

POLYTECHNIQUE MONTRÉAL

affiliée à l'Université de Montréal

**Assessment of dermal exposure to metal(loid)s from contaminated soils
using in vitro methods**

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using in vitro methods**

présentée par **Carlos Augusto MARIN VILLEGAS**

en vue de l'obtention du diplôme de *Philosophiae Doctor*

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DEDICATION

In memory of my mother.

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RÉSUMÉ

La contamination des sols par les métaux (loïdes) générés par les activités anthropiques est un problème persistant, car elle peut constituer un risque pour la santé humaine. Ces contaminants peuvent être transmis à l'homme par ingestion, inhalation ou exposition cutanée. Cette dernière a reçu moins d'attention que les deux autres voies, ce qui justifie des études visant à améliorer la caractérisation de l'exposition cutanée aux sols contaminés.

La fraction du contaminant qui est dissoute dans les liquides du film cutané de surface (sueur et sébum) et disponible pour la pénétration à travers la peau est la fraction bioaccessible par voie cutanée. Cette fraction peut être utilisée avec les données de perméation pour calculer la biodisponibilité. En outre, la bioaccessibilité in vitro devrait être préférée aux tests in vivo en raison de considérations éthiques et de coûts associés moins élevés. En outre, les évaluations des risques qui prennent en compte les concentrations bioaccessibles donneraient un résultat plus représentatif de l'exposition puisque la surestimation serait évitée et que l'interaction avec les fluides biologiques serait simulée.

La composition des liquides du film cutané de surface (SSFL) est essentielle car elle peut affecter la bioaccessibilité, la toxicité, la mobilité et la perméabilité des métaux(loïdes). Il est donc nécessaire d'évaluer les performances des différentes compositions de SSFL utilisées dans les études d'exposition cutanée.

Les objectifs de cette étude sont les suivants : i) évaluer la bioaccessibilité cutanée de l'arsenic (As), du chrome (Cr), du cuivre (Cu), du nickel (Ni), du plomb (Pb) et du zinc (Zn) présents dans les sols contaminés par l'arséniate de cuivre et de chrome (CCA) et dans les sols de référence certifiés par le biais d'essais in vitro utilisant diverses formulations de sueur et de sébum synthétiques ; ii) comparer les compositions de sueur synthétiques en tant que sources de bioaccessibilité cutanée pour les sols contaminés par l'arséniate de cuivre ; ii) comparer les compositions de sueur synthétique en tant que solutions donneuses et évaluer l'influence du sébum dans la diffusion des métaux(loïdes) à travers une membrane synthétique (agissant comme substitut de la peau) en utilisant la méthodologie de la cellule de Franz ; iii) effectuer une modélisation de l'équilibre chimique pour évaluer la spéciation de la fraction soluble des métaux(loïdes) dans les compositions synthétiques de sueur et évaluer son influence sur la bioaccessibilité et la perméation ; et iv) caractériser les risques pour la santé humaine associés aux métaux(loïdes) potentiellement toxiques

présents dans le sol en utilisant une approche d'exposition à voies multiples, en tenant compte de la fraction bioaccessible des contaminants et de deux scénarios différents : des enfants jouant (exposition résidentielle) et une exposition typique d'un travailleur (exposition industrielle).

Les résultats montrent que la bioaccessibilité cutanée des métaux(loïdes) dépend des propriétés du sol et de l'agent d'extraction. Les sols dont le pH, le carbone organique total et la capacité d'échange cationique sont plus élevés présentent généralement une bioaccessibilité cutanée plus faible. La bioaccessibilité est globalement faible dans le cas du sébum par rapport à la sueur (sauf pour le Zn), probablement en raison de la faible affinité des métaux pour les lipides non polaires. La bioaccessibilité des métaux(loïdes) dans la sueur synthétique était généralement inversement proportionnelle au pH.

