17.98±21.64
Fluoranthene	8.14±3.68	17.31±10.80	5.94±3.34	10.64±6.47	28.97±21.61	86.46±58.37
Pyrene	14.54±8.66	23.46±13.86	7.89±4.66	14.54±9.98	47.60±32.34	73.72±52.40
Benzo(a)Anthracene	3.61±1.47	14.89±21.25	2.20±1.08	12.51±26.00	13.46±5.74	28.82±26.40
Chrysène + Triphenylene	8.92±2.78	29.43±35.68	4.50±2.61	18.62±41.01	21.35±8.49	58.17±39.64
Benzo(b)Fluoranthene + Benzo(j)Fluoranthene + Benzo(k)Fluoranthene	17.69±8.01	70.63±85.67	7.35±5.07	47.47±106.83	94.44±42.40	212.42±134.66
Benzo(e)Pyrene	11.16±5.02	30.81±31.95	4.43±2.80	17.89±38.31	58.46±31.13	109.00±72.49
Benzo(a)Pyrene	8.29±4.85	28.26±33.32	2.74±1.93	20.10±49.84	28.15±23.04	51.80±49.57
Perylene	1.92±1.05	4.92±5.95	0.74±0.43	4.70±9.44	6.54±3.95	13.25±11.56
Indeno(1,2,3 -c,d)Pyrene	13.25±5.99	37.68±36.97	6.04±4.49	26.05±53.97	126.56±66.72	179.93±146.73
Dibenzo(a,h)Anthracene + Dibenzo(a,c)Anthracene	2.00±1.10	3.87±4.67	1.23±0.74	5.59±8.73	12.62±5.05	22.28±15.66
Benzo(g,h,i)Perylene	21.97±12.71	48.72±46.62	7.40±5.25	28.31±51.57	152.68±101.60	196.68±160.64

QL: quantification limits

End of the Manuscript

General conclusion



Source: International Energy Agency (IEA, 2017) & Organization of the Petroleum exporter countries (OPEC, 2017)

There is an endless concern for safety during oil production, transport and refining because of oil spills or leakages from the pipelines or direct atmospheric emissions from gas flares. Besides, the emissions of contaminants that can seriously damage air, soil and water quality and consequently the health of the local population have driven policies towards renewable and less harmful energy sources.

However, in Ecuador this resource has significantly contributed to the recent economic development of the country as crude oil represents almost 34% of the total exported products and about 20% of the government's budget. According to National Ministry of Hydrocarbons (ARCH, 2016), Ecuadorian crude oil reserves would last at least 45 years, depending on the price of the oil barrel on the global market and the available extraction technologies allowing the discovery of new oil fields.

Even if the National Oil Companies, Petroamazonas (oil exploration and production) and Petroecuador (oil refining, transport, storage and commercialization), as well as the transnational consortiums are now supposed to use cutting edge technology and respect environmental policies, no one can guarantee that major accidents would never happen again. Recently, after the rehabilitation of the Esmeraldas National Oil Refinery that cost the country more than 2,200 million USD, several technical problems have been recorded in the catalytic cracking unit and even a major fire has occurred in one of the turbo-generators (Orozco and Viveros, 2017). Therefore, if almost half of Ecuador's economy is based on oil exploitation and exportation, a past and present assessment of oil pollution is absolutely necessary for the government to take further measures to ensure the quality of life of local populations by protecting their natural environment and avoiding social conflicts.

While oil wastes contain mainly polycyclic aromatic hydrocarbons (PAHs) and metal(loid)s, both toxic for humans and for the environment because they can be easily transferred into waters, soils, atmosphere and then into crops, any literature on the quantification of these toxic compounds in the environment was unavailable before the beginning of the MONOIL Research Program.

The main objectives of this study were to:

1. Assess the levels of metal(loid) contamination in soils, crops and air and the associated human health risks (depending on the exposure pathway) in three study areas impacted by oil activities: Orellana and Sucumbíos provinces, in the North Amazon Region (oil production area), and in Esmeraldas city on the North Pacific Coast (oil refining area).

2. Determine if Cd, a non-essential and toxic metal, is easily up taken up by cacao crops growing in oil contaminated sites and if it also implies health risks by the ingestion of cacao-based products.

3. Estimate if the cocktail of metal(loid)s and PAHs in the air (PM_{10}) induce the formation of reactive oxygen species (ROS) in synthetic lung fluids responsible for oxidative stress, a key parameter in cardio-respiratory diseases due to atmospheric pollution.

Several biogeochemical analytical methods as well as a sociological approach were combined in the frame of the MONOIL ANR Research Program to conduct, for the first time, a state of the art study of the social and environmental impacts of oil activities in Ecuador. Besides, two advanced metrics, highly recommended by the geochemical and atmospheric community, were considered but that until now were not included in worldwide standards: the gastric bioaccessibility fraction and the oxidative potential of PM.

The major results of this research are:

- **In soils**: From a total of 15 small-scale farms in the NAR and 6 in the NPC, 72% showed that from three to eight metal(loid)s exceeded the Ecuadorian standards for soil quality. These metals were: Ba (13 farms), V (21 farms), Zn (20 farms), Cr (16 farms), Cu (14 farms), Ni and Co (18 farms) and Pb (2 farms).

  In contrast, Mo and As were below the Ecuadorian thresholds in all sites, whereas Cd concentrations in soils exceeded the limit value in only five small farms of the Amazon region. Regarding the type of oil infrastructure, soils located close to oil waste pools (open pits), reinjection wells and in the vicinity of the oil refinery recorded higher concentrations of Ba than those located in the vicinity of gas open flares, oil platforms or pipelines. Chromium and Ni also displayed important contents close to the refinery. No evident pattern was identified for the distribution of the other metal(loid)s in the soil compartment.

  The combination of soil enrichment factor calculations and elementary analysis of crude oil samples was used as an approach to source identification. Nevertheless, in soils, it is still difficult to determine which metals come only from oil activities given the multiple natural and anthropogenic sources co-existing in the study area.

- **In crops**: Concentrations of V, Cr, Ni, Co, Pb, As and Mo in crops collected in the NAR were below the quantification limits, except in some cacao and manioc samples. Thus, phytoavailability of metal(loid)s not only depends on the concentration and mobility in the soil solution, but also on soil physico-chemical properties (pH, CEC, TOC, clay content) and on plant physiology (Kabata-Pendias and Szteke, 2015). Some metals can be easily taken up (as Mn, Cu, Zn, Ba, Cd) and some cannot (V, Cr, Ni, Co, Pb, As, Ba).

  In contrast, high contents of Cd were measured in cacao plants and liquor. Bioaccumulation of Cd in cacao pods and leaves depended on geographical, geological and biological parameters (i.e., plant varieties) as well as on agricultural practices. High concentrations of Cd found in cacao samples from Manabí, not impacted by oil activities, have highlighted the use of agrochemical products as an important source of Cd.

  Other non-essential metals, such as Ni and Ba, were also found in cacao and cocoa liquor samples. Despite the fact that studies in the literature of trace metals in cacao are scarce, cocoa could fit in the category of metallophytes species, but not hyperaccumulators (Pollard et al., 2014).

  Similar concentrations of Cd found in cacao raw beans and cocoa liquor samples allowed the performance of the BARGE test in both types of samples which showed that almost 100% of the total Cd content was bioaccessible after ingestion.

Consumption of cacao-based products made with cacao beans (or cocoa liquor) which contain up to 1.6 mg kg^{-1} of Cd represent a risk for human health if the daily chocolate consumption reaches 100 g per week. Both of the cocoa liquor samples from the NAR showed concentrations exceeding (by 2-fold) this content.

- **In PM$_{10}$**: Aerosols mass composition depends on the oil activity developed in the sampling areas. In oil extraction sites (NAR), organic matter and soil elements accounted for 80% of the mass concentration and for 50% in the refining site (NPC).

  Concentrations of metal(loid)s were always higher in the NPC than in the NAR, although As, Cd, Ni and Pb concentrations were below the limits established by the European Union in the three sampling areas. Based on the concentrations found in PM$_{10}$, Ba and Mo were proposed as potential metallic tracers of oil activities in aerosols. Both showed high concentrations in the same range as in highly industrialized sites of the Northern hemisphere.

  Regarding PAHs, they barely contribute with 1.7 and 10% of the organic matter fraction in the NAR and in the NPC, respectively. However, despite their low concentrations, oxy-PAHs such as 9,10-anthraquinone, 1,4-naphtoquinone or benzanthrone display high redox reactivity and are known to increase oxidative damage in the lungs (Wei et al., 2010).

  The three acellular DTT, AA and DCFH assays indicated that Ba, the main oil tracer element, was significantly correlated with ROS generation for all sampling sites. In addition, other compounds also contribute to the ROS exposure of the local population: sugars from biomass burning and some oxy-PAHs (from oil or biomass burning) in the NAR and metals (i.e. As, Ni, Zn) from industrial emissions in association with an oxy-PAH (anthraquinone) in the NPC.

- **Health risk** assessment based on the diet of farmers from the NAR, inhalation of PM and dermal contact with soil particles and water showed that the population (adults and children) is vulnerable to contamination by metal(loid)s. Ingestion (water and crops) was the main exposure pathway for non-carcinogenic effects of metal(loid)s. Inhalation was the major pathway for carcinogenic elements exposure such as As, Ni, Cd and Cr in adults and children for both sampling areas. TCR index in the NPC was 1.6-fold higher than in the NAR.

  However, freshwater fish and shrimp are also important products of the human diet in the Amazon region. These additional data were collected in the frame of the MONOIL Program and published in the Masters reports of two Ecuadorian students. Their manuscripts for publication in international journals are in preparation and will allow a more realistic approach to investigating human exposure to metal(loid)s in the Ecuadorian Amazon region.

Perspectives

All work, even hard, is perfectible. Hereafter are listed some experiments, analysis or complementary approaches that would improve our first results.

About transfer of metal(loid)s in the soil-plant continuum:

- Metal(loid)s total concentrations were determined in soil samples as shown In **Chapter 1** and **Chapter 2.** However, it would also be interesting to perform leaching tests and to determine the chemical speciation of these inorganic compounds. Natural processes such as weathering, drainage, microbial activity and the production of exudates by plant roots can modify soil chemical properties, therefore affecting metal(loid) speciation and, *a fortiori*, their phytoavailability.

About health risk assessment (HRA):

- Integrate recent PAHs results in PM_{10} of both study areas in the HRA calculation to assess the potential impacts of these compounds on human health.

About Cd and other metals in cacao beans and end-products:

- As stated in **Chapter 2,** sources of Cd in soil are still not identified. It would be important to determine the elementary composition of agrochemical products or organic amendments. Besides, it would be interesting to collect and analyze samples from farms outside of oil activity areas.
- Cacao varieties and cultivars could be a key parameter in Cd bioaccumulation. Identifying which variety exhibits the highest Cd content in beans is of great importance to cacao producers. Such information could be used to cross varieties less tolerant to Cd and other metals.
- In addition to the preliminary analysis in bean mucilage, we recommend determining Cd levels in roots and stems in order to provide more information about the complex process of translocation in cacao trees.
- Finally, Cd isotopes could be used as tracers of contamination sources but also to understand the mechanisms of Cd uptake by roots or leaves and its translocation to the rest of the plant under field conditions.

About PM_{10} and ROS generation:

- From the results obtained in **Chapter 3** about ROS generation in biological media induced by PM_{10}, the next step will be to evaluate the biological effects in pulmonary cells analyzing the cytotoxicity, inflammatory response and DNA damage. Further cross-over studies between toxicological markers, oxidative potential and PM chemical composition would allow us to assign to each emission source its health impact contribution.

- This study focused on particulate air pollution but PAHs quantification in the gas-phase could reveal meaningful information about oil contamination. Concentrations of PAHs bound on particles could be lower than those in the gaseous phase depending on their volatility. Thus, these data could be helpful to calculate diagnostic ratios used for source identification.

Conclusion Générale

En raison de déversements ou de fuites provenant d'oléoducs ou d'émissions atmosphériques provenant des torchères, les risques associés à la production, au transport et au raffinage du pétrole sont courants et constants, et malheureusement encore très difficiles à maitriser. Comme les émissions de contaminants nuisent gravement à la qualité de l'air, des sols et de l'eau et, par conséquent, à la santé de la population locale, les pouvoirs publics se tournent désormais vers le développement de nouvelles technologies d'énergies renouvelables, moins nocives.

Cependant, en Équateur, le pétrole brut représente près de 34% de l'ensemble des produits exportés et environ 50% du budget national. Cette ressource a donc contribué de manière significative au développement économique récent du pays.

Selon le Ministère National des Hydrocarbures de l'Equateur (ARCH, 2016), les réserves en pétrole brut sont estimées à l'équivalant d'environ 45 ans, en fonction des possibilités de découvrir de nouveaux champs pétroliers, mais aussi en fonction du prix du pétrole et des technologies d'extraction disponibles.

Même si en Equateur les sociétés pétrolières nationales, Petroamazonas (production et extraction) et Petroecuador (raffinage, transport, stockage et commercialisation), et les consortiums transnationaux sont désormais supposés utiliser des technologies de pointe et respecter les politiques environnementales, personne ne peut garantir que la tragédie Chevron-Texaco ne se reproduira pas.

Récemment, après la réhabilitation de la Raffinerie Nationale d'Esmeraldas, qui a pourtant coûté au pays plus de 2.200 millions de dollars, plusieurs problèmes techniques ont été répertoriés dans l'unité de craquage catalytique et même un incendie dans l'un des turbogénérateurs (Orozco et Viveros, 2017). Par conséquent, comme la moitié de l'économie équatorienne repose sur l'exploitation et l'exportation du pétrole, une évaluation passée et actuelle de la pollution par les technologies pétrolières est absolument nécessaire afin que le gouvernement puisse garantir de nouvelles mesures pour assurer la qualité de vie des populations locales en protégeant leur environnement naturel et en évitant les conflits sociaux.

Les déchets du pétrole contiennent principalement des métaux et métalloïdes co-émis avec des HAPs, toxiques pour l'Homme et l'environnement. Etant donné qu'ils peuvent être facilement transférés dans les eaux, les sols, l'atmosphère et ensuite dans les cultures, leur quantification dans les principaux compartiments environnementaux semble essentielle.

Ainsi, les principaux objectifs de notre étude étaient:

1. Évaluer les niveaux de contamination en métaux et métalloïdes dans les sols, les cultures et l'air mais aussi les risques impliqués pour la santé humaine (selon la voie d'exposition considérée) dans 3 zones d'étude impactées par les activités pétrolières: les provinces de Orellana et Sucumbíos, dans le nord de la région amazonienne, et la ville d'Esmeraldas, sur la côte Pacifique.

2. Déterminer si le Cd, un métal non essentiel et toxique, est facilement absorbé par les cultures de cacaoyers dans les sites impactés par les activités pétrolières, et si cela implique également des risques pour la santé humaine après ingestion de produits à base de cacao.

3. Estimer si le cocktail de métaux, métalloïdes et HAPs présents dans l'air (fraction PM_{10}) induit la formation d'espèces réactives de l'oxygène (ROS) dans les poumons, contribuant ainsi à l'exposition par inhalation des populations vivant dans ces zones impactées.

Plusieurs méthodes analytiques biogéochimiques, ainsi qu'une approche sociologique, ont été combinées dans le cadre du programme de recherche ANR MONOIL pour fournir un état de l'art sur les impacts de la contamination pétrolière en Équateur. Par ailleurs, nous avons inclus deux mesures fortement recommandées par la communauté géochimique et atmosphérique, mais qui ne sont pas encore prises en compte dans les normes internationales: la fraction bioaccessible gastrique et le potentiel oxydatif des PM.

Les principaux résultats de cette recherche sont:

- **Dans les sols:** sur un total de 15 petites fermes étudiées dans le NAR et 6 dans le NPC, 72% ont mis en évidence le fait que 3 métaux avaient des teneurs au-dessus des normes équatoriennes de qualité du sol. Ces métaux sont le Ba (13 fermes), le V (21 fermes), le Zn (20 fermes), le Cr (16 fermes), le Cu (14 fermes), le Ni (18 fermes), le Co (18 fermes) et le Pb (2 fermes).

  En revanche, le Mo et l'As avaient des concentrations inférieures aux seuils équatoriens dans tous les sites, alors que la valeur pour le Cd dépassait la limite uniquement sur 5 fermes dans le NAR. Concernant le type d'infrastructure pétrolière, il apparait que les sols situés à proximité des puits de pétrole, des puits de réinjection ou situés à proximité de la raffinerie possèdent des concentrations plus élevées en Ba que ceux situés à proximité des torchères, des plates-formes pétrolières ou des oléoducs.

  Le Cr et le Ni ont également montré des teneurs importantes à proximité de la raffinerie. Aucune tendance n'a cependant pu être clairement identifiée quant à la distribution des autres métaux et métalloïdes dans le compartiment sol de l'écosystème.

  La combinaison des calculs des facteurs d'enrichissement du sol et de l'analyse élémentaire des échantillons de pétrole brut a été utilisée comme approche d'identification des sources.

Néanmoins, dans les sols, il est encore difficile de prouver que les métaux proviennent uniquement des activités pétrolières, compte-tenu des multiples sources naturelles et anthropiques coexistant dans la zone d'étude.

- **Dans les cultures:** les concentrations en V, Cr, Ni, Co, Pb, As et Mo dans les cultures collectées dans le NAR étaient inférieures aux limites de quantification, sauf dans certains échantillons de cacao et manioc.

  Ainsi, la phytodisponibilité des métaux dépend non seulement de leur concentration et de leur mobilité dans la solution du sol, mais aussi des propriétés physico-chimiques du sol (pH, CEC, TOC, teneur en argiles) et de la physiologie de la plante (Kabata-Pendias et Szteke, 2015). Certains métaux seront facilement absorbés (Mn, Cu, Zn, Ba, Cd) tandis que d'autres ne le seront pas (V, Cr, Ni, Co, Pb, As, Ba).

  En revanche, des teneurs élevées en Cd ont été mesurées dans les organes des plants de cacao et dans les pâtes et produits qui en sont issus. La bioaccumulation de Cd dans les cabosses et les feuilles de cacao dépend de paramètres géographiques, géologiques et biologiques (c.-à-d. des variétés végétales considérées) ainsi que des pratiques agricoles en vigueur. Des concentrations en Cd élevées ont été retrouvées dans des échantillons de cacaoyers de la région de Manabí, une zone pourtant non impactée par les activités pétrolières, mais pour laquelle il a été mis en évidence l'utilisation de produits agrochimiques comme source importante de Cd.

  D'autres métaux non essentiels, tels que le Ni et le Ba, ont également été retrouvés dans des échantillons de cacao (graines et pâtes de cacao). Bien que les données sur les métaux traces dans le cacao soient rares dans la littérature, ce végétal pourrait s'inscrire dans la catégorie des espèces métallophytes, mais ne serait cependant pas une plante hyperaccumulatrice (Pollard et al., 2014).

  Comme les concentrations en Cd retrouvées dans les pâtes de cacao sont très voisines de celles retrouvées dans les fèves, le test BARGE a pu être mis en œuvre sur ces deux types d'échantillons, montrant alors que près de 100% de la teneur totale en Cd est bioaccessible après ingestion.

  La consommation de produits fabriqués à base de cacao (ou pâtes de cacao) contenant jusqu'à 1,6 mg kg^{-1} de Cd présente un risque pour la santé humaine, si la consommation quotidienne de chocolat atteint 100 g par jour. Enfin, les deux échantillons de pâte de cacao provenant du NAR ont montré des concentrations dépassant jusqu'à 2 fois ce contenu.

- **Dans les PM$_{10}$:** la composition chimique des aérosols dépend de l'activité pétrolière développée dans les zones d'échantillonnage. Dans les sites d'extraction de pétrole (NAR), la matière organique et les éléments du sol représentent 80% de la masse contre 50% dans les sites de raffinage (NPC).

Les concentrations en métaux étaient toujours plus élevées dans le NPC que dans le NAR, bien que les concentrations en As, Cd, Ni et Pb étaient inférieures aux limites établies par l'Union Européenne dans les trois zones d'échantillonnage. Sur la base des concentrations observées dans les PM_{10}, le Ba et le Mo sont proposés comme des traceurs métalliques potentiels des activités pétrolières dans les aérosols. Ces deux éléments présentent des concentrations dans la même gamme que celles relevées dans les sites fortement industrialisés de l'hémisphère nord.

Les HAPs contribuent faiblement à la fraction de matière organique dans le NAR et dans le NPC avec des valeurs de 1.7 et 10%, respectivement. Cependant, malgré leurs faibles concentrations, les oxy-HAPs tels que la 9,10-anthraquinone, la 1,4-naphtoquinone ou le benzanthrone, présentent une réactivité redox élevée et sont connus pour augmenter les dommages oxydatifs dans les poumons (Wei et al., 2010).

Les trois tests acellulaires réalisés (DTT, AA et DCFH) ont démontré que les teneurs en Ba, principal traceur métallique des activités pétrolières, étaient significativement corrélées avec la génération de ROS pour tous les sites d'échantillonnage. D'autres composés contribuent également à l'exposition de la population locale aux ROS: les sucres provenant de la combustion de la biomasse et les oxy-HAPs (provenant du pétrole ou de la combustion de biomasse) dans le NAR mais aussi les métaux (principalement As, Ni, Zn) provenant d'émissions industrielles en association avec oxy-HAPs (anthraquinone) dans le NPC.

- **L'évaluation des risques pour la santé humaine,** basée sur la diète des agriculteurs du NAR, l'inhalation de PM et le contact dermique avec les particules du sol et de l'eau, montre que la population (aussi bien les adultes que les enfants) est vulnérable à la contamination par les métaux et métalloïdes. L'ingestion d'eau et la consommation de végétaux est la principale voie d'exposition aux effets non cancérogènes.

  L'inhalation demeure la voie principale d'exposition pour les effets cancérogènes de l'As, le Ni, le Cd et le Cr chez les adultes et les enfants, dans nos deux zones d'étude. L'indice TCR dans le NPC était 1,6 fois plus élevé que dans le NAR.

  Cependant, les poissons et les crevettes ont été également identifiés comme des produits majeurs dans la diète de la région amazonienne. Ces données supplémentaires sont désormais recueillies dans le cadre du programme MONOIL et publiées dans les rapports de Master de 2 étudiants équatoriens. Leurs publications dans des revues internationales sont en préparation et permettront une approche plus réaliste de l'exposition humaine aux métaux et métalloïdes dans la région amazonienne Equatorienne.

Perspectives

Chaque étude, même si elle a été menée avec rigueur et assiduité, est perfectible. Dès lors, des expériences, analyses et approches complémentaires, envisagées en complément des investigations déjà menées, sont listées ci-après, dans l'objectif d'améliorer encore la qualité de nos premiers résultats et d'aller plus loin sur cette problématique des contaminations pétrolières en Equateur.

Concernant le transfert des métaux et métalloïdes dans le continuum sol-plante:

- Les concentrations totales des métaux et métalloïdes ont été déterminées dans les sols (**Chapitres 1 et 2**). Cependant, il serait également intéressant d'effectuer des tests de lixiviation afin de déterminer la spéciation chimique des composés inorganiques. Les processus naturels tels que le lessivage, le drainage, l'activité microbienne et la production d'exsudats racinaires peuvent modifier les propriétés chimiques du sol, affectant alors la phytodisponibilité des métaux.

Concernant l'évaluation des risques sanitaires (HRA):

- L'évaluation des risques sanitaires proposée dans le **Chapitre 1** ne considère que l'exposition aux composés métalliques. Malheureusement, par manque de temps, les résultats des HAPs dans les PM_{10} des deux zones d'étude n'ont pas pu être intégrés dans l'HRA. Il serait alors judicieux de pouvoir en tenir compte par la suite afin d'évaluer les impacts potentiels de ces composés sur la santé humaine.

Concernant le Cd et les autres métaux dans les fèves de cacao et les produits finis:

- Comme indiqué dans le **Chapitre 2**, les sources de Cd dans le sol ne sont pas encore clairement identifiées. Il serait alors important de déterminer la composition élémentaire des produits agrochimiques ou des engrais organiques appliqués sur les champs de cacao. En outre, il serait intéressant de collecter et d'analyser des échantillons provenant de fermes hors des zones d'activités pétrolières.
- Les variétés et les cultivars de cacao pourraient être un paramètre clé de la bioaccumulation du Cd. L'identification de la variété bioaccumulant le plus le Cd dans les fèves serait d'une haute importance pour les producteurs de cacao. Ces informations pourraient également être utilisées pour réaliser des croisements entre des variétés moins tolérantes au Cd et aux autres métaux.
- En plus de l'analyse préliminaire réalisée au niveau du mucilage des fèves de cacao, nous recommandons de déterminer les teneurs en Cd accumulé dans les racines et les tiges, afin de

fournir de plus amples informations sur le processus complexe de translocation des nutriments et polluants dans les cacaoyers.

- Enfin, les isotopes du Cd pourraient être utilisés comme traceurs de sources de la contamination, mais aussi pour comprendre les mécanismes de l'absorption du Cd par les racines ou les feuilles et leur translocation vers les autres organes de la plante, et ce dans des conditions contrôlées mais aussi en plein champ.

Concernant la génération PM_{10} et des ROS:

- À partir des résultats présentés dans le **Chapitre 3** sur la génération de ROS induits par les PM_{10} dans les milieux biologiques, la prochaine étape consistera à évaluer les effets biologiques dans les cellules pulmonaires en analysant la cytotoxicité, la réponse inflammatoire et les dommages à l'ADN. D'autres études transversales entre les marqueurs toxicologiques, le potentiel oxydatif et la composition chimique des PM permettraient également d'attribuer à chaque source d'émission son impact sur la santé.
- Pour finir, cette étude a porté sur la pollution atmosphérique des particules, mais la quantification des HAPs en phase gazeuse pourrait révéler des informations significatives sur la contamination pétrolière. En effet, les concentrations en HAPs liés aux particules pourraient être inférieures à celles de la phase gazeuse selon leur volatilité. Ainsi, ces données pourraient être utiles pour calculer des rapports de diagnostic, généralement utilisés pour l'identification des sources de contamination.

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Appendix A. Crude oil

Section A.1: Oil formation and production process.

(Text adapted from Union Pétrolière, 2003)

Oil has been formed in the shallow sedimentary basins of the oceans 20 to 350 millions of years ago. Living organisms (sea plants, animals, microorganisms and plankton) floated in the upper layers of the water bodies looking for light, indispensable to survive. When they died, their rests laid down, accumulated and mixed with the underwater mud to form sediments layers enriched in organic matter.

In the seabed, poor in oxygen, the rests of plankton which could be no longer decomposed have been transformed into a complex mixture of hydrocarbons: petroleum (Figure A.1). This transformation has been possible only through continuous chemical and biological processes at temperatures of 65 to 120 ° C and under high pressure (Figure A.2).

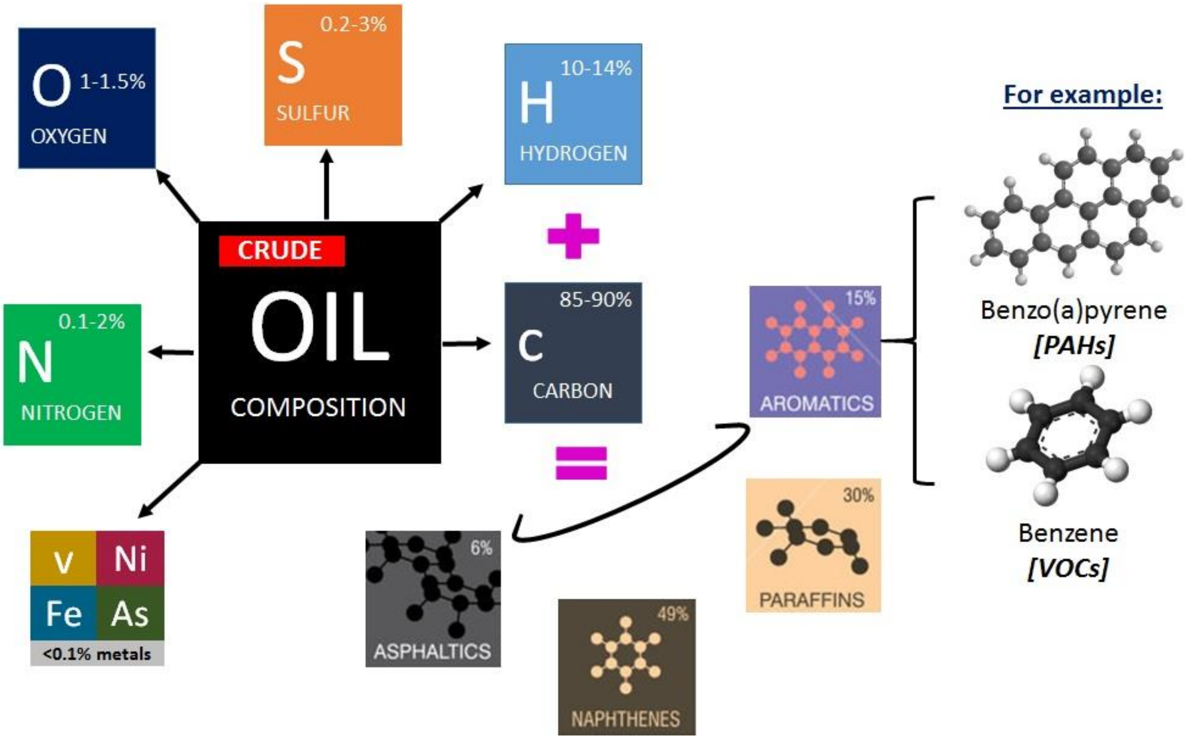

Figure A.1. Crude oil composition. PAHs: polycyclic aromatic hydrocarbons, VOCs: volatile organic compounds, benzene is one of the compounds of the commonly named BTEX (Benzene, Toluene, Ethylene and Xylene). (Source: Speight and Özüm, 2002)

Thus, the formed oil, with a density lower than the surrounding rock formations, naturally ascend towards the surface through the pores of the rock and sometimes rises to the surface and escapes in the form of bitumen seeps. However, in many places, layers of impermeable rock stop the progression of oil. And when it cannot escape laterally, the oil accumulates there. These geological formations are called oil traps.

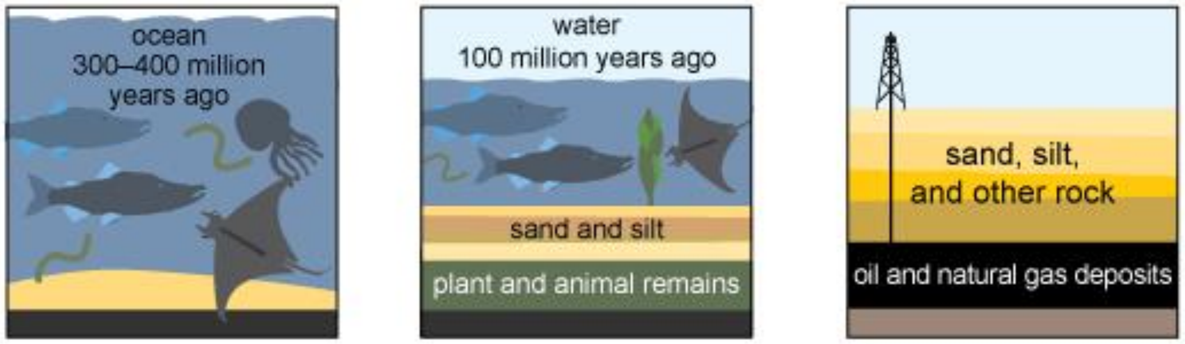

Figure A.2. Oil formation (Source: EIA, 2017)

Oil production process is briefly described in Figure A.3. The search for oil is called exploration. To detect rock formations that may contain oil, geologists uses two methods: seismic surveys and exploration drillings. The first one consists on sending vibrations into the soil, then seismic waves are recorded and provide a 3D image of the geological basement. But only exploration drilling can reveal whether the presumed rock structure actually contains hydrocarbons.

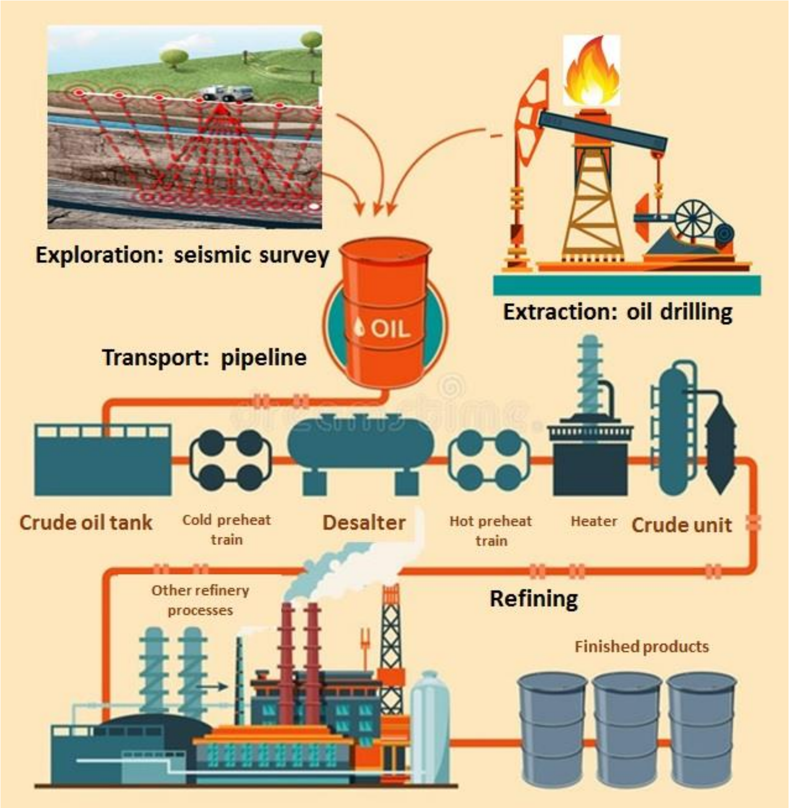

Figure A.3. Oil production process (Modified from: www.dreamstime.com)

In the case of successful drilling, the extent, quality and yield of the discovered deposit must be assessed. Initial production drilling will only occur if commercial exploitation of the deposit is established. Oil extraction varies from one deposit to another. In some cases fluid oil, which is subjected to high pressure and contains large amounts of released gas, rises in the borehole without any assistance. In others, is necessary to add chemical products and inject steam to reduce the oil surface tension.