Seuls le chrome (Cr) et le cuivre (Cu) ont traversé la membrane synthétique. Les coefficients de perméation étaient plus élevés pour le Cr dans la sueur à pH élevé que dans la sueur à pH plus bas, en raison d'une teneur plus élevée en Cr(VI), qui est plus toxique, plus mobile et plus perméable que le Cr(III). Il est intéressant de noter que la composition de la sueur qui a produit une bioaccessibilité plus élevée pour le Cr a montré une perméation plus faible et vice versa. La présence de sébum déposé sur la peau synthétique pourrait interagir avec le Cr et favoriser la formation de composés plus perméables. La présence de sébum a augmenté les temps de latence pour la perméation du Cu. En revanche, l'As, le Ni, le Pb et le Zn n'ont pas pénétré la peau synthétique. Il est recommandé d'effectuer des tests supplémentaires pour évaluer l'influence du sébum sur la perméation des métaux(loïdes). L'analyse de l'équilibre chimique réalisée avec VMINTEQ montre que la composition de la sueur synthétique joue un rôle crucial dans la spéciation des métaux dans la sueur et influence la bioaccessibilité et la perméation des métaux. Des tests analytiques de spéciation sont suggérés pour valider les résultats.

Enfin, le danger non cancérigène a été évalué pour les scénarios résidentiel et industriel. Les voies orale et cutanée ont donné un indice de danger supérieur à la recommandation de la ligne directrice de l'USEPA, avec et sans considérations de bioaccessibilité. Le danger par inhalation était inférieur à la recommandation de la ligne directrice. Pour la voie cutanée, le danger était plus élevé lorsque la bioaccessibilité était prise en compte dans le scénario industriel. En ce qui concerne le risque cancérigène, la voie orale est la plus importante, suivie de la voie cutanée. Le risque cancérigène global est supérieur au risque acceptable recommandé par la ligne directrice, avec ou sans prise en

compte de la bioaccessibilité pour les voies orale et cutanée. Toutefois, étant donné que des valeurs K_p génériques ont été utilisées, une grande incertitude a été associée au calcul du risque par voie cutanée. Il est donc recommandé de mesurer la biodisponibilité pour les évaluations des risques.

Bien que les outils et protocoles *in vitro* évalués dans cette étude se soient révélés utiles pour prédire l'exposition cutanée aux contaminants dans des études préliminaires, il est impératif de procéder à des essais supplémentaires, y compris des essais *in vivo* sur la peau humaine pour corréler et valider la bioaccessibilité, ainsi que des essais de perméation, afin que les résultats puissent être utilisés efficacement dans la caractérisation des risques.

ABSTRACT

Contamination of soils by metal(loid)s generated by anthropogenic activities is a persistent problem as it can pose a risk to human health. These contaminants can be transferred to humans by soil ingestion, inhalation, or dermal exposure. The latter has received less attention than the other two pathways, warranting studies to improve the characterization of dermal exposure to contaminated soils.

The fraction of the contaminant that is dissolved in surface skin film liquids (sweat and sebum) and available for penetration through the skin is the dermal bioaccessible fraction. This fraction can be used alongside permeation data to calculate bioavailability. Moreover, *in vitro* bioaccessibility should be preferred over *in vivo* testing due to ethical considerations and lower associated costs. Furthermore, risk assessments that consider the bioaccessible concentrations would yield a more representative result of the exposure since overestimation would be prevented and interaction with biological fluids simulated.

The composition of surface skin film liquids (SSFL) is critical since it can affect metal(loid)s' bioaccessibility, toxicity, mobility, and permeability. Therefore, there is a need to assess the performance of different SSFL compositions involved in dermal exposure studies.

The objectives of this study are to i) evaluate the dermal bioaccessibility of arsenic (As), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) from chromium copper arsenate (CCA) contaminated soils and certified reference soil materials via *in vitro* assays using various synthetic sweat and sebum formulations; ii) compare synthetic sweat compositions as donor solutions and evaluate the influence of sebum in the diffusion of metal(loid)s through a synthetic membrane (acting as skin surrogate) using the Franz cell methodology; iii) perform chemical equilibrium modelling to assess speciation of the soluble fraction of metal(loid)s in synthetic sweat compositions and assess its influence in bioaccessibility and permeation; and iv) characterize human health risks associated with potentially toxic metal(loid)s present in the soil using a multi-pathway exposure approach, taking into account the bioaccessible fraction of the contaminants and two different scenarios: children playing (residential exposure) and a typical worker exposure (industrial exposure).