After extraction, oil is usually transported by pipelines to refineries or to the next oil port. Contrarily to a preconceived idea, the pipeline is the safest mean of oil's transport.

To obtain the final products **(Table A.1)**, crude oil must be fractionated and refined. The main processes during oil refining are: distillation, cracking, refining and desulfurization.

Table A.1. Some of the final products obtained after refining in Ecuador (EP PetroEcuador, 2013).

Local Consumption	Exportation
Asphalt	Fuel Oil N° 6
Naphtha (high octane)	Naphtha (low octane)
Diesel (1 and 2)	“Oriente” crude oil
LPG (liquefied petroleum gas)	“Napo” crude oil

Appendix B. Trace elements toxicity.

Table B.1. Trace elements classification according to their toxicity, human exposure pathways and most common health effects.

Trace metal	IARC[a] / U.S. EPA[b] classification	Main exposure pathway	Health Effects	Reference
As	1 : Carcinogenic to humans	• Diet: drinking water with high inorganic As content, food (rice, fish, seafood) • Inhalation *Cigarette smoking*	• Non carcinogenic risk: Blackfoot disease, skin lesions • Carcinogenic risk: Lung cancer (exposed primarily through inhalation). Multiple internal organ cancers (liver, kidney, lung, and bladder) Increased incidence of skin cancer in populations consuming drinking water enriched in inorganic arsenic.	IRIS (2015)[c] Mulware (2013)
Ba	Not classifiable as to human carcinogenicity [b] Not likely to be carcinogenic to humans (Oral route) [b]	• Diet • Inhalation	• Renal intoxication, hypertension, cardiac malfunction, and hearing loss (in experimental animals). • Poisoning: causing gastrointestinal, cardiac and skeletomuscular stimulation followed by paralysis • Benign pneumoconiosis	IRIS (2015)[c] Oskarsson (2015)
Cd	1 : Carcinogenic to humans	• Inhalation *Cigarette smoking* • Diet: drinking water, food (seafood, milk, fruits, chocolate)	• Non carcinogenic risk: Itai-itai disease (renal impairment and osteomalacia) Renal cortex. • Carcinogenic risk: Prostate, kidney and lung cancer	Mulware, (2013) Nogawa and Suwazono (2011)
Co	2B: possible carcinogenic to humans- based on sufficient evidence of carcinogenicity in animals	• Inhalation • Diet: drinking water and food • Dermal contact	• Non carcinogenic risk: Asthma, deficits on the peripheral and central nervous system (cognitive function), skin allergies, myocardial degeneration. • Possible Carcinogenic risk: Sarcomas and blood neoplasm, lung cancer	Leyssens et al. (2017) Lison (2015)

[a] IARC. International Agency for Research on cancer. World Health Organization.

[b,c] IRIS & U.S. EPA. Integrated Risk Information System. U.S. Environmental Protection Agency

n.a: information not available

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Table B.1. (continued). Trace elements classification according to their toxicity, human exposure pathways and most common health effects.

Trace metal	IARC[a] / U.S. EPA[b] classification	Main exposure pathway	Health Effects	Reference
Cu	Not classifiable as to human carcinogenicity [b]	• Diet: drinking water • Inhalation	• Gastro-intestinal disturbances • May cause irritation of the respiratory tract and metal fume fever	Ellingsen et al. (2015)
Cr (VI)	1 : Carcinogenic to humans	• Inhalation • Diet: drinking water and food • Dermal contact	• Non carcinogenic risk: Sores in the mouth, diarrhea, stomachache, indigestion, vomiting • Carcinogenic risk: Lung, nose and nasal sinuses cancer	Mulware (2013) Langård and Costa (2015)
Mn	Not classifiable as to human carcinogenicity [b]	• Diet: drinking water and food • Inhalation	• Neurotoxicity , associated with "manganese madness" or "Parkinson-like diseases" • Main toxic effects on the central nervous system, including impairment of motor and cognitive function	Pinsino et al. (2012) Lucchini et al., (2015)
Mo	Not assessed	• Diet	• "Teart disease" *(diet high in Mo, low in Cu)* : anemia, gastrointestinal disturbances, bone disorders and growth retardation • Pneumoconiosis	Tallkvist and Oskarsson, (2015)
Ni	A : human carcinogen	• Inhalation *Cigarette smoking* • Diet: drinking water and food • Dermal contact	• Non carcinogenic risk: Hepatotoxic • Carcinogenic risk: Nasal, larynx, prostate and lung cancer	Klein and Costa (2015) Mulware (2013)
Pb	2B: possible carcinogenic to humans- based on sufficient evidence of carcinogenicity in animals	• Inhalation	• Non carcinogenic risk: Effects on the mental development of infants and children • Possible Carcinogenic risk: Lung, stomach, kidney and bladder cancers	Mulware (2013) Skerfving and Bergdahl (2015)

[a] IARC. International Agency for Research on cancer. World Health Organization.
[b,c] IRIS & U.S. EPA. Integrated Risk Information System. U.S. Environmental Protection Agency
n.a: information not available

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Table B.1. (continued). Trace elements classification according to their toxicity, human exposure pathways and most common health effects.

Trace metal	IARC[a] / U.S. EPA[b] classification	Main exposure pathway	Health Effects	Reference
Sb	2B: possible carcinogenic to humans (SbO_3) 3: Not classifiable as to its carcinogenicity to humans (HS_3Sb)	• Inhalation	• Irritation in the respiratory tract • Pneumoconiosis • Cardiovascular effects	Tylenda et al. (2015)
Sn (inorganic compounds)	n.a	• Inhalation • Ingestion • Dermal contact	• "Stannosis," a benign pneumoconiosis • Gastrointestinal illness, liver and kidney problems • Skin irritations	Ostrakhovitch (2015)
Ti (TiO_2)	2B: possible carcinogenic to humans	• Inhalation	• Possible carcinogenic risk: Fibrosarcomas and lymphosarcoma (in rats)	Jin and Berlin (2015)
V (V_2O_5)	2B: possible carcinogenic to humans	• Inhalation • Dermal contact	• Non carcinogenic risk: Irritation of the respiratory tract, coughing, bronchospasm, wheezing, and diarrhea, eye irritation and conjunctivitis • Possibly Carcinogenic risk: lung neoplasms, and some lung tumors (experiments in rats and mice)	Assem and Oskarsson (2015) IRIS (2015)[c] NTP (2002)
Zn	n.a	• Diet • Inhalation	• Gastrointestinal illness • Flu-like metal fume fever (poisoning)	Sandstead (2015)

[a] IARC. International Agency for Research on cancer. World Health Organization.

[b,c] IRIS & U.S. EPA. Integrated Risk Information System. U.S. Environmental Protection Agency

n.a: information not available

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Appendix C. Health risk assessment data set

Table C.1. Crops ingestion: Reference dose (RfD), Average daily dose (ADD), Hazard quotient (HQ) and Hazard index (HI) for adults and children in the North Amazon Region (NAR).

		ADD $_{adults}$ (mg kg^{-1} day^{-1})									
Metal(loid)s	**RfD (mg^{-1} kg^{-1} day^{-1})**	**Banana**	**Manioc**	**Pineapple**	**Lemon**	**Peach palm**	**Abiu**	**Soursop**	**Cacao**	**TOTAL**	**HQ**
Mn	1.40E-01	1.32E-01	5.44E-03	1.52E-02	5.33E-03	9.04E-03	8.63E-03	1.68E-03	1.62E-03	1.79E-01	1.28E+00
Ba	2.00E-01	1.63E-02	3.45E-03	6.89E-04	2.75E-02	4.43E-03	4.22E-03	2.45E-03	4.56E-04	5.95E-02	2.98E-01
V	9.00E-03	6.99E-04	9.66E-05		6.04E-05					8.56E-04	9.52E-02
Zn	3.00E-01	1.87E-02	2.58E-03	1.16E-03	3.24E-03	2.65E-03	5.56E-03	2.33E-03	9.26E-04	3.72E-02	1.24E-01
Ni	2.00E-02								5.96E-05	5.96E-05	2.98E-03
Co	2.00E-02				3.24E-05				1.52E-05	4.76E-05	2.38E-03
Pb	3.50E-03		2.86E-04							2.86E-04	8.17E-02
Mo	5.00E-03	8.05E-04				1.65E-05		2.88E-04	6.18E-06	1.12E-03	2.23E-01
Cd	1.00E-03	2.34E-06	2.95E-06	8.80E-07	1.25E-06	1.77E-05	3.94E-05	5.75E-06	9.36E-06	7.96E-05	7.96E-02
										HI	2.19E+00

		ADD $_{children}$ (mg kg^{-1} day^{-1})									
Metal(loid)s	**RfD (mg^{-1} kg^{-1} day^{-1})**	**Banana**	**Manioc**	**Pineapple**	**Lemon**	**Peach palm**	**Abiu**	**Soursop**	**Cacao**	**TOTAL**	**HQ**
Mn	1.40E-01	4.02E-01	1.57E-02	6.57E-02	1.73E-02	3.92E-02	5.50E-02	7.29E-03	7.01E-03	6.09E-01	4.35E+00
Ba	2.00E-01	4.94E-02	9.96E-03	2.98E-03	8.95E-02	1.92E-02	2.69E-02	1.06E-02	1.98E-03	2.11E-01	1.05E+00
V	9.00E-03	2.12E-03	2.79E-04		1.96E-04					2.60E-03	2.89E-01
Zn	3.00E-01	5.67E-02	7.46E-03	5.04E-03	1.05E-02	1.15E-02	3.54E-02	1.01E-02	4.01E-03	1.41E-01	4.69E-01
Ni	2.00E-02								2.58E-04	2.58E-04	1.29E-02
Co	2.00E-02				1.05E-04				6.58E-05	1.71E-04	8.56E-03
Pb	3.50E-03		8.26E-04							8.26E-04	2.36E-01
Mo	5.00E-03	2.44E-03				7.17E-05		1.25E-03	2.68E-05	3.79E-03	7.57E-01
Cd	1.00E-03	7.09E-06	8.53E-06	3.81E-06	4.07E-06	7.68E-05	2.51E-04	2.49E-05	4.06E-05	4.17E-04	4.17E-01
										HI	7.59E+00

Table C.2. Water and soil ingestion: Reference dose (RfD), Slope Factor (SF), Average daily dose (ADD), Lifetime average daily dose (LADD), Hazard quotient (HQ),Hazard index (HI), Cancer Risk (CR) and Total Cancer Risk (TCR) for adults and children in the North Amazon Region (NAR).

	Water ingestion					Soil ingestion				
		ADD (mg kg^{-1} day^{-1})		HQ			ADD (mg kg^{-1} day^{-1})		HQ	
Metal(loid)s	**RfD (mg^{-1} kg^{-1} day^{-1})**	**Adults**	**Children**	**Adults**	**Children**	**RfD(mg^{-1} kg^{-1} day^{-1})**	**Adults**	**Children**	**Adults**	**Children**
Mn	1.40E-01	1.30E-02	5.62E-02	9.26E-02	4.01E-01	1.40E-01	1.31E-03	1.14E-02	9.38E-03	8.13E-02
Ba	2.00E-01	4.43E-03	1.92E-02	2.21E-02	9.59E-02	2.00E-01	3.70E-04	3.20E-03	1.85E-03	1.60E-02
V	9.00E-03	2.78E-04	1.20E-03	3.09E-02	1.34E-01	9.00E-03	2.47E-04	2.14E-03	2.74E-02	2.37E-01
Zn	3.00E-01	5.43E-02	2.35E-01	1.81E-01	7.84E-01	3.00E-01	1.45E-04	1.26E-03	4.85E-04	4.20E-03
Cr	3.00E-03	2.09E-04	9.06E-04	6.97E-02	3.02E-01	3.00E-03	5.61E-05	4.86E-04	2.98E-02	2.59E-01
Ni	2.00E-02	6.49E-05	2.81E-04	3.24E-03	1.41E-02	2.00E-02	8.95E-05	7.76E-04	1.74E-03	1.51E-02
Co						2.00E-02	3.48E-05	3.01E-04	1.18E-03	1.02E-02
Pb	3.50E-03	1.73E-04	7.50E-04	4.94E-02	2.14E-01	3.50E-03	2.36E-05	2.04E-04	5.76E-03	4.99E-02
Mo	5.00E-03	2.72E-04	1.18E-03	5.45E-02	2.36E-01	5.00E-03	2.02E-05	1.75E-04	6.71E-04	5.81E-03
As	3.00E-04	8.11E-05	3.51E-04	2.70E-01	1.17E+00	3.00E-04	3.35E-06	2.91E-05	2.94E-02	2.54E-01
Cd	5.00E-04	5.35E-06	2.32E-05	1.07E-02	4.63E-02	1.00E-03	8.81E-06	7.63E-05	7.82E-04	6.78E-03
			HI	7.84E-01	3.40E+00			**HI**	1.08E-01	9.40E-01
	SF (mg^{-1} kg day)	**LADD (mg kg^{-1} day^{-1})**		**CR**		**SF (mg^{-1} kg day)**	**LADD (mg kg^{-1} day^{-1})**		**CR**	
As	1.50E+00	3.47E-05	3.01E-05	5.21E-05	4.52E-05	1.5	1.44E-06	2.49E-06	2.52E-06	4.36E-06
			TCR	5.21E-05	4.52E-05			**TCR**	2.52E-06	4.36E-06

Table C.3 Inhalation: Reference concentration for inhalation (RfC), Slope Factor (SF), Concentration non-cancer adjusted (Ca-adj-nc), Lifetime average daily dose (LADD), Hazard quotient (HQ),Hazard index (HI), Cancer Risk (CR) and Total Cancer Risk (TCR) for adults and children in the North Amazon region (NAR).

		Ca-adj-nc (mg m^{-3})		HQ	
Metal(loid)s	**RfC (mg m^{-3})**	**Adults**	**Children**	**Adults**	**Children**
Mn	5.00E-05	1.87E-06	9.35E-06	3.74E-02	1.87E-01
Ba	1.43E-04	3.06E-06	1.53E-05	2.14E-02	1.07E-01
Cr	8.00E-06	8.49E-06	1.70E-06	1.06E+00	2.12E-01
Co	5.71E-06	7.59E-08	3.79E-07	1.33E-02	1.33E-02
Pb	3.52E-03	3.75E-07	1.88E-06	1.07E-04	5.33E-04
As	5.00E-05	2.55E-07	5.09E-08	5.09E-03	1.02E-03
Sb	2.00E-04	1.06E-07	5.30E-07	5.30E-04	2.65E-03
			HI	1.14E+00	5.24E-01
	SF (mg^{-1} kg day)	**LADD (mg kg^{-1} day^{-1})**		**CR**	
Cr	47.6	4.48E-06	3.88E-06	2.13E-04	1.85E-04
Ni	0.84	8.60E-07	7.46E-07	7.23E-07	6.26E-07
As	15.1	1.34E-07	1.16E-07	2.03E-06	1.76E-06
Cd	6.1	3.00E-08	2.60E-08	4.52E-07	3.92E-07
			TCR	2.16E-04	1.87E-04

Table C.4. Soil and water dermal contact: Reference dose (RfD), Slope Factor (SF), Average daily dose (ADD), Lifetime average daily dose (LADD), Hazard quotient (HQ), Hazard index (HI), Cancer Risk (CR) and Total Cancer Risk (TCR) for adults and children in the North Amazon Region (NAR)

	Soil dermal contact					**Water dermal contact**				
		ADD (mg kg^{-1} day^{-1})		**HQ**			**ADD (mg kg^{-1} day^{-1})**		**HQ**	
Metal(loid)s	**RfD (mg^{-1} kg^{-1} day^{-1})**	**Adults**	**Children**	**Adults**	**Children**	**RfD (mg^{-1} kg^{-1} day^{-1})**	**Adults**	**Children**	**Adults**	**Children**
Mn	1.84E-03	4.14E-06	4.51E-07	2.25E-03	2.45E-04	1.40E-01	1.83E-05	6.99E-06	1.31E-04	5.00E-05
Ba	4.90E-03	1.16E-06	1.27E-07	2.38E-04	2.59E-05	2.00E-01	6.26E-06	2.39E-06	3.13E-05	1.19E-05
V	7.00E-05	7.77E-07	8.46E-08	1.11E-02	1.21E-03	9.00E-03	3.93E-07	1.50E-07	4.36E-05	1.66E-05
Zn	6.00E-02	4.58E-07	4.99E-08	7.63E-06	8.32E-07	3.00E-01	4.61E-05	1.76E-05	1.54E-04	5.86E-05
Cr	6.00E-02	2.82E-07	3.07E-08	4.70E-06	5.12E-07	2.00E-02	5.91E-07	2.26E-07	1.97E-04	7.52E-05
Ni	5.40E-03	1.10E-07	1.19E-08	2.03E-05	2.21E-06	3.50E-03	1.84E-08	7.00E-09	9.18E-07	3.50E-07
Co	1.60E-02	7.43E-08	8.09E-09	4.64E-06	5.06E-07					
Pb	5.25E-04	6.35E-08	6.92E-09	1.21E-04	1.32E-05	5.00E-03	3.18E-08	1.21E-08	9.09E-06	3.47E-06
Mo	1.90E-03	1.06E-08	1.15E-09	5.56E-06	6.06E-07					
As	1.23E-04	8.33E-07	3.17E-07	6.77E-03	2.58E-03	3.00E-04	1.15E-07	4.37E-08	3.82E-04	1.46E-04
Cd	1.00E-05	2.46E-09	2.68E-10	2.46E-04	2.68E-05	5.00E-04	8.32E-09	3.17E-09	1.66E-05	6.34E-06
			HI	2.08E-02	4.11E-03			**HI**	9.66E-04	3.68E-04
	SF (mg^{-1} kg day)	**LADD (mg kg^{-1} day^{-1})**		**CR**			**LADD (mg kg^{-1} day^{-1})**		**CR**	
As	3.66E+00	3.57E-07	1.36E-07	1.31E-06	4.98E-07	1.58E+00	4.91E-08	1.87E-08	7.76E-08	6.90E-08
			TCR	1.31E-06	4.98E-07			**TCR**	7.76E-08	6.90E-08

Table C.5. Water and soil ingestion: Reference dose (RfD), Slope Factor (SF), Average daily dose (ADD), Lifetime average daily dose (LADD), Hazard quotient (HQ), Hazard index (HI), Cancer Risk (CR) and Total Cancer Risk (TCR) for adults and children in the North Pacific Coast (NPC).

		Water ingestion					**Soil ingestion**			
		ADD (mg kg^{-1} day^{-1})		**HQ**			ADD (mg kg^{-1} day^{-1})		**HQ**	
Metal(loid)s	**RfD (mg^{-1} kg^{-1} day^{-1})**	**Adult**	**Children**	**Adult**	**Children**	**RfD (mg^{-1} kg^{-1} day^{-1})**	Adult	Children	Adult	Children
Mn	1.40E-01	3.31E-04	1.43E-03	2.36E-03	1.02E-02	1.40E-01	6.14E-04	5.32E-03	4.39E-03	3.80E-02
Ba	2.00E-01	1.16E-03	5.02E-03	5.79E-03	2.51E-02	2.00E-01	4.97E-04	4.31E-03	2.48E-03	2.15E-02
V	9.00E-03	2.17E-04	9.41E-04	2.41E-02	1.05E-01	9.00E-03	2.12E-04	1.83E-03	2.35E-02	2.04E-01
Zn	3.00E-01	8.54E-03	3.70E-02	2.85E-02	1.23E-01	3.00E-01	1.38E-04	1.20E-03	4.60E-04	3.99E-03
Cr	3.00E-03	1.50E-05	6.52E-05	5.01E-03	2.17E-02	3.00E-03	1.40E-04	1.21E-03	4.65E-02	4.03E-01
Ni	2.00E-02	3.71E-05	1.61E-04	1.85E-03	8.03E-03	2.00E-02	7.74E-05	6.71E-04	3.87E-03	3.35E-02
Co						2.00E-02	2.32E-05	2.01E-04	1.16E-03	1.01E-02
Pb	3.50E-03	2.34E-05	1.01E-04	6.68E-03	2.89E-02	3.50E-03	1.16E-05	1.00E-04	3.30E-03	2.86E-02
Mo	5.00E-03	6.13E-05	2.66E-05	1.23E-02	5.31E-02	5.00E-03	2.93E-06	2.54E-05	5.86E-04	5.07E-03
As	3.00E-04	1.99E-05	8.63E-05	6.64E-02	2.88E-01	3.00E-04	1.27E-05	1.10E-04	4.23E-02	3.67E-01
Cd	5.00E-04	8.53E-05	3.70E-04	1.71E-01	7.39E-01	1.00E-03	5.66E-07	4.90E-06	5.66E-04	4.90E-03
			HI	3.21E-01	1.40E-00			**HI**	1.29E-01	1.12E+00
	SF (mg^{-1} kg day)	**LADD (mg kg^{-1} day^{-1})**		**CR**		**SF (mg^{-1} kg day)**	**LADD (mg kg^{-1} day^{-1})**		**CR**	
As	1.5	8.53E-05	7.39E-05	1.28E-05	1.11E-05	1.50E+00	5.44E-06	9.43E-06	3.63E-06	6.29E-06
			TCR	1.28E-05	1.11E-05			**TCR**	3.63E-06	6.29E-06

Table C.6 Inhalation: Reference concentration for inhalation (RfC), Slope Factor (SF), Concentration non-cancer adjusted (Ca-adj-nc), Lifetime average daily dose (LADD), Hazard quotient (HQ),Hazard index (HI), Cancer Risk (CR) and Total Cancer Risk (TCR) for adults and children in the North Pacific Coast (NPC).

		Ca-adj-nc (mg m^{-3})		HQ	
Metal(loid)s	**RfC (mg m^{-3})**	**Adult**	**Children**	**Adult**	**Children**
Mn	5.00E-05	3.24E-06	1.62E-05	6.48E-02	3.24E-01
Ba	1.43E-04	1.65E-05	8.24E-05	1.15E-01	5.76E-01
Cr	8.00E-06	1.36E-05	2.71E-06	1.70E+00	3.39E-01
Ni					
Co	5.71E-06	1.04E-07	5.19E-07	1.82E-02	1.82E-02
Pb	3.52E-03	7.37E-07	3.69E-06	2.09E-04	1.05E-03
As	5.00E-05	8.40E-07	1.68E-07	1.68E-02	3.36E-03
Sb	2.00E-04	3.91E-07	1.96E-06	1.96E-03	9.79E-03
			HI	1.91E+00	1.27E+00
	SF (mg^{-1} kg day)	**LADD (mg kg^{-1} day^{-1})**		**CR**	
Cr	47.6	7.16E-06	6.20E-06	3.41E-04	2.95E-04
Ni	0.84	4.16E-06	3.60E-06	3.49E-06	3.03E-06
As	15.1	4.43E-07	3.84E-07	6.69E-06	5.80E-06
Cd	6.1	9.17E-08	7.94E-08	1.38E-06	1.20E-06
			TCR	3.52E-04	3.05E-04

Table C.7. Water and soil dermal contact: Reference dose (RfD), Slope Factor (SF), Average daily dose (ADD), Lifetime average daily dose (LADD), Hazard quotient (HQ), Hazard index (HI), Cancer Risk (CR) and Total Cancer Risk (TCR) for adults and children in the North Pacific Coast (NPC).

	Water dermal contact					**Soil dermal contact**				
		ADD (mg kg^{-1} day^{-1})		**HQ**			ADD (mg kg^{-1} day^{-1})		**HQ**	
Metal(loid)s	**RfD (mg^{-1} kg^{-1} day^{-1})**	**Adult**	**Children**	**Adult**	**Children**	**RfD (mg^{-1} kg^{-1} day^{-1})**	Adult	Children	Adult	Children
Mn	1.40E-01	4.68E-07	1.78E-07	1.05E-03	1.15E-04	1.84E-03	1.93E-06	2.11E-07	1.05E-03	1.15E-04
Ba	2.00E-01	1.64E-06	6.25E-07	3.19E-04	3.48E-05	4.90E-03	1.56E-06	1.70E-07	3.19E-04	3.48E-05
V	9.00E-03	3.07E-07	1.17E-07	9.52E-03	1.04E-03	7.00E-05	6.66E-07	7.26E-08	9.52E-03	1.04E-03
Zn	3.00E-01	7.24E-06	2.76E-06	7.25E-06	7.89E-07	6.00E-02	4.35E-07	4.74E-08	7.25E-06	7.89E-07
Cr	3.00E-03	4.25E-08	1.62E-08	7.33E-06	7.98E-07	6.00E-02	4.40E-07	4.79E-08	7.33E-06	7.98E-07
Ni	2.00E-02	1.05E-08	4.00E-09	4.51E-05	4.92E-06	5.40E-03	2.44E-07	2.66E-08	4.51E-05	4.92E-06
Co	--	--	--	--	--	1.60E-02	7.31E-08	7.97E-09	4.57E-06	4.98E-07
Pb	3.50E-03	4.30E-09	1.64E-09	6.94E-05	7.56E-06	5.25E-04	3.64E-08	3.97E-09	6.94E-05	7.56E-06
Mo	--	--	--	--	--	1.90E-03	9.22E-09	1.00E-09	4.85E-06	5.29E-07
As	3.00E-04	1.20E-08	1.07E-07	9.75E-03	3.72E-03	1.23E-04	1.20E-06	4.58E-07	9.75E-03	3.72E-03
Cd	5.00E-04	1.78E-07	5.06E-08	1.78E-04	1.94E-05	1.00E-05	1.78E-09	1.94E-10	1.78E-04	1.94E-05
			HI					**HI**	2.10E-02	4.94E-03
	SF (mg^{-1} kg day)	**LADD (mg kg^{-1} day^{-1})**		**CR**		**SF (mg^{-1} kg day)**	**LADD (mg kg^{-1} day^{-1})**		**CR**	
As	1.50E+00	1.21E-08	1.07E-08	1.90E-08	1.70E-08	3.66E+00	5.14E-07	1.96E-07	1.88E-06	7.18E-07
			TCR	1.90E-08	1.70E-08			**TCR**	1.88E-06	7.18E-07

Table C.8. Metal(loid)s concentration (μg L^{-1}) in drinking water* in the North Pacific Coast (NPC).

	Mn	Ba	V	Zn	Cr	Cu	Ni	Co	Pb	Mo	Cd	As
WHO[a]	400	700	--	3000	50	2000	70	--	10	70	3	10
Mean	6.41	22.44	4.21	165.34	0.29	3.16	0.72	0.08	0.45	1.19	1.65	0.39
Min	0.80	18.18	3.14	1.57	0.13	0.86	0.15	0.03	0.03	0.19	0.01	0.29
Max	34.62	25.65	9.32	1161.46	0.76	6.08	2.60	0.18	2.51	3.85	16.34	0.51

*from Esmeraldas water supply network

[a] World Health Organization (2011). Guidelines for Drinking-Water Quality.

Appendix D. Published article from Chapter 2

Cadmium bioaccumulation and gastric bioaccessibility in cacao: A field study in areas impacted by oil activities in Ecuador

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Cadmium bioaccumulation and gastric bioaccessibility in cacao: A field study in areas impacted by oil activities in Ecuador☆

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ABSTRACT

Cacao from South America is especially used to produce premium quality chocolate. Although the European Food Safety Authority has not established a limit for cadmium (Cd) in chocolate raw material, recent studies demonstrate that Cd concentrations in cacao beans can reach levels higher than the legal limits for dark chocolate (0.8 mg kg^{-1}, effective January 1st, 2019). Despite the fact that the presence of Cd in agricultural soils is related to contamination by fertilizers, other potential sources must be considered in Ecuador. This field study was conducted to investigate Cd content in soils and cacao cultivated on Ecuadorian farms in areas impacted by oil activities. Soils, cacao leaves, and pod husks were collected from 31 farms in the northern Amazon and Pacific coastal regions exposed to oil production and refining and compared to two control areas. Human gastric bioaccessibility was determined in raw cacao beans and cacao liquor samples in order to assess potential health risks involved. Our results show that topsoils (0–20 cm) have higher Cd concentrations than deeper layers, exceeding the Ecuadorian legislation limit in 39% of the sampling sites. Cacao leaves accumulate more Cd than pod husks or beans but, nevertheless, 50% of the sampled beans have Cd contents above 0.8 mg kg^{-1}. Root-to-cacao transfer seems to be the main pathway of Cd uptake, which is not only regulated by physico-chemical soil properties but also agricultural practices. Additionally, natural Cd enrichment by volcanic inputs must not be neglected. Finally, Cd in cacao trees cannot be considered as a tracer of oil activities. Assuming that total Cd content and its bioaccessible fraction (up to 90%) in cacao beans and liquor is directly linked to those in chocolate, the health risk associated with Cd exposure varies from low to moderate.

1. Introduction

Ecuador is ranked as the sixth largest oil producing country in South America and 27th in the world (Ecuador Oil Production, 2016). Since the 1960s, most extraction activities have taken place in the northeastern Amazonian region, while refining occurs in Esmeraldas along the Pacific coast. During operations of the Texaco Oil company (1960s–1992), outdated practices and technologies used for oil production (Buccina et al., 2013) generated millions of gallons of untreated toxic waste, gas and oil, which were partially released into the environment (San Sebastián and Hurtig, 2005). Local populations were and continue to be exposed to a mixture of toxic compounds, such as trace metals and polycyclic aromatic hydrocarbons (PAHs), from water consumption, inhalation of airborne particles or ingestion of contaminated crops.

Although the economy of the country revolves around the oil industry, agriculture is the second most important activity in exportation. Ecuador is currently the fourth largest cacao producer in the world from which 87% is directly exported as cacao beans to Europe, mainly France, Germany and England, as well as to the United States (Anecacao, 2015; ProEcuador, 2013). *Theobroma cacao* is mostly cultivated in the western part of the country but the growing area now extends to the Amazon region, covering a total area of 433,978 ha. Cacao plantations utilize a wide variety of soils: from clayey highly eroded soils to volcanic sands and silty soils,

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with pH varying between 4 and 7. Nevertheless, cacao requires depth, well drained and clay loamy soils, with high organic matter content in order to grow in optimal conditions (Quiroz and Agama, 2006).

The cocoa tree, as a plant species, includes a wide range of vastly different varieties. In order to characterize its forms and cultivars, morphological (e.g. flowers), agronomic (e.g. resistance to diseases, fruit shape and grain size) and molecular (e.g. enzymes) properties are frequently used (Dostert et al., 2012). Of all the varieties cultivated in Ecuador, the 'Nacional fino de aroma' and CCN-51 are the most appreciated on the international market (Amores et al., 2009; Loor et al., 2009).

However, there is recent international concern regarding the presence of trace metals in cacao tissues. Recent studies have shown that As, Bi, Cr, Cd, and Pb can be accumulated in cacao beans, pod husks, and cacao-based products (Bertoldi et al., 2016; Chavez et al., 2015; Huamani et al., 2012). Among these elements, cadmium (Cd), a non-essential trace metal, seems to accumulate mainly in the edible parts of cacao, which entails potential risks for human health by ingestion of contaminated products. It has been also reported that cacao beans have different Cd concentrations depending not only on the variety but also on the geographical site, with mean concentrations reaching 1.4 mg kg^{-1} in South America, 0.5 mg kg^{-1} in East Africa and Central America, 0.3 mg kg^{-1} in Asia, and 0.09 mg kg^{-1} in West Africa (Bertoldi et al., 2016).

Indeed, Cd is considered to be one of the most toxic metals that exhibit adverse effects on all biological processes. It reveals very harmful impacts on the environment and food quality (Kabata-Pendias and Szteke, 2015). Based on its carcinogenic effects and, more precisely, the adverse effects observed in the brain, kidneys and bones, the European Union classified Cd and its chlorinated, oxygenated, sulfurated, and sulfate derivatives, into category 1B. Similarly, the International Agency of Research on Cancer (IARC) and the United States Environmental Protection Agency (US EPA) classified Cd into Group 1 and class B, respectively.

In a global context, the major sources of Cd seem to be atmospheric deposition from industries, sewage sludge, and P fertilizers (Kabata-Pendias, 2011). But in Ecuador, Cd can be present in the environment due to natural sources such as volcanic eruptions, which are particularly frequent in the Andes, or the leaching of volcanic rocks, as well as a result of anthropogenic activities including industrial and oil processing (FAO and WHO, 2015). Indeed, it is well known that crude oil can naturally contain metal(oid)s (e.g. As, Cr, Cd, Cu, Co, Ni, Pb, Ti, V, Zn) as a result of the mineral composition of the source rock, or these elements may be added during oil production (e.g. Ba and Mo), refining, transportation, or storage (Fu et al., 2014a; Khuhawar et al., 2012; Lienemann et al., 2007). As a consequence, soils near oil fields can be contaminated by trace metals that are easily transferred to crops (Fu et al., 2014b).

However, to our knowledge, no previous study has investigated the link between Cd bioaccumulation in cacao plants and the proximity of oil production or refinement infrastructures. The Ecuadorian Amazon region houses a vast network of roads, pipelines, and oil facilities (San Sebastián and Hurtig, 2005) that are located close to both large- and small-scale farms, called '*fincas*.' Along the Pacific coast, the city of Esmeraldas is also exposed to air contamination by organic and inorganic compounds due to emissions from the national oil refinery, while cacao crops are cultivated in the adjacent areas. Even though it is currently well known that cacao tissues can contain high concentrations of Cd, the sources and transfer mechanisms (e.g. by foliar and/or root uptake) and the bioaccumulation processes remain poorly described in the literature and several questions remain unsettled.

It should be noted that 90% of human exposure to Cd for the non-smoking population is related to food products (European Food Safety Agency EFSA, 2012). Cadmium dietary exposure is estimated to about 2.04 μg kg^{-1} of body weight per week over a lifetime assuming an average life span of 77 years. Within the 20 main groups described in the Foodex (food classification system), vegetables and confectionary products account for 11 and 3% of food exposure to Cd, respectively, and more precisely, cacao beans and chocolate contribute from 4 to 26% and from 15 to 92%, respectively, depending on the consumer's age (European Food Safety Agency EFSA, 2012).