Results show that the dermal bioaccessibility of metal(loid)s depends on the soil and extractant properties. Soils with higher pH, total organic carbon, and cation exchange capacity generally

produced lower dermal bioaccessibility. Overall low bioaccessibility was yielded in the case of sebum when compared with sweat (except for Zn), possibly due to the low affinity of metals for non-polar lipids. Metal(loid) bioaccessibility in synthetic sweat was generally inversely proportional to the pH.

Only chromium (Cr) and copper (Cu) permeated the synthetic membrane. Permeation coefficients were higher for Cr in sweat with higher pH than in sweat with lower pH due to a higher content of Cr(VI), which is more toxic, mobile, and pervious than Cr(III). Interestingly, the sweat composition that yielded a higher bioaccessibility for Cr showed lower permeation and vice versa. Presence of sebum deposited on the synthetic skin might interact with Cr promoting the formation of more permeable compounds. The presence of sebum increased lag times for Cu permeation. However, As, Ni, Pb, and Zn did not permeate the synthetic skin. Additional testing to assess the influence of sebum on the permeation of metal(loid)s is recommended. Chemical equilibrium analysis performed with VMINTEQ shows that synthetic sweat composition plays a crucial role in the speciation of metal(loid)s in sweat and influences the bioaccessibility and permeation of metal(loid)s. Analytical speciation testing is suggested to validate the results.

Finally, the non-carcinogenic hazard was evaluated for the residential and industrial scenarios. The oral and dermal pathways yielded a hazard index over the USEPA guideline recommendation with and without bioaccessibility considerations. The inhalation hazard was under the guideline's recommendation. For the dermal pathway, the hazard was higher when bioaccessibility was considered for the industrial scenario. Regarding carcinogenic risk, the oral pathway is the major contributor, followed by the dermal pathway. The overall carcinogenic risk is higher than the acceptable risk recommended by the guideline with and without bioaccessibility considerations for the oral and dermal pathways. However, since generic K_p values were utilized, high uncertainty was associated with the calculation of dermal risk. Therefore, it is recommended to measure bioavailability for risk assessments.

Although the *in vitro* tools and protocols evaluated in this study have proven helpful in the prediction of dermal exposure to contaminants in early-stage studies, additional testing, including *in vivo* tests with human skin to correlate and validate bioaccessibility, as well as permeation tests, are imperative so that results can be effectively used in risk characterization.

TABLE OF CONTENTS

DEDICATION	III
ACKNOWLEDGEMENTS	IV
RÉSUMÉ.....	V
ABSTRACT	VIII
TABLE OF CONTENTS	X
LIST OF TABLES	XV
LIST OF FIGURES.....	XVII
LIST OF SYMBOLS AND ABBREVIATIONS.....	XIX
LIST OF APPENDICES	XXI
PREFACE	XXII
CHAPTER 1 INTRODUCTION.....	1
1.1 State of knowledge and research needs.....	2
1.2 Objectives and methodology	3
1.3 Research hypotheses	4
1.4 Originality	5
1.5 Plan of dissertation	5
CHAPTER 2 LITERATURE REVIEW.....	7
2.1 Exposure of metal(loid)s from contaminated soil	7
2.2 Metal(loid)s in chromated copper arsenate (CCA) contaminated soils	7
2.3 Bioavailability and bioaccessibility of contaminated soils	10
2.3.1 Contaminants in soils	10
2.3.2 Speciation of metal(loid)s in soils.....	14
2.4 Health risk assessment	15

2.4.1	Exposure assessment	15
2.4.2	Estimation of dermal exposure to chemicals in water.....	15
2.4.3	Estimation of dermal exposures to chemicals in soil	16
2.4.4	Toxicity assessment.....	18
2.4.5	Risk characterization	19
2.4.6	Bioavailability in health risk assessment	20
2.5	Skin surface film liquids (SSFL).....	21
2.5.1	Sweat	22
2.5.2	Sebum.....	23
2.5.3	Synthetic sweat formulations	24
2.6	Dermal permeation.....	24
2.6.1	The skin as a permeate layer	24
2.6.2	Dermal absorption.....	26
2.6.3	Franz diffusion cell system	27
CHAPTER 3 ARTICLE 1 - COMPARISON OF FIVE ARTIFICIAL SKIN SURFACE FILM LIQUIDS FOR ASSESSING DERMAL BIOACCESSIBILITY OF METALS IN CERTIFIED REFERENCE SOILS.....		38
3.1	Introduction	39
3.2	Materials and methods	42
3.2.1	Characterization of certified reference soils.....	42
3.2.2	Artificial SSFLs.....	43
3.2.3	In vitro bioaccessibility tests	45
3.2.4	<i>In vitro</i> tests with spiking solution	47
3.3	Results and discussion.....	47
3.3.1	Soil characterization.....	47