Nevertheless, the determination of the fraction of total Cd concentration that may potentially affect human health after ingestion has not yet been studied in cacao-based products. Beyond the determination of total trace metal concentrations within a given food product, health risk assessments must also consider their gastric bioaccessibility (Caboche, 2009). This parameter indicates the maximum amount of a compound that, after being released from its matrix during digestion, can be absorbed by the human intestinal epithelium and then enter the blood stream (Hu et al., 2013; Intawongse and Dean, 2006; Peixoto et al., 2016).

A large-scale field study in two areas highly impacted by oil activities in Ecuador was therefore conducted in order to determine Cd concentrations in soils from small-scale farms in the northern Amazon region and near the national oil refinery along the Pacific coast. The aims of this study are to (i) investigate if oil activities constitute a real source of Cd contamination in cacao tissues, (ii) better understand the mechanisms of Cd transfer from soils to cacao plants and its potential bioaccumulation in edible parts, and finally (iii) assess the potential health risk involved after ingestion of cacao-based products, taking the gastric bioaccessibility of Cd in raw materials (cacao beans and liquor) into account.

2. Materials and methods

2.1. Global localization and site descriptions

Cacao samples and associated soils were collected between 2014 and 2016 in the Ecuadorian provinces of Orellana and Sucumbíos in the northern Amazon region (NAR), close to oil production fields, and in Esmeraldas, along the north Pacific coast (NPC), in a two km radius from the national oil refinery. For comparison, two other provinces were studied: Manabí (MC) in the south of the Pacific coast and Morona-Santiago (MS) in the southern Ecuadorian Amazon. These additional sites were considered control areas in the absence of any oil production or mining extraction activities.

A total of 31 small-scale farms (Fig. 1) were chosen within the large area of oil environmental liability, according to the Program of Social and Environmental Reparation and the Ministry of the Environment (PRAS and MAE, 2010), where oils spills, accumulation of drilling muds, formation of water in open pits, and emissions of waste gas into the air are the most common sources of contamination (Fig. SI-1, Supplementary Information). Sampling sites in Esmeraldas were chosen because of their proximity to the national oil refinery, that refines 110,000 barrels of oil per day (EP PetroEcuador, 2016), and the central thermoelectric power plant, which has 12 smokestacks (Ministry of Electricity and Renewable Energy of Ecuador, 2016).

The wet season in the Pacific coastal region usually occurs from mid-December until May, whereas the dry season begins in June and lasts until mid-November. By contrast, precipitation in the Amazon region is continuous over the year but more pronounced between June and September. The total annual precipitation can reach more than 3000 mm in both areas, ranging between 200 and 500 mm per month. The annual mean temperature is around 25 °C, and ranges from 13 °C to 38 °C (FAO, 2006; INAMHI, 2014; Pourrut,

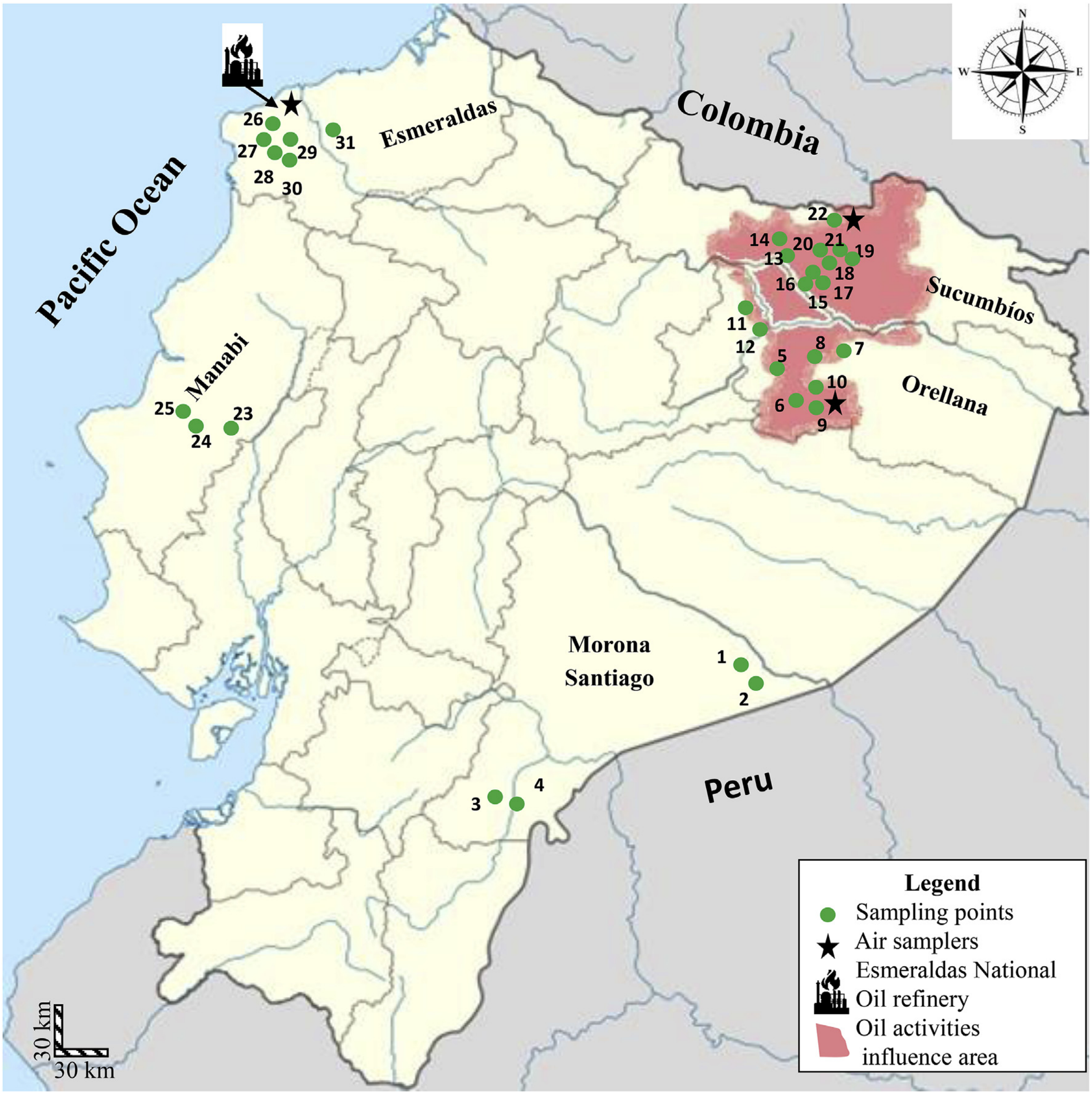

Fig. 1. Sampling sites location in the Ecuadorian provinces of Sucumbíos, Orellana, Esmeraldas, Manabí and Morona-Santiago. Each sampling point is materialized by a green circle and its associated number. The pink area represents the large zone impacted by oil production activities (according to Pérez Naranjo et al. (2015)), whereas the Esmeraldas National Oil Refinery is described by a little pattern. Air samplers are depicted by black stars. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1995). Farm crops are not artificially irrigated except for those located in Portoviejo (MC).

2.2. Soil, cacao, and aerosols sampling

For each sampling point, one mature and healthy cacao pod and ten leaves were selected from three different trees. Three soil samples around each cacao tree were also collected from the surface to 60 or 80 cm depth, according to the site, and to 1 m depth in the control area, namely between three and five horizons. Different cacao varieties (Nacional, CCN-51, Forastero) were collected whenever possible, depending on their availability in the selected *finca*. Three genotypes of Nacional cacao (EET103, EET116, EET576) were also provided by the National Institute of Agriculture and Livestock Research of Ecuador (INIAP-Portoviejo). Amazon Native, Zamora, and CCN-51 cacao varieties were also collected in the control areas of Gualaquiza and San Miguel (MS). Additionally, two cocoa liquor samples from Pacayacu (NAR) were collected to verify Cd gastric bioaccessibility in cacao beans and mass, and to assess health risks involved by their consumption. Detailed information of the cocoa liquor procedure is provided in Section 1 of the Supplementary Information.

Aerosol samples, and more precisely PM_{10} (particulate matters of 10 μm or less in diameter), were collected monthly on pre-calcined quartz fiber filters using a low volume air sampler (3 L min^{-1}, Microvol® devices) in three sites. Two air samplers were settled in the NAR on the roof of two houses located 300 m away from gas burning open flares (15 °E and 15 °S, downwind direction), and one was placed in the NPC in an elementary school, 1500 m east of the national oil refinery and downwind of the major wind directions, S-SE, S, and S-SW (EP PetroEcuador, 2013).

All samples were freeze-dried and transported to France for chemical analysis. Soil composite samples were then air-dried, sieved at 2 mm, and ground with agate ring mills. Cacao beans for the same site and variety were separated from their pod husks, mixed together, air-dried in Teflon trays for three days before being oven-dried at 40 °C for two days. Finally, cacao leaves were oven-dried at 40 °C and ground using an agate mortar and pestle and liquid nitrogen.

2.3. Soil physico-chemical properties

Soil morpho-pedological descriptions were performed in the field and soil classes were determined according to the United States Soil Taxonomy (USDA, 1999), and to the soil maps of the Ecuadorian Ministry of Agriculture, Livestock, Aquaculture and Fisheries and the General Coordination of the National Information System (MAGAP and CGSIN, 2012). Soil pH was measured in the laboratory using a high precision pH meter (744 Metrohm pH meter) using a 1:5 dilution. The cation exchange capacity (CEC) was measured only for the topsoil samples (i.e. top layers of 0–5 cm and 5–20 cm) by spectrocolorimetry using cobalt hexamine trichloride (Fallavier et al., 1985). The total organic carbon (TOC) was determined by combustion using a HORIBA EMIA-320 V carbon/sulfur analyzer.

2.4. Cadmium content determination in soil, aerosols, and cacao samples

For each soil sample, 0.1 g was digested with a mixture of bi-distilled and suprapur acids (11 ml of aqua regia solution and 3 ml of HF) using a Mars 5-CEM microwave. Aerosol samples on quartz fiber filters were digested on a hotplate using 9 ml of aqua regia, 1 ml of H_2O_2 and 2 ml of HF. Mineralization solutions were then completely evaporated at 60 °C on a hotplate and the dry residue was resuspended by adding 0.5 ml of HNO_3.

For plant samples (leaves, pod husks, beans), 0.125 g were mineralized with a hotplate system (Digripep, SCP Science) using 5 ml of HNO_3 and 5 ml of H_2O_2. After digestion, samples were diluted with bi-distillated water and filtered through a 0.22 μm cellulose acetate membrane. The same protocol was applied for the two cocoa liquor samples.

All samples were diluted with HNO_3 0.37 N before ICP-MS (inductively coupled plasma mass spectrometry) analyses (Agilent 7500) in order to determine trace metal, and especially Cd, concentrations. Certified reference materials, SRM 2709 San Joaquin Soil, SRM1648a Urban Dust and SRM1515 apple leaves, were used to validate the mineralization methods for soil, aerosols, and plant samples, respectively. Recoveries of 90–110% were calculated for Cd in all sample types. Each composite sample was analyzed in triplicate and measured values are presented as the mean ± standard deviation (SD).

2.5. Cadmium transport and bioaccumulation in edible parts

The global enrichment factor (GEF) was calculated to determine the degree of metal accumulation in leaves and fruits of cacaos grown on a contaminated site with respect to control zones (Schreck et al., 2014). The GEF method calculation normalizes the measured metal on contaminated sites to the metal content contribution from the biogeochemical background according to

Equation (1):

$$\mathrm{GEF} = \frac{[Cd]\ cacao\ beans\ grown\ on\ contaminated\ sites}{[Cd] cacao\ beans\ from\ control\ site} \tag{1}$$

where [Cd] is the Cd concentration of dry weight (d.w.) in mg kg^{-1}.

The Cd concentration in cacao beans samples from control sites was calculated as the mean value of Cd results in samples 3 and 4 (0.14 ± 0.01 mg kg^{-1}), corresponding to the CCN-51 variety, collected in the MS area of the Amazon region. These sites were chosen because there were not significant variations between Cd concentrations in topsoils and deep soils, and because they were located in the Amazon control area (MS). Even if CCN-51 can differ from the Nacional variety when unhybridized, we decided to use it for our estimations because it is more representative of our sampling (13 of 34 cacao samples) compared to the Native species or Zamora variety. Besides, CCN- 51 is often used as a parent in crosses on the breeding programs in Ecuador (Boza et al., 2014). It is also common to find Nacional fino de aroma and CCN-51 hybrids, which can favor natural hybridization, on local farms.

Cadmium transport from soil to cacao beans was also measured by the bioaccumulation factor (BF) (Mombo et al., 2016), which is defined as

Equation (2):

$$\mathrm{BF} = \frac{[Cd] cacao\ beans}{[Cd]\ soil(5 - 20\ cm)} \tag{2}$$

In essence, the BF represents the ratio between the total Cd concentration in the cacao beans compared to that in the surface soil (5–20 cm), as the root system in cacao trees is almost completely concentrated in the first 20 cm of the soil layer with most of the fine roots in the first 10 cm (De Almeida and Valle, 2007). GEF and BF factors were calculated only for samples for which Cd concentrations in beans exceeded 0.8 mg kg^{-1}, according to the legal Cd limit established by the European Union for dark chocolate (i.e. contains more than 50% of total dry cocoa solids), beginning January 1st, 2019 (European Commission, 2014).

2.6. Human gastric bioaccessibility of Cd

Gastric bioaccessibility (GB) was only determined for cacao beans (i.e. edible part of cacao tree) and cocoa liquor samples with Cd concentrations up to 0.8 mg kg^{-1} in order to detect a potential risk to human health. GB was measured according to the BARGE (Bioaccessibility Research Group of Europe) unified method (Denys et al., 2012; Uzu et al., 2014; Mombo et al., 2016). Using synthetic digestive solutions, this two-step extraction procedure mimics predigestive chemical processes that occur in the mouth and stomach. Digestive fluids were prepared as described by Denys et al. (2009).

Dried and ground samples (0.6 g) were mixed with 9 ml of saliva (pH 6.5) and 13.5 mL of gastric solution (pH 1.1), as reported by Denys et al. (2012) and Wragg et al. (2011). This range of sample-to-fluid ratios is assumed to cover all physiological conditions *in vivo*, as described by Oomen et al. (2003) for soil samples. If the pH of the final solution was not in the range of 1.15–1.25, HCl was added to adjust it. After 1 h of agitation at 37 °C using and end-over-end rotation agitator, the pH was checked again to ensure a value under 1.5 before centrifugation at 3000 rpm for 15 min. Finally, the supernatant was filtrated with cellulose acetate membranes (0.45 μm porosity) and acidified with HNO_3.

Cadmium concentrations in the gastric phase were also measured by ICP-MS (Agilent 7500). The Cd bioaccessible fraction was expressed as a percentage, dividing the total Cd concentration by the gastric bioaccessible Cd concentration.

3. Results

3.1. Physico-chemical properties and total Cd contents in soils

As shown in Table 1, soils from the sampling areas are divided into three types according to the USDA classification typically used in Latin America: inceptisols in NAR and its control area (MS); entisols in NPC; and alfisols in the MC control area.

In the MS area, the soil pH varied between 4.15 ± 0.01 and

Table 1
Physico-chemical properties and total Cd concentrations (in mg kg^{-1} d.w.) measured in soil samples collected in the North Amazon Region (NAR), the North Pacific Coast (NPC) and in the control areas of Morona-Santiago (MS) and in Manabi (MC), in Ecuador.

Sample Code	Site location (sampling area)	Description	Depth (cm)	Soil type (USDA classification)	pH water	CEC (cmol kg^{-1})	TOC (g kg^{-1})	Total Cd (mg kg^{-1} DW soil)
1	Yawi - Gualaquiza (MS)	Control area Amazon Region	0–5	Inceptisol	4.15 ± 0.01	17.4	6.99	0.25 ± 0.01
			5–20		4.32 ± 0.01	17.5	15.21	0.24 ± 0.01
			20–60		4.37 ± 0.04		8.99	0.25 ± 0.03
			60–80		4.69 ± 0.02		6.42	0.23 ± 0.02
			80–100		4.68 ± 0.02		3.65	0.21 ± 0.03
2	Yawi - Gualaquiza (MS)	Control area Amazon Region	0–5	Inceptisol	6.19 ± 0.06	7.1	2.83	0.50 ± 0.01
			5–20		5.33 ± 0.01	15.1	12.66	0.27 ± 0.01
			20–60		5.22 ± 0.13		48.2	0.27 ± 0.02
			60–80		4.82 ± 0.01		10.35	0.24 ± 0.02
			80–100		4.70 ± 0.02		9.11	0.25 ± 0.03
3	San Miguel (MS)	Control area Amazon Region	0–5	Inceptisol	5.09 ± 0.02	16.4	5.23	0.27 ± 0.02
			5–20		4.95 ± 0.02	17.7	5.19	0.24 ± 0.01
			20–60		5.31 ± 0.13		11.52	0.25 ± 0.01
			60–80		5.08 ± 0.04		4.38	0.24 ± 0.04
			80–100		5.10 ± 0.01		3.10	0.28 ± 0.03
4	San Miguel (MS)	Control area Amazon Region	0–5	Inceptisol	4.22 ± 0.01	13.2	1.82	0.49 ± 0.02
			5–20		4.22 ± 0.02	13.9	1.70	0.50 ± 0.01
			20–60		4.41 ± 0.05		20.33	0.51 ± 0.01
			60–80		4.40 ± 0.00		8.15	0.39 ± 0.04
			80–100		4.49 ± 0.02		6.58	0.48 ± 0.03
5	Dayuma NAR)	Auca 9 Oil Well	0–5	Inceptisol	4.51 ± 0.09	16.7	18.12	2.23 ± 0.07
			5–20		4.61 ± 0.01	11.7	7.62	0.79 ± 0.03
			20–60		5.00 ± 0.02		2.76	0.57 ± 0.01
6	Com.Cristal (NAR)	Auca 22 Oil well	0–5	Inceptisol	5.10 ± 0.03	14.3	7.43	0.29 ± 0.03
			5–20		5.11 ± 0.02	14.8	4.11	0.26 ± 0.03
			20–60		5.01 ± 0.03		1.64	0.25 ± 0.01
			60–80		5.29 ± 0.03		0.84	0.24 ± 0.04
7	Com.Shuar Tzakim (NAR)	Hormiguero Oil block	0–5	Inceptisol	4.57 ± 0.03	17.2	17.38	0.34 ± 0.02
			5–20		4.72 ± 0.01	18.7	11.79	0.25 ± 0.01
			20–60		4.72 ± 0.01		4.85	0.31 ± 0.01
			60–80		4.79 ± 0.02		3.09	0.28 ± 0.04
8	Com.Shuar Rio Tiputini (NAR)	Hormiguero Oil block	0–5	Inceptisol	5.49 ± 0.06	14.1	18.17	0.54 ± 0.02
			5–20		5.60 ± 0.02	16.4	7.81	0.40 ± 0.01
			20–60		5.50 ± 0.04		5.26	0.35 ± 0.01
			60–80		5.50 ± 0.06		2.25	0.28 ± 0.04
9	Auca Sur (NAR)	Auca Sur Oil Platform	0–5	Inceptisol	4.86 ± 0.01	17.0	6.67	0.18 ± 0.03
			5–20		4.86 ± 0.04	16.9	3.04	0.19 ± 0.03
			20–60		5.03 ± 0.01		1.44	0.16 ± 0.01
			60–80		4.98 ± 0.00		1.16	0.17 ± 0.04
10	Auca (NAR)	Influence Area of oil activities	0–5	Inceptisol	5.35 ± 0.04	13.3	13.78	0.32 ± 0.02
			5–20		4.87 ± 0.01	15.1	2.68	0.27 ± 0.01
			20–60		5.04 ± 0.01		2.21	0.28 ± 0.01
11	Com.Kichwa M. Yaku (NAR)	Gacela Oil Platform	0–5	Inceptisol	4.44 ± 0.01	17.8	10.4	0.33 ± 0.01
			5–20		4.37 ± 0.01	18.0	7.54	0.32 ± 0.01
			20–60		4.71 ± 0.01		7.25	0.31 ± 0.01
12	Flor de Manduro (NAR)	Gacela Oil Platform	0–5	Inceptisol	5.44 ± 0.02	16.2	14.16	0.60 ± 0.02
			5–20		5.08 ± 0.08	18.3	4.09	0.35 ± 0.01
			20–60		5.11 ± 0.00		3.82	0.36 ± 0.01
13	Shushufindi (NAR)	Aguarico 3 Oil Platform	0–5	Inceptisol	5.99 ± 0.05	14.0	15.75	0.54 ± 0.03
			5–20		6.09 ± 0.01	16.0	8.11	0.34 ± 0.03
			20–60		5.95 ± 0.00		6.54	0.42 ± 0.04
14	Shushufindi (NAR)	Aguarico 3 Oil Platform	0–5	Inceptisol	6.00 ± 0.02	15.5	10.41	0.51 ± 0.01
			5–20		5.91 ± 0.04	16.6	6.45	0.48 ± 0.03
			20–60		5.54 ± 0.04		3.33	0.28 ± 0.01
15	Coop. Nueva Aurora (NAR)	Cobra 1 Oil Platform Well 43	0–5	Inceptisol	6.15 ± 0.01	8.8	4.23	1.09 ± 0.02
			5–20		5.48 ± 0.04	16.9	25.47	0.39 ± 0.01
			20–60		5.39 ± 0.09		8.99	0.48 ± 0.01
16	Nueva Quevedo (NAR)	Oil activities influence area	0–5	Inceptisol	5.12 ± 0.01	16.7	10.55	0.40 ± 0.03
			5–20		5.10 ± 0.01	16.7	7.22	0.37 ± 0.05
			20–60		5.28 ± 0.01		4.13	0.31 ± 0.01
17	Nueva Quevedo (NAR)	Oil activities influence area	0–5	Inceptisol	5.86 ± 0.04	15.77	20.13	0.51 ± 0.02
			5–20		5.93 ± 0.03	17.0	15.00	0.49 ± 0.01
			20–60		6.06 ± 0.06		7.35	0.44 ± 0.01

Table 1 (*continued*)

Sample Code	Site location (sampling area)	Description	Depth (cm)	Soil type (USDA classification)	pH water	CEC (cmol kg^{-1})	TOC (g kg^{-1})	Total Cd (mg kg^{-1} DW soil)
18			0–5		6.38 ± 0.01	14.9	14.66	0.65 ± 0.03
	Coop.	Pichincha 2	5–20	Inceptisol	6.57 ± 0.01	17.3	7.49	0.45 ± 0.03
	Pacayacu	Oil well	20–60		6.65 ± 0.01		7.55	0.44 ± 0.01
	(NAR)		60–80		6.81 ± 0.35		3.79	0.43 ± 0.09
19			0–5		6.39 ± 0.01	14.3	13.24	1.49 ± 0.03
	Coop.	Pichincha 2	5–20	Inceptisol	6.23 ± 0.04	15.4	14.22	1.02 ± 0.03
	Pacayacu	Oil well	20–60		6.61 ± 0.03		5.5	0.64 ± 0.01
	(NAR)		60–80		6.98 ± 0.18		2.53	0.39 ± 0.04
20			0–5		6.61 ± 0.07	17.9	8.58	0.48 ± 0.03
	Coop.	Pichincha 2	5–20	Inceptisol	6.52 ± 0.10	18.3	7.05	0.46 ± 0.03
	Pacayacu	Oil well	20–60		6.47 ± 0.11		4.16	0.33 ± 0.01
	(NAR)		60–80		6.60 ± 0.00		3.62	0.37 ± 0.04
21			0–5		6.65 ± 0.03	9.0	10.66	0.88 ± 0.03
	Coop.	Pichincha 2	5–20	Inceptisol	6.32 ± 0.02	18.0	28.56	0.42 ± 0.03
	Pacayacu	Oil well	20–60		6.42 ± 0.04		7.88	0.47 ± 0.01
	(NAR)		60–80		6.15 ± 0.21		10.95	0.32 ± 0.04
22	Nuevo		0–5		4.65 ± 0.21	18.1	5.86	0.23 ± 0.02
	Oriental	Shuara 9	5–20	Inceptisol	4.57 ± 0.04	18.4	9.45	0.25 ± 0.01
	(NAR)	Oil Platform	20–60		4.60 ± 0.01		6.43	0.25 ± 0.01
23			0–5		7.24 ± 0.01	5.3	4.59	0.46 ± 0.02
	Santa Ana	Control area	5–20	Alfisol	7.14 ± 0.01	5.6	3.6	0.44 ± 0.03
	(MC)	NPC	20–60		7.17 ± 0.01		1.09	0.40 ± 0.01
			60–80		7.53 ± 0.06		2.45	0.23 ± 0.04
24			0–5		6.94 ± 0.01	2.5	6.43	1.00 ± 0.05
	Portoviejo	INIAP	5–20	Alfisol	6.65 ± 0.00	2.3	4.29	0.77 ± 0.03
	(MC)	Control area	20–60		6.47 ± 0.01		4.12	1.02 ± 0.01
		NPC	60–80		7.10 ± 0.02		2.16	0.62 ± 0.04
25			0–5		7.14 ± 0.30	4.7	6.79	0.74 ± 0.03
	Portoviejo	INIAP	5–20	Alfisol	7.14 ± 0.16	4.8	5.36	0.72 ± 0.03
	(MC)	Control area	20–60		7.20 ± 0.23		3.93	0.74 ± 0.01
		NPC	60–80		7.17 ± 0.17		3.08	0.73 ± 0.01
26	Lucha de los	Esmeraldas	0–5		7.44 ± 0.13	7.3	2.29	0.40 ± 0.02
	Pobres	National	5–20	Entisol	7.55 ±0.01	3.8	9.51	0.50 ±0.01
	(NPC)	Oil Refinery	20–60		7.78 ± 0.03		5.75	0.47 ± 0.01
27	Lucha de los	Esmeraldas	0–5		7.23 ± 0.01	1.3	10.19	0.34 ± 0.02
	Pobres	National	5–20	Entisol	7.20 ± 0.00	1.0	5.70	0.40 ± 0.01
	(NPC)	Oil Refinery	20–60		7.48 ± 0.05		5.34	0.45 ± 0.02
28	Lucha de los	Esmeraldas	0–5		7.43 ± 0.01	1.0	9.16	0.42 ± 0.02
	Pobres	National	5–20	Entisol	6.87 ± 0.01	1.7	6.20	0.35 ± 0.01
	(NPC)	Oil Refinery	20–60		6.91 ± 0.01		5.66	0.33 ± 0.01
29	Lucha de los	Esmeraldas	0–5		8.00 ± 0.04	2.7	1.45	0.37 ± 0.03
	Pobres	National	5–20	Entisol	7.60 ± 0.01	4.7	3.84	0.27 ± 0.03
	(NPC)	Oil Refinery	20–60		7.64 ± 0.01		3.35	0.25 ± 0.01
30	Lucha de los	Esmeraldas	0–5		7.50 ± 0.01	3.2	6.43	0.47 ± 0.03
	Pobres	National	5–20	Entisol	7.66 ± 0.02	3.2	4.35	0.43 ± 0.03
	(NPC)	Oil Refinery	20–60		7.90 ± 0.01		2.18	0.39 ± 0.01
31		Esmeraldas	0–5		7.27 ± 0.04	8.3	4.07	0.32 ± 0.03
	Tiwinza	National	5–20	Entisol	7.44 ± 0.01	8.1	3.05	0.33 ± 0.03
	(NPC)	Oil Refinery	20–60		7.35 ± 0.04		2.85	0.34 ± 0.01

Note. The criteria of elaboration of this table was to separate the NAR from the NPC regions, starting with the control areas for both sites.

6.19 ± 0.06 and was more acidic in the first 20 cm. CEC values showed no difference between the first two horizons (0–5 cm and 5–20 cm), varying between 13.2 and 17.5 cmol kg^{-1}, except for the sampling point 2 at Yawi-Gualaquiza (CEC = 7.1 cmol kg^{-1} with a slightly less acidic pH). The TOC contents ranged between 1.71 and 20.33 g kg^{-1} of d.w. soil.

In the NAR, the pH ranged from 4.44 ± 0.05 at the Manduro Yaku Kichwa community to 6.98 ± 0.18 at Pacayacu. The pH and CEC trends were the same as observed in the MS control area. TOC contents varied between 4.23 and 20.13 g kg^{-1} of DW soil with higher concentrations in the topsoils. In contrast to NAR, NPC and MC control areas exhibited slightly neutral to basic pH values (from 6.47 ± 0.01 to 8.00 ± 0.04) that increased slightly with depth. CEC and TOC values were also lower in these areas: between 1.0 and 7.3 cmol kg^{-1} and 1.45 and 10.19 g kg^{-1}, respectively.

In general, the total Cd concentrations decreased with depth, excluding Pacific coast soil sample 24 in MC and sample 26 in the NPC. In the Amazonian control area (MS), Cd remained below 0.27 mg kg^{-1} for points 1 and 3, and 0.5 mg kg^{-1} for points 2 and 4. Nevertheless, some points in the NAR exhibit lower Cd concentrations in all the horizons: about 0.18 and 0.24 mg kg^{-1} (0–60 cm depth) in Auca Sur and Nuevo Oriental, respectively. The highest concentrations in the topsoil measured in the NAR were: 2.23 ± 0.07; 1.49 ± 0.03; 1.09 ± 0.02; and 0.88 ± 0.03 mg kg^{-1} corresponding to points 5, 19, 15 and 21, respectively.

Additionally, Cd concentrations were higher (between 0.46 ± 0.02 and 1.00 ± 0.05 mg kg^{-1}) in the Pacific coast control area, MC, than in NPC area, within the first 5 cm of soil. Finally, 12 of the 31 (39%) investigated sites exceeded the maximum Cd level in soils of 0.5 mg kg^{-1} as established by the Ecuadorian legislation (TULSMA, 2015). The highest concentrations were measured in topsoils sampled in Dayuma in the oil block Auca 09 and Pacayacu in the oil block Pichincha 2. Both sites are characterized by intense oil activity and widespread oil spills. In the coastal control area, both soils sampled in the INIAP-Portoviejo research station showed Cd concentrations two times higher than the recommended level by the Ecuadorian legislation.

3.2. Cadmium concentrations in cacao leaves, pod husks, and beans

Cadmium concentrations in cacao tissues are given in the Supplementary Information (Table SI-1). In general, Cd concentrations are higher in leaves than in pod husks or beans (Fig. 2), except for the points 13, 18 and 22 in NAR, and 29 in NPC. In Dayuma (sample 5, CCN-51 cacao variety), Cd concentrations are the highest of the NAR, reaching values of 7.87 ± 0.05, 2.81 ± 0.02 and 3.50 ± 0.16 mg kg^{-1} in leaves, pod husks, and raw beans, respectively. In the Amazonian control area (MS), the native cacao specie 1a and the Zamora variety (sample 2) exhibited higher Cd concentrations in beans, pod husks, and leaves than the CCN-51 varieties (samples 3 and 4).

Cadmium concentrations in leaves from the NPC study area varied between 0.34 ± 0.05 and 1.96 ± 0.05 mg kg^{-1}, whereas values in the pod husks and beans ranged from 0.34 ± 0.05 to 1.96 ± 0.05 and 0.24 ± 0.19 to 1.30 ± 0.19 mg kg^{-1}, respectively. Additionally, the highest concentrations of Cd were found in the coastal area in Portoviejo at the INIAP station with 6.43 ± 0.05, 4.09 ± 0.02 and 1.66 ± 0.19 mg kg^{-1} for leaves, pod husks and beans, respectively, corresponding to the “EET 116” Nacional cacao genotype. Depending on the sampling site, cacao beans from oil production areas (extraction or refinement activities) reached mean Cd concentrations of 1.21 ± 0.87 and 0.89 ± 0.64 mg kg^{-1} for the CCN-51 and Nacional varieties, respectively, whereas Cd concentrations were 1.71 ± 1.26 for the Nacional variety in the MC control area and 0.14 ± 0.01 mg kg^{-1} for the CCN-51 variety in the MS control area. Finally, even if the Cd in cacao beans could not be compared to a reference value set by worldwide legislation, 18 of the 34 (53%) cacao samples exhibited Cd concentrations above the threshold of

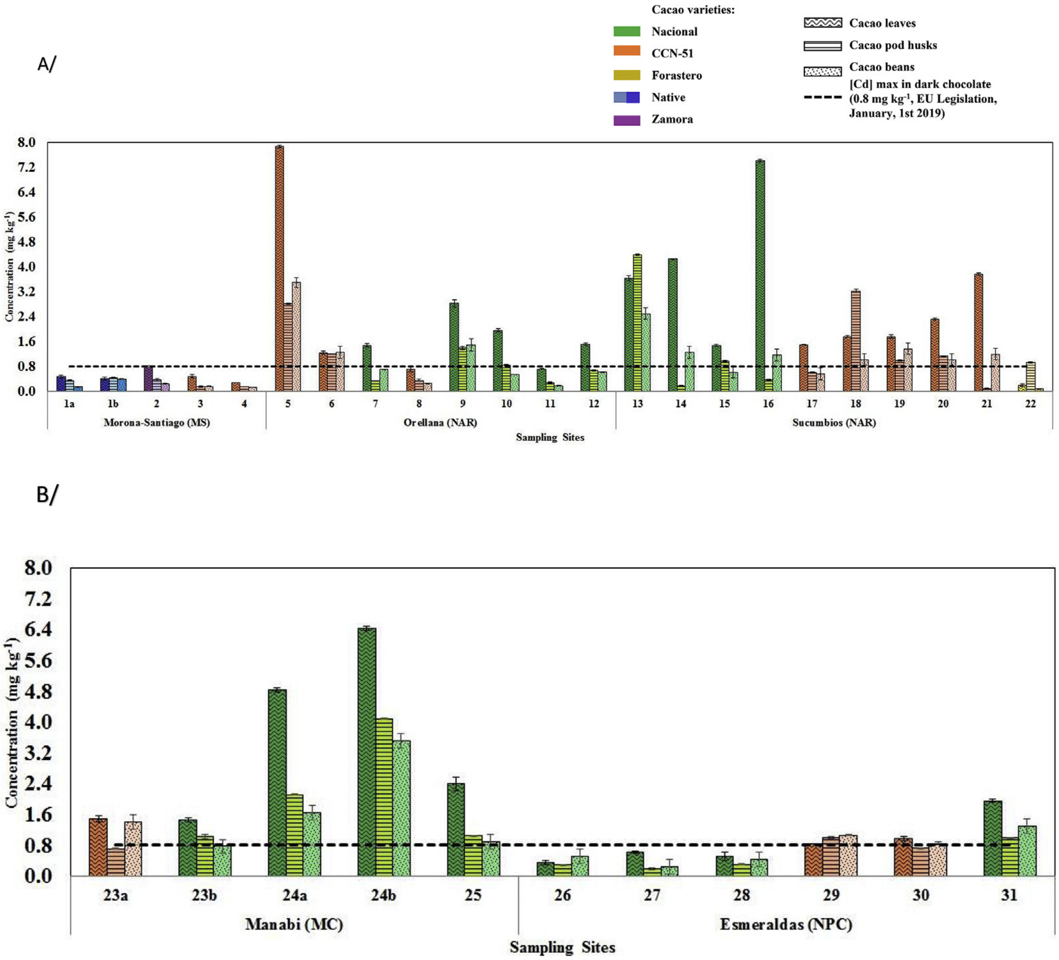

Fig. 2. Total Cd concentrations (mg kg^{-1} d.w.) in cacao leaves, pod husks and beans from 31 small-scale farms in Ecuador. Sampling points are reported in the graphs according to their localization (A: MS and NAR; B: MC and NPC). The control areas (MS and MC) are presented on the left side of the graphs, separated by a black solid line. On the right side, sampling sites are located closed to oil infrastructures (A: wells, oil pools, platforms with open flares, B: refining facilities). Dotted black line represents the Cd threshold for dark chocolate (>50% cacao mass) according to European Legislation.