3.3.2	Metal recoveries in in vitro tests performed with spiking solutions	47
3.3.3	Dermal bioaccessibility of metals	50
3.4	Conclusion.....	53
CHAPTER 4 ARTICLE 2 - COMPARISON OF SYNTHETIC SWEAT AND INFLUENCE OF SEBUM IN THE PERMEATION OF BIOACCESSIBLE METAL(LOID)S FROM CONTAMINATED SOILS THROUGH A SYNTHETIC SKIN MEMBRANE		
4.1	Introduction	56
4.2	Materials and methods	58
4.2.1	Soil sampling and characterization	58
4.2.2	Artificial SSFLs.....	59
4.2.3	In vitro dermal bioaccessibility test	60
4.2.4	Permeation test	61
4.3	Results and discussion.....	62
4.3.1	Soil characterization.....	62
4.3.2	Dermal bioaccessibility of metal(loid)s	63
4.3.3	Permeation test	65
4.4	Future research and study limitations.....	71
CHAPTER 5 ARTICLE 3 - METAL(LOID) SPECIATION IN DERMAL BIOACCESSIBILITY EXTRACTS FROM CONTAMINATED SOILS AND PERMEATION THROUGH SYNTHETIC SKIN.....		
5.1	Introduction	74
5.2	Materials & methods	76
5.2.1	Soil sampling and sample characterization	76
5.2.2	Artificial SSFLs.....	77
5.2.3	<i>In vitro</i> dermal bioaccessibility test	77

5.2.4	Geochemical modelling.....	78
5.2.5	Permeation test	79
5.3	Results and discussion.....	80
5.3.1	Soil characterization.....	80
5.3.2	Bioaccessibility of metal(loid)s.....	81
5.4	Chemical equilibrium modeling.....	82
5.4.1	Arsenic	82
5.4.2	Chromium.....	83
5.4.3	Copper	85
5.4.4	Nickel	86
5.4.5	Lead.....	86
5.4.6	Zinc.....	86
5.5	Permeation.....	86
5.5.1	Chromium.....	87
5.5.2	Copper	88
5.6	Study limitations	90
5.7	Conclusions	91
5.8	Appendix A. Supplementary material	92
CHAPTER 6 ARTICLE 4 - INCORPORATING ORAL, INHALATION AND DERMAL BIOACCESSIBILITY INTO HUMAN HEALTH RISK CHARACTERIZATION FOLLOWING EXPOSURE TO CHROMATED COPPER ARSENATE (CCA)-CONTAMINATED SOILS..		
6.1	Introduction	96
6.2	Materials & methods	98
6.2.1	Soil sampling.....	98
6.2.2	Soil physico-chemical characterization.....	99

6.2.3	Bioaccessibility tests	100
6.2.4	Risk characterization	103
6.3	Results and discussion.....	107
6.3.1	Soil characterization.....	107
6.3.2	Bioaccessibility	109
6.3.3	Risk characterization	110
6.4	Conclusions	117
6.5	Appendix A. Supplementary material	118
CHAPTER 7	GENERAL DISCUSSION.....	120
7.1	Influence of soil properties in bioaccessibility.....	120
7.2	Influence of SSFL in <i>in vitro</i> bioaccessibility.....	120
7.3	Factors Influencing the permeation of metal(loid)s through the membrane	121
7.4	Speciation of the bioaccessible fraction of metalloids in sweat.....	122
7.5	Risk characterization with bioaccessibility considerations	123
CHAPTER 8	CONCLUSION AND RECOMMENDATIONS.....	125
8.1	Conclusions	125
8.2	Recommendations	127
8.2.1	Main recommendations	127
8.2.2	Other recommendations	128
BIBLIOGRAPHY		129
APPENDIX		149

LIST OF TABLES

Table 2.1. Common inorganic contaminants in soils, their sources and major health hazards.....	11
Table 3.1 Composition of three synthetic sweat formulations used in in vitro tests.....	43
Table 3.2 Composition of two synthetic sebum formulations used in in vitro tests.	45
Table 3.3 Total concentrations of Cr, Ni, Pb, and Zn ($\text{mg.kg}^{-1} \pm$ relative standard deviation in %), pH, total organic carbon (TOC, w/w %), and cation exchange capacity (CEC, meq.100 g^{-1}) of certified reference soils tested.....	46
Table 3.4 Recovery percentages (%) for Cr, Ni, Pb, and Zn following spiking (in absence of soil) after 2 h of in vitro dermal bioaccessibility testing using artificial sweat and sebum.....	48
Table 3.5 Recovery percentages (%) for Cr, Ni, Pb, and zinc following spiking (in the presence of reference soils SQC001 and BGS 102) after 2 h of in vitro dermal bioaccessibility testing using artificial sweat and sebum.	49
Table 4.1 Composition of artificial SSFLs.....	60
Table 4.2 Total concentrations of As, Cr, Cu, Ni, Pb, and Zn (mg kg^{-1}), pH, total organic carbon (TOC, w/w %), and cation exchange capacity (CEC, meq 100 g^{-1}) of soils. Precision is expressed as mean $\pm$ relative standard deviation %.	63
Table 4.3 Bioaccessibility of As, Cr, Cu, Ni, Pb, and Zn in soils using synthetic sweat formulations A and B: (a) bioaccessibility expressed in percentage (%) and (b) bioaccessible concentration (mg L^{-1})	64
Table 4.4 Flux ($\text{g cm}^{-2} \text{ h}^{-1}$), lag time (h), and permeation coefficient (cm h^{-1}) for (a) Cr and (b) Cu (data expressed as mean $\pm$ standard deviation).	67
Table 5.1 Total Concentrations of As, Cr, Cu, Ni, Pb, and Zn (mg/kg), pH, Total Organic Carbon (TOC, w/w %), and Cation Exchange Capacity (CEC, meq 100/g) of Soils. Precision is Expressed as Mean $\pm$ Relative Standard Deviation %.	80
Table 5.2 Bioaccessible Concentration (mg/L) of As, Cr, Cu, Ni, Pb, and Zn in Soils Using Synthetic sweat Formulations A and B.	82

Table 5.3 Flux ($\mu\text{g}/\text{cm}^2 \text{ h}$), Lag Time (h), and Permeation Coefficient (cm/h) for Cr and Cu (Data Expressed as Mean $\pm$ Standard Deviation)	88
Table 5.4 Composition of artificial SSFLs formulations	92
Table 5.5. Bioaccessible percentage (%) of As, Cr, Cu, Ni, Pb, and Zn in Soils Using Synthetic sweat formulations A and B	93
Table 6.1 Parameters values for estimating human exposure to CCA-contaminated soils.....	104
Table 6.2 Toxicological properties of metal(oids) for health risk assessments.	106
Table 6.3 Total As, Cr, Cu, Pb, and Zn concentrations (mean $\pm$ standard deviation ($n = 2$)) in CCA-contaminated soils in 250–90 μm , and $< 20 \mu\text{m}$ fractions.	108
Table 6.4 Physicochemical properties (mean $\pm$ standard deviation ($n = 2$)) of different fractions of CCA-contaminated soil samples.	108
Table 6.5. Bioaccessible concentrations ($\text{mg}/\text{L} \pm \text{RSD in } \%$) following dermal bioaccessibility tests using artificial sweat solution NIHS-9610 for 2 h	118
Table 6.6. Bioaccessible concentrations ($\text{mg}/\text{L} \pm \text{RSD in } \%$) following dermal bioaccessibility tests using artificial sweat solution NIHS-9610 for 8 h	119