0.8 mg kg^{-1} set for dark chocolate (i.e. contains more than 50% of total dry cocoa solids) established by the European Union to be effective from 2019 (European Commission, 2014).

3.3. *Cadmium concentrations in aerosols*

Mean annual Cd concentrations from the PM_{10} samples collected during the 2015 monitoring season (Table SI-2, Supplementary Information) ranged between 0.11 ± 0.03 and 0.17 ± 0.09 ng m^{-3} in the NAR (sampling sites 9 and 22, respectively), and averaged 0.44 ± 0.13 ng m^{-3} in the NPC in front of the most important refinery of Ecuador. These values were below the mean annual Cd concentration in PM_{10} of 0.5 ng m^{-3} according to the Ecuadorian Legislation (TULSMA, 2015). A maximum concentration of 0.57 ng m^{-3} was observed in the NPC, while a minimum concentration of 0.07 ng m^{-3} was observed at both of the NAR sampling sites. No marked short-term variations were observed for the three sites.

3.4. *Gastric bioaccessibility of Cd*

Total and bioaccessible Cd contents in cacao beans and cocoa liquor are extremely similar and even equal in some cases (Table 2). Between 90 and 100% of the total Cd is bioaccessible by human ingestion after digestion by gastric fluids. No differences were observed between the CCN-51 and Nacional fino de aroma cacao varieties.

4. Discussion

4.1. *Cadmium bioaccumulation in cacao pods: uptake pathways and influence of external parameters*

The mobility of trace metals, including Cd, in soils and their absorption by plants depends on many factors such as soil texture, pH, CEC, organic matter content, total concentrations in the soil, chemical speciation, plant species and varieties, and farming practices (Qasim et al., 2015; Song et al., 2015; Yang et al., 2016). Wang et al. (2012) reported that Cd uptake by plant roots and its transfer to fruits are influenced by soil properties, plant species and varieties, and also by crop management practices.

In this study, soils displayed the same physico-chemical properties: acidic pH; low CEC; and variable TOC content, which fit with two categories of tropical soils: inceptisols and entisols. Due to the pedogenesis process that performs total hydrolysis of the bedrock in tropical environments, soils are naturally enriched in secondary minerals such as clays and Al and Fe oxy-hydroxides (e.g. gibbsite, goethite, kaolinite), which explains their low CEC and acidic pH (Juo and Franzluebbers, 2003). All of these factors are clearly in accordance with a high bioavailability of Cd in cacao plants. As reported by He et al. (2015), there is an indirect linear relationship between soil pH and phytoavailability of Cd in soils; when pH decreases, Cd uptake by plant increases. Sekara et al. (2004) reported that Cd uptake by plants can be important even at low Cd concentrations in the soil, which explains the high Cd contents in plant tissues.

A correlation matrix was established to identify the potential relationships between Cd contents in cacao plants, soils, and soil physico-chemical parameters (Table SI-3, Supplementary Information). A significant correlation was found for the entire dataset between Cd concentrations in beans and those in pods or leaves, but not with Cd concentrations in soils, except for the NPC samples. Moreover, in the same NPC topsoil samples, Cd concentrations were negatively correlated with pH, indicating that acidic pH can favor the solubilization of this cation and its transfer by the roots to the cocoa plant. For the whole dataset, the Cd concentrations in topsoils did not depend on TOC or CEC.

In this field study, cacao leaves were found to have higher Cd concentrations than beans and pod husks, as reported in section 3.2. This trend has also already been observed in two previous studies. Gramlich et al. (2017) reported Cd average concentrations of 0.91, 0.54, and 0.21 mg kg^{-1} d.w. in leaves, pod husks, and beans, respectively, for two different cacao cultivars in Bolivia. Ramtahal et al. (2016) also found that Cd concentrations in leaves, pods, shells and nibs (beans without shell) were 2.9, 2.5, 2.4, and 2.1 mg kg^{-1}, respectively. Sekara et al. (2004) explained that Cd is transported via the transpiration stream of the plant and accumulates primarily in the leaves. As such, pods and seeds contain much less Cd than the leaves as they evaporate less water. But, in contrast, in a recent study conducted in southern Ecuador, Chavez et al. (2015) showed that Cd contents in leaves were below the detection limit while Cd was detected only in shells when its concentration in beans was above 1 mg kg^{-1}. All differences in the trends of Cd concentrations within cacao tissues might be explained, at least in part, as a function of crop species and cultivars. Hence, the genetic variation among cultivars means there is great potential for utilizing plant breeding as a way to select low-Cd characteristics in crops (Roberts, 2014). In our study, 17 of the 34 cacao samples belonged to the Nacional fino de aroma variety and 13 to the CCN-51 variety. Average Cd concentrations in leaves, pod husks, and beans for the two varieties are quite similar, however, they are slightly higher in the National variety, suggesting a potential impact of the cacao variety on Cd uptake and bioaccumulation in edible parts.

Table 2
Gastric bioaccessibility (GB) of Cd in cacao beans and cocoa liquor after ingestion (% ± SD obtained in replicates).

Sampling point	NAR									
	5	6	9	13	14	16	18	19	20	21
Cacao variety	CCN-51	CCN-51	Nacional	Nacional	Nacional	Nacional	CCN-51	CCN-51	CCN-51	CCN-51
Total $[Cd]_{beans}$ (mg kg^{-1})	3.50 ± 0.16	1.25 ± 0.19	1.49 ± 0.32	2.50 ± 0.19	1.25 ± 0.19	1.16 ± 0.19	1.02 ± 0.19	1.37 ± 0.19	1.02 ± 0.19	1.20 ± 0.19
GB [Cd] (mg kg^{-1})	3.45 ± 0.05	1.19 ± 0.02	1.43 ± 0.02	2.27 ± 0.08	1.29 ± 0.03	1.20 ± 0.05	1.00 ± 0.06	1.30 ± 0.03	0.97 ± 0.02	1.25 ± 0.02
GB Cd (%)	98.7 ± 0.1	95 ± 0.2	96.2 ± 0.2	91.0 ± 0.1	102.8 ± 0.2	103.6 ± 0.2	97.3 ± 0.2	94.8 ± 0.1	94.7 ± 0.2	104.7 ± 0.2

Sampling point	MC 23a	23b	24a	24b	25	NPC 29	30	31	Cocoa liquor samples (NAR)	
Cacao variety	CCN-51	Nacional	ETT103	ETT116	ETT576	CCN-51	CCN-51	Nacional	Nacional-CCN51 Hybrid	
Total $[Cd]_{beans}$ (mg kg^{-1})	1.06 ± 0.19	0.77 ± 0.19	1.66 ± 0.19	3.51 ± 0.19	0.90 ± 0.19	1.06 ± 0.02	0.85 ± 0.05	1.30 ± 0.19	1.47 ± 0.19	3.88 ± 0.19
GB [Cd] (mg kg^{-1})	1.08 ± 0.01	0.80 ± 0.03	1.63 ± 0.06	3.51 ± 0.05	0.86 ± 0.02	1.08 ± 0.05	0.88 ± 0.02	1.38 ± 0.06	1.44 ± 0.05	3.76 ± 0.07
GB Cd (%)	102.0 ± 0.2	103.8 ± 0.3	98.3 ± 0.1	100.1 ± 0.1	95.1 ± 0.2	102.0 ± 0.1	103.0 ± 0.1	106.2 ± 0.2	98.2 ± 0.1	97 ± 0.1

Table 3
Global Enrichment Factor (GEF) and Bioaccumulation Factor (BF) values (mean ± SD) for sampling sites in which Cd concentrations in cacao beans exceeded 0.8 mg kg^{-1}.

Sampling site	NAR									
	5	6	9	13	14	16	18	19	20	21
Cacao variety	CCN-51	CCN-51	Nacional	Nacional	Nacional	Nacional	CCN-51	CCN-51	CCN-51	CCN-51
GEF	24.38 ± 1.86	8.73 ± 2.30	10.38 ± 1.47	17.41 ± 1.70	8.75 ± 1.42	8.11 ± 1.41	7.15 ± 1.39	9.57 ± 1.44	7.14 ± 1.39	8.34 ± 1.42
BF	4.42 ± 0.24	4.80 ± 1.32	8.01 ± 1.51	7.33 ± 0.79	2.59 ± 0.41	3.18 ± 0.68	2.29 ± 0.44	1.34 ± 0.19	2.22 ± 0.43	2.87 ± 0.49

Sampling site	MC					NPC		
	23a	23b	24a	24b	25	29	30	31
Cacao variety	CCN-51	Nacional	ETT103	ETT116	ETT576	CCN-51	CCN-51	Nacional
GEF	9.78 ± 1.45	5.37 ± 1.36	11.58 ± 1.50	24.47 ± 2.01	6.27 ± 1.37	7.42 ± 0.48	5.93 ± 0.49	9.07 ± 1.43
BF	3.17 ± 0.47	2.34 ± 0.60	4.89 ± 0.67	10.33 ± 0.97	1.25 ± 0.27	3.89 ± 0.38	1.97 ± 0.16	3.95 ± 0.65

In Table 3, we report values of GEF and BF factors higher than 1, which suggests a bioaccumulation of Cd in the cacao plant and root transfer of Cd. The impact of cacao variety on Cd bioaccumulation in beans could not be clearly established because these factors are only based on the cacao varieties found in each farm, which makes it difficult to strictly compare the same field conditions.

Cadmium can be released into the atmosphere through metal production activities, fossil fuel combustion, or waste incineration, and can also be enriched in soils by fertilizers or other phytosanitary products (European Food Safety Agency EFSA, 2012; IARC, 2009; Kirkham, 2006). Elevated Cd levels in topsoils and water, even in remote locations, can sometimes result in the long-distance transport of contaminated aerosols (ATSDR, 2012) or by direct anthropogenic input. Since Cd is readily available from air sources, the contribution of airborne Cd to Cd in crops cannot be neglected (Kabata-Pendias, 2011). In order to consider atmospheric deposition as a possible source of trace elements, the natural concentration in the soil substrate must be low and its uptake and accumulation by plants cannot be related to soil borne elements (De Temmerman et al., 2015). It has been demonstrated that dust particles accumulated in the foliar surface can enter the plant as soluble ions through stomata, cuticular cracks, lenticels, ectodesmata, and aqueous pores (Shahid et al., 2017; Uzu et al., 2010). Once trace metals are accumulated inside the plant, they can be transported to the rest of the tissues contaminating edible parts and become bioaccumulated (Schreck et al., 2012; Xiong et al., 2014). In a review of three case studies of the contribution of airborne metal to plant Cd in agricultural crops, Smolders (2001) concluded that atmospheric Cd may be a significant source of Cd for crops grown in areas where its concentration is at least tenfold higher than 2 g Cd ha^{-1} y^{-1}, and where Cd content in soil is not high, or not available.

However, in this field study, as described in sections 2.2 and 3.3, aerosol samples were collected for two years but a complete dataset is still not yet currently available. Although air monitoring was not possible in the entire sampling area, these data provide insight into the Cd levels in air, highlighting that the atmosphere is not polluted by Cd. Furthermore, analyses were performed using SEM-EDX (Scanning electron microscopy with energy dispersive X-ray spectroscopy) and observations did not show any trace of Cd particles deposited on leaf surfaces (Fig. SI-2), suggesting that, in the present study, atmosphere-plant is not a pathway for Cd uptake by leaves or that Cd is quickly dissolved on the leaf surface and incorporated through the cuticle. Hence, we propose that soils remains the primary pathway of Cd uptake by plants cultivated on small-scale farms in areas impacted by oil activities and/or specific agricultural practices.

4.2. Potential sources of cadmium in soils of small-scale Ecuadorian farms

Cadmium is introduced into the soil through geogenic and anthropogenic processes (He et al., 2015). In uncontaminated soils, Cd contents are highly governed by soil texture and range generally from 0.01 to 0.3 and from 0.2 to 0.8 mg kg^{-1} in sandy and loamy soils, respectively (Kabata-Pendias, 2011). In a study on heavy metals in Brazilian soils in areas with minimal or no anthropogenic activity, Fadigas et al. (2006) recommended a Cd reference value, depending on the soil type, from 0.3 to 1.6 mg kg^{-1} (1 mg kg^{-1} for inceptisols). Even if higher Cd values in superficial horizons (compared to background soils) usually reflect anthropogenic input, it can also be derived from the parent material (Caridad-Cancela et al., 2005; Kabata-Pendias, 2011). However, the present study shows that average Cd concentrations in the deepest soil layers from the NAR and NPC (0.36 ± 0.09 mg kg^{-1}) remain low and comparable with the pedogenic background measured in the MC area (0.30 ± 0.12 mg kg^{-1}).

Nevertheless, a trend in the contaminated sampling points (i.e. where soil Cd concentrations exceed 0.5 mg kg^{-1}) is observed: Cd concentrations generally decrease from the top to deeper soil horizons, suggesting anthropogenic inputs instead of natural geological processes. It should be noted that in soils developed in humid climates, migration of Cd down the profile is more likely to occur than its accumulation in the surface horizon. Thus, the enrichment of Cd observed so commonly in topsoils should be related to contamination effects (Kabata-Pendias, 2011), instead of natural sources of heavy metals (i.e. from the parent rock material).

In order to confirm the initial hypothesis in which crude oils naturally contain Cd and other trace metals, four crude oil samples from the national oil pipeline, Auca 3 and Auca 8 oil camps, and from a natural outcrop of bituminous sandstones, all collected in the NAR, were analyzed (Table SI-4, in Supplementary Information). As these concentrations were found to be below the detection limit as well as below the concentrations in soils or air, the possibility to link the Cd content in cacao samples to oil spills or refining activities is quite limited, even if some sampling points of the NAR showed the highest Cd contents in topsoils. However, the existence of another anthropogenic source of Cd in soils is suggested. For example, the control area chosen along the Pacific coast showed high Cd levels in cacao tissues (mean concentrations of 1.65, 1.80, and 3.32 mg kg^{-1} in beans, pod husks, and leaves, respectively) in the absence of oil activities, suggesting a rather key role of agricultural practices in the Cd contamination of cacao plants compared to the impact of mining or oil activities. As highlighted by Zarcinas et al. (2004), the excessive application of inexpensive and contaminated phosphate fertilizers may have contributed to elevated levels of Cd in soil and cacao crops. In Ecuador, the total fertilizer consumption reached 368,370 tons in 2014 (FAO, 2017). The indiscriminate use of phosphate fertilizers and other chemical inputs, or crop irrigation with wastewater, can be considered as important Cd sources to topsoils that are partly responsible for cacao contamination in Ecuador (Mite et al., 2010). In fact, Cd concentrations in P fertilizers depend on the phosphate source rock (He et al., 2015; Jiao et al., 2012), varying from 2 to more than 100 mg kg^{-1} in triple super phosphate products (Roberts, 2014).

Most of the local farmers from the NAR and NPC did not indicate the use of phosphate fertilizers. Nevertheless, manuals of cacao crop management in Ecuador recommend the application of NPK (nitrogen, phosphorus and potassium) fertilizers in combination with micronutrient solutions or ammonia sulfate in controlled doses, and if not, they also promote the use of organic fertilizers (Amores et al., 2009; Quiroz and Agama, 2006; Rodriguez et al., 2010). Chicken manure, locally known as ‘gallinaza,’ fermented organic fertilizers known as ‘bocashi,’ and compost are some of the products named by Ecuadorian farmers but their Cd concentrations generally remain below 2.5 mg kg^{-1} (Carrillo, 2016). However, it is also well known that long-term application will result in Cd accumulation in soils (Nziguheba and Smolders, 2008; Salmanzadeh et al., 2016).