LIST OF FIGURES

Figure 2.1 Schematic of Franz cell.....	28
Figure 2.2 Comparison of 8-h permeation of saturated caffeine in PG through human cadaver skin samples and synthetic membranes. A) Strat-M and human skin; B) CA and human skin. (Haq et al., 2018).....	30
Figure 2.3 Comparison of 8-h permeation of caffeine patch through human cadaver skin samples and synthetic membranes. A) Strat-M and human skin; B) CA and human skin (Haq et al., 2018).....	31
Figure 2.4 Correlation of flux between Strat-M synthetic membrane and human (Haq et al., 2018)	31
Figure 2.5 Diffusion of aspirin (A), nicotine (B), and hydrocortisone (C) through the synthetic membrane and comparison with various skin models used for in vitro diffusion studies (Joshi et al., 2012).....	32
Figure 2.6 Cumulative amount of chemical fluxing out of the stratum corneum as a function of time (EPA, 1992).....	37
Figure 3.1 Bioaccessibility (%) of Cr, Ni, Zn, and Pb in certified reference soils using synthetic sweat formulations A, B, and C: a) SQC001, b) BGS 102. The error bars represent the % of difference between duplicates.	51
Figure 3.2 Bioaccessibility (%) of Cr, Ni, Zn, and Pb in certified reference soils using synthetic sebum formulations A and B: a) SQC001, b) BGS 102 (<DL: concentrations below detection limit). The error bars represent the % of difference between duplicates.	53
Figure 4.1 Mean cumulative mass per area and standard deviation of metal in sweat A (pH = 6.5) that permeated through Strat-M membrane for (a) chromium (not coated with sebum), (b) chromium (coated with sebum), (c) copper (not coated with sebum), and (d) copper (coated with sebum).....	66
Figure 4.2 Mean cumulative mass per area and standard deviation of chromium in sweat B (pH = 4.7) that permeated through Strat-M membrane when (a) not coated with sebum and (b) coated with sebum.	66

Figure 5.1 Speciation of As (V) (%): (a) in sweat A, (b) in sweat B	83
Figure 5.2. Chromium speciation (%): (a) Cr(III) in sweat A, (b) Cr(VI) in sweat A, (c) Cr(III) in sweat B, and (d) Cr(VI) in sweat B.....	84
Figure 5.3 Copper speciation (%): (a) Cu(II) in sweat A (b) Cu(II) in sweat B	85
Figure 5.4 Mean Cumulative Mass per Area and Standard Deviation of Metal in sweat A (pH = 6.5) That Permeated Through the Strat-M Membrane for (a) Chromium (Not Coated with Sebum), (b) Chromium (Coated with Sebum), (C) Copper (Not Coated with Sebum), and (d) Copper (Coated With Sebum). Results of S7, S8, And SQC001 are Extracted from Marin Villegas & Zagury (2021)	90
Figure 5.5 Nickel speciation (%) in: (a) Sweat A, (b) Sweat B	93
Figure 5.6 Lead speciation (%) in: (a) Sweat A, (b) Sweat B.....	94
Figure 5.7 Zinc speciation (%) in: (a) Sweat A, (b) Sweat B	94
Figure 6.1 Boxplot of bioaccessibility % of metal(loid)s (n = 10). Dermal bioaccessibility % following 2 h extraction (representing children's exposure scenario) is plotted in this graph.	110
Figure 6.2 Boxplot of contribution of the different exposure pathways and combined metal(loid)s to hazard index (HI) without bioaccessibility considerations and considering bioaccessibility for (A) residential scenario, (B) industrial scenario.....	112
Figure 6.3 Percentage of non-carcinogenic risk contribution per pathway for residential and industrial scenarios.....	113
Figure 6.4 Boxplot of carcinogenic risk for combined metal(loid)s (As and Pb) for each pathway and for cumulative multi-pathway following exposure to total metal(loid) content and to bioaccessible fraction of CCA-contaminated soils.	114
Figure 6.5 Boxplot of carcinogenic risks for As, Pb and Cr(VI) in CCA-contaminated soils, through oral, inhalation and dermal pathway for total metal(loid) content and bioaccessible fraction.	115
Figure 6.6 Tornado plot of the parameters to evaluate (A) HI for the residential scenario, (B) HI for the industrial scenario.....	117

LIST OF SYMBOLS AND ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometry
ABSd	Dermal Absorption Factor
AEC	Anion Exchange Capacity
AF	Soil to Skin Adherence Factor
ALF	Artificial Lysosomal Fluid
As	Arsenic
AT	Averaging Time
BGS	British Geological Survey
Br	Bromine
BW	Body Weight
C	Concentration Of Metal(loid) In Soil
CCA	Chromated Copper Arsenate
Cd	Cadmium
CEC	Cation Exchange Capacity
Cr	Chromium
CSF	Carcinogenic Slope Factor
Cu	Copper
DAD	Dermal Absorbed Dose
ED	Exposure Duration
EF	Exposure Frequency
ET	Exposure Time
EV	Event Frequency
F	Fluorine
GS	Gamble's Solution
HDPE	High-Density Polyethylene
HQ	Hazard Quotient
I	Iodine
IBM	Inhalation Bioaccessibility Method
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
IngR	Soil Ingestion Rate
IVG-GI	In Vitro Gastrointestinal Model Including the Intestinal Phase
Kp	Permeability Coefficient
Mn	Manganese
Ni	Nickel
NSERC	Natural Sciences and Engineering Research Council of Canada
OECD	Organisation for Economic Co-Operation and Development
P	Phosphorus

Pb	Lead
PBS	Phosphate-Buffered-Saline
PEF	Soil-to-Air Particulate Emission Factor
PTMs	Potentially Toxic Metal(loid)s
RBA	Relative Oral Bioavailability
SA	Skin Surface Area Available for Exposure
SC	Stratum Corneum
SSFL	Skin Surface Film Liquids
TC	Total Carbon Content
TOC	Total Organic Carbon
Zn	Zinc

LIST OF APPENDICES

Appendix A – In vitro evaluation of dermal bioaccessibility of metals using two synthetic sebum and three synthetic sweat formulations	149
Appendix B –Dermal bioaccessibility of metals: In Vitro permeation of metals from soils through synthetic skin (Strat M. membrane)	168

PREFACE

Research activities realized for the completion of this dissertation have led to four articles in journals with peer review. The details of the articles are given below:

Marin Villegas, C. A., Guney, M., & Zagury, G. J. (2019). Comparison of five artificial skin surface film liquids for assessing dermal bioaccessibility of metals in certified reference soils. *Science of The Total Environment*, 692, 595-601.

Marin Villegas, C. A., & Zagury, G. J. (2021). Comparison of synthetic sweat and influence of sebum in the permeation of bioaccessible metal (loid) s from contaminated soils through a synthetic skin membrane. *Environmental science & technology*, 55(12), 8215-8222.

Marin Villegas, C. A., & Zagury, G. J. (2023). Incorporating oral, inhalation and dermal bioaccessibility into human health risk characterization following exposure to Chromated Copper Arsenate (CCA)-contaminated soils. *Ecotoxicology and Environmental Safety*, 249, 114446.

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CHAPTER 1 INTRODUCTION

Metal(loid)s are arguably the most important group of environmental contaminants (Adriano et al., 2004). Six out of eleven of the most common contaminants at U.S priority list sites are metal(loid)s. This list includes As, Pb, Cr, Ni, and Zn (USEPA, 1995). Their frequent occurrence in the environment can be attributed to their essential role in the world economy. There is a high diversity of manufactured products and processes which use these elements. Methods for disposal and containment also vary largely (Adriano et al., 2004). Consequently, there are diverse ways in which poorly managed metal(loid)s can reach the soil and affect human health.

Over the last decades, the previous belief that the biological response of an organism could be predicted based on the total concentration of contaminants in an environment has been replaced by bioavailability as an input parameter for risk assessments. The bioavailability of metal(loid)s from geological materials is highly dependent on specific characteristics of the soil and the speciation and origin of the metal(loid)s in question. Factors such as the source and concentration of contaminants, and soil characteristics such as Eh, pH, soil cation exchange capacity, organic carbon content, soil metal oxide concentration, and clay fraction all influence bioavailability. For this reason, to refine risk assessments, site-specific studies need to consider the bioavailability of contaminants.

Absorption of contaminants through the skin is a relatively new issue in medical geology even though dermatologists and pharmacologists have, since the 1960s, developed methods to assess dermal absorption to study the transit of drugs and cosmetics through the skin (Sartorelli et al., 2000). Much is known regarding the penetration of drugs and cosmetics through the skin, but little has been established concerning this route for metal(loid) contaminants of geological origin. However, techniques used to study the dermal absorption of drugs and cosmetics can be used to assess the same transport