Additionally, in order to increase the organic matter content in soils and to contribute to the decomposition of plant litter, pod husks are left scattered or stacked around other trees after cacao harvest (Ardila and Carreño, 2011; Larrea, 2008). This procedure is not only considered inadequate because it becomes a source of inoculum of harmful pathogens, but it also becomes a pathway to reincorporate Cd already present in cacao pods into the topsoil. The same can occur with the accumulation of cacao leaves in the surface soil. The practice of not raking fallen leaves can favor Cd leaching into the soil solution or become absorbed by organic matter during litter decomposition (Scheid et al., 2009). As an example to illustrate this hypothesis, the highest Cd concentration in the topsoil was found in sampling point 5, and also in cacao beans and leaves. Later, the low soil pH increases Cd availability to the cacao plant roots.

In tropical lowlands with temperatures higher than 30 °C, annual precipitation above 2000 mm, wild winds and intense solar radiation, it is necessary to protect crops against fungal and pest diseases (De Almeida and Valle, 2007; Norgrove, 2007; Pico et al., 2012). Since cacao crops are extremely vulnerable to black pod, frosty pod rot, and cocoa witches' broom diseases (Norgrove, 2007; Pico et al., 2012), pesticides may be considered as another source of trace metal contamination (Castro et al., 2015; Khan et al., 2017; Kirkham, 2006). While these practices are not widespread on small-scale farms because farmers do not necessarily have sufficient income to afford such assorted chemical products, they are well developed on experimental farms managed by the Ministry of Agriculture, Livestock, Aquaculture and Fisheries (MAGAP) and research stations (INIAP) like those in Portoviejo (samples 24 and 25).

Finally, other Cd sources are mentioned in the literature. For example, Chavez et al. (2015) pointed out that elevated Cd levels have been reported in rivers near the study area of southern Ecuador, and consequently the irrigation of cacao fields with Cd-enriched water might have raised soil Cd to the current levels. But in the present study, cacao trees are not manually irrigated, except in Portoviejo (MC). In brief, the combination of many industrial and agricultural practices in Ecuador would confirm multiple sources of Cd in soils and cacao tissues, with agriculture practices probably being the main source of Cd bioaccumulated in cacao plants.

4.3. Human health risk assessment

Half of the investigated cacao samples exhibit Cd concentrations above the critical threshold of 0.8 mg kg^{-1} for dark chocolate (see section 3.2). Even if we assume that food quality standards for dark chocolate cannot be directly applied to raw cacao beans, Cd contents in cacao beans have often been compared to those in chocolate by several authors (Chavez et al., 2015; Gramlich et al., 2017; Pereira de Araújo et al., 2017; Takrama et al., 2015). In fact, cadmium content in chocolate appears to be directly related to the percentage of cocoa mass or cocoa liquor and cocoa powder (FAO and WHO, 2015). For example, in a premium chocolate with a cocoa mass of 70%, the Cd content would be more than 0.8 mg kg^{-1} if the level in the raw beans were higher than 1.15 mg kg^{-1}, a value found in 34% of our samples.

Moreover, the human risk assessment may now focus on the oral bioaccessible fraction, measured here on 18 raw cocoa beans and two cocoa liquor samples. Most studies on Cd gastric bioaccessibility have been performed using soil samples (Qin et al., 2016; Xia et al., 2016), with only a few studies on atmospheric particles (Goix et al., 2016), seafood (Ju et al., 2012), vegetables (Hu et al., 2013; Uzu et al., 2014), and mushrooms (Sun et al., 2012). Depending on the matrix, percentages can vary from 45 to 100, 35 to 99, 73 to 84, 14 to 71 and 30 to 70 in PM, soils, seafood, vegetables, and mushrooms, respectively.

In the present study, more than 90% of the Cd contained in cacao beans or cocoa liquor samples was bioaccessible after ingestion regardless of which cultivar was considered. According to Peixoto et al. (2016), the intestinal bioaccessibility of Cd ranged from 3 to 11% in five types of Brazilian chocolate drink powder, which contain cacao mixed with other ingredients like artificial sweeteners that could affect the chemistry of the whole process by the formation of soluble or insoluble complexes in the gastrointestinal tract. Moreover, the differences between gastric and intestinal bioaccessibility results are explained by the different analytical protocols used to estimate these percentages.

In our study, we have chosen to measure only the gastric bioaccessibility of Cd in order to maximize the health risks (Xiong et al., 2016). Even if the main part of Cd absorption occurs in the jejunum and ileum, the duodenum is a non-negligible absorption site (Denys et al., 2009). As described by Caboche (2009), at the exit of the stomach, a small amount of Cd contained in the gastric phase may be absorbed in the duodenum by passive paracellular diffusion or passive transcellular diffusion. For cationic elements like Cd, an increase of pH in the gastrointestinal phase induces precipitation and/or resorption of a part of the Cd solubilized in the gastric phase. This precipitated Cd will not be absorbed and is then eliminated via the feces (Denys et al., 2009). Cadmium absorption at these levels has been estimated to be 1–7% and is usually characterized by a high accumulation within the mucosa and low transfer rate into the organism (Vázquez et al., 2015).

Several authors have described a decrease of cationic element bioaccessibility in the gastrointestinal phase (Denys et al., 2009; Pascaud et al., 2014; Pelfrêne et al., 2015; Zhuang et al., 2016). As highlighted by Zhuang et al. (2016), a significant decrease of the gastrointestinal bioaccessibility of Cd in rice (63–72%) was observed compared to its gastric bioaccessibility (81–89%). This may be attributed to the pH increase and to the addition of organic components, such as bile extract and pancreatin, in the gastrointestinal phase. We therefore also suggest that gastric bioaccessibility is the most suitable method to assess the risks posed by ingestion of Cd-enriched cacaos.

Moreover, taking into account the Cd tolerable weekly intake (TWI) of 2.5 μg kg^{-1} of body weight (European Food Safety Agency EFSA, 2012) and supposing that Cd content in cacao beans do not suffer any change during chocolate elaboration processes, human exposure to this trace element by chocolate consumption was calculated according to:

Equation (3):

$$\textbf{\textit{Weekly Cd exposure level}} = \frac{\textit{Cacao content in chocolate} \times \textit{Total Cd in beans (and GB Cd in beans)}}{\textit{Average adult body weight}} \tag{3}$$

where the weekly Cd exposure level is expressed in μg of Cd per kg^{-1} of body weight per week. Chocolate consumption was considered on a weekly basis. Cacao content in chocolate was expressed in μg. Total Cd concentrations and GB of Cd in beans, expressed in mg kg^{-1}, were divided by 1000 to transform into units of μg kg^{-1} Average adult body weight was expressed in kg.

Considering a worldwide average chocolate consumption of 5.72 kg year^{-1} person^{-1} (Nieburg, 2014), equivalent to 110 g of chocolate per week, the weekly Cd intake for the cacao bean samples was calculated for two types of chocolate: a dark chocolate containing 70% cacao liquor; and a milk chocolate with only 40%. Results are presented in Table 4 as the maximum, minimum, and mean Cd concentration measured in cacao beans and liquor from our study. Even though the average concentration of Cd measured in the samples selected for GB (described on Section 3.4, Table 2), is 1.63 mg kg^{-1}, it would not represent a risk for human health after ingestion of dark or milk chocolate made from Ecuadorian cacao beans. Conversely, if we consider the highest value reported in this field study (cocoa liquor samples), the weekly Cd intake from dark chocolate would raise to 4.48 μg kg^{-1} b.w., exceeding the TWI by a factor of 1.8.

After human ingestion, Cd is accumulated in the kidneys and liver and has a biological half-life between 10 and 30 years (European Food Safety Agency EFSA, 2012; FAO and WHO, 2015; MAK Value Documentation, 2012). In general, the digestive system of children and adolescents can more easily absorb Cd than adults (Yanus et al., 2014), so that the susceptibility of adverse effects in these groups is higher compared to adults. But, fortunately, the premium chocolate (i.e. containing a high percentage of cacao) manufactured from these Ecuadorian cacao beans is primarily consumed by adults and in small quantities due to its strong flavor and high price (FAO and WHO, 2015).

5. Conclusion

Cadmium distribution in Ecuadorian soils from the Amazon and Pacific coastal regions depends on natural (e.g. soil properties, geochemical background conditions) and anthropogenic (e.g. agricultural practices) factors. Cd concentrations in the first 0–20 cm of soil were typically higher than those in deeper layers, and 39% exceeded the Ecuadorian permissible limit, which suggests anthropogenic sources. Despite its low concentration in most of the study soils, Cd is easily uptaken by roots and accumulates in shoots and fruits. Leaf cadmium levels were always higher than pod husks or beans, however foliar uptake was not considered as the main pathway because of Cd low concentrations in PM_{10}.

Even if oil activities, which are widely spread across the study area, were initially considered a relevant source of Cd in soils and cacaos, our results suggest that Cd contents are more likely related to crop management practices including the extensive use of fertilizers and pesticides, and the natural decomposition of pods and leaves on the soil surface. A first approach to measure gastric bioaccessibility in chocolate raw material (cacao beans and liquors) revealed that almost 100% of the total Cd content is bioaccessible after ingestion. However, a risk assessment calculation shows that levels of Cd exposure by chocolate consumption (eventually processed from these samples) varies from low to high depending on the total Cd concentration, percentage of total cacao solids, and other factors concerning consumers (i.e. age, body weight, etc.).

Additional chemical analyses are currently being performed on raw and processed beans, as well as cocoa liquors and final chocolate products, in order to control each step of the fabrication process. We aim to determine if different steps of chocolate elaboration (e.g. fermentation, roasting, mucilage removal, etc.) can influence the Cd content of the final product and its gastric bioaccessibility. Further studies are also in progress to enhance the knowledge of Cd biological uptake mechanisms (e.g. sequestration and transport) for each commercial variety, as well as tracing its sources in Ecuadorian soils.

Table 4
Weekly Cd intake for dark and milk chocolate consumption elaborated from Ecuadorian cacao beans and cocoa liquor.

Type of cacao-based products	Average chocolate consumption(g week^{-1})	Average cacao consumption from chocolate(g week^{-1})	TWI-EFSA(μg kg^{-1} bw)	Weekly Cd consumption from chocolate (μg)	Weekly Cd intake for an adult of 65 kg (μg kg^{-1} body weight)	Weekly bioaccessible Cd intake for an adult of 65 kg (μg kg^{-1} body weight)*	Risk for human health
	≈ 1 chocolate bar week^{-1}						
Dark chocolate (70% of cocoa liquor)	110	77.3	2.5	Mean: 125.9 Max: 299.7 Min: 59.5	1.94 4.61 0.92	1.88 4.48 0.89	Low High Very low
Milk chocolate (40% of cocoa liquor)	110	44.2	2.5	Mean: 71.9 Max: 171.3 Min: 34.0	1.11 2.63 0.52	1.07 2.56 0.51	Low Moderate Very low

Note. *A mean GB of 97% was considered for these calculations.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.07.080.

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Appendix E.

Table E.1. Localization, GPS coordinates, codification and equivalences for between chapters and sampling sites.

Province	Sampling site	UTM coordinates		HRA codes (Chapter 1)	Cd in cacao codes (Chapter 2)
Morona-Santiago	Com. Yawi (Gualaquiza)	17M 0769614	9611154	nn	1a
	Com. Yawi (Gualaquiza)	17M 0769600	9511154	nn	1b
	Com. Yawi (Gualaquiza)	17M 0769617	9611157	nn	2
	San Miguel (Morona)	18M 0172629	9666674	nn	3
	San Miguel (Morona)	18M 0172253	9665191	nn	4
Orellana	Dayuma	18M 0290721	9926366	5	5
	Com.Cristal	18M 0290805	9917733	2a	6
	Com.Cristal	18M 0290793	9917712	2b	nn
	Com. Shuar Tzakim	18M 0298581	9917079	7	7
	Com.Shuar Rio Tiputini	18M 0299241	9917461	8	8
	Auca Sur	18M 0289898	9922049	1a	nn
	Auca Sur	18M 0289883	9922064	1b	9
	Auca	18M 028778	99220563	6	10
	Com.Kichwa Manduro Yaku	18M 0271565	9945014	9	11
	Com. Flor de Manduro	18M 0270210	9943874	10	12
Sucumbíos	Shushufindi	18M 0314476	9991258	11b	13
	Shushufindi	18M 0314428	9991247	11a	14
	Coop. Nueva Aurora	18M 0319424	9980010	12	15
	Coop. Nueva Quevedo	18M 0312284	9979708	13a	16
	Coop. Nueva Quevedo	18M 0312453	9979651	13b	17
	Coop. Pacayacu	18M 0324986	9996216	3	18
	Coop. Pacayacu	18M 0325025	9996216	14	19
	Coop. Pacayacu	18M 0325309	9996156	15a	20
	Coop. Pacayacu	18M 0325405	9996064	15b	21
	Coop.Nuevo Oriente	18N 0326332	0006536	4a	nn
	Coop.Nuevo Oriente	18N 0326456	0006653	4b	nn
	Coop.Nuevo Oriente	18N 0326531	0006760	4c	22
Manabí	Sta Ana	17M 0572404	9865628	nn	23a
	Sta Ana	17M 0572404	9865628	nn	23b
	Portoviejo	17M 0568081	9870966	nn	24a
	Portoviejo	17M 0568098	9870950	nn	24b
	Portoviejo	17M 0568714	9871287	nn	25
Esmeraldas	Lucha de los Pobres	17N 0645083	101687	16	26
	Lucha de los Pobres	17N 0644961	0101613	17	27
	Lucha de los Pobres	17N 0644921	0101610	18	28
	Lucha de los Pobres	17N 0645139	0101601	19	29
	Lucha de los Pobres	17N 0644859	0101265	20	30
	Tiwinza	17N 0645355	0101624	21	31

*nn: sampling site not included on the chapter

The Earth has a skin and that skin has diseases, one of its diseases is called man.

– Friedrich Nietzsche, Philosopher

Sooner or later, we will have to recognize that the Earth has rights, too, to live without pollution. What mankind must know is that human beings cannot live without Mother Earth, but the planet can live without humans.

– Evo Morales, President of Bolivia

Fire made us human, fossil fuels made us modern, but now we need a new fire that makes us safe, secure, healthy and durable.

– Amory Lovins, Physicist

BARRAZA CASTELO Fiorella

TITLE: Human exposure assessment related to oil activities in Ecuador: from the air quality monitoring to the study of metallic contaminants transfer in the soil-plant continuum

ABSTRACT:

Oil production in Ecuador, for over 40 years, generates waste containing not only polycyclic aromatic hydrocarbons (PAHs) but also co-emitted metals, known for their carcinogenic and toxic effects in humans.

This study aims to determine the distribution of metals in the environmental comparments (air, soil and crops) on extraction (Amazon) and refining sites (Esmeraldas, Pacific coast) in Ecuador, to better understand their transfer in the environment and their bioaccumulation into plants (especially cocoa trees). The impact of anthropogenic activities on human health is also assessed through ingestion, inhalation and dermal contact.

Although some metals are closely related to oil activities, others may come from other anthropogenic or natural sources. But both children and adults are exposed by ingestion and inhalation.

KEYWORDS:

Ecuador; oil activities; metal(loid)s; soil-plant continuum; air quality; health risk assessment.

AUTEUR : BARRAZA CASTELO Fiorella

TITRE : Évaluation de l'exposition humaine liée aux activités pétrolières en Équateur : de la surveillance de la qualité de l'air à l'étude du transfert des contaminants métalliques dans le continuum sol-plante

DIRECTRICES DE THESE : Laurence MAURICE, Eva SCHRECK, Gaëlle UZU

LIEU ET DATE DE SOUTENANCE : Toulouse, le 13 octobre 2017.

RESUME :

Depuis plus de 40 ans, la production de pétrole en Equateur génère des déchets contentant non seulement des hydrocarbures aromatiques polycycliques (HAPs) mais aussi des métaux, connus pour leurs effets cancérogènes et toxiques chez l'Homme.
Cette étude vise à déterminer la distribution des métaux dans l’environnement (air, sols et cultures) sur des sites d’extraction (Amazonie) et de raffinage (Esmeraldas, côte Pacifique), dans l’objectif de mieux comprendre leur transfert dans l’environnement et leur bioaccumulation dans les plantes (cacaoyers notamment). L'impact sanitaire des activités anthropiques a été évalué par ingestion, inhalation et contact dermique.
Bien que certains métaux soient étroitement liés aux activités pétrolières, d’autres sources anthropiques ou naturelles demeurent. Les enfants comme les adultes sont exposés par ingestion et inhalation.

MOTS-CLES : Equateur; Activités pétrolières; Métaux; Continuum sol-plante; Qualité de l'air; Évaluation des risques sanitaires.

DISCIPLINE ADMINISTRATIVE : SURFACES ET INTERFACES CONTINENTALES, HYDROLOGIE

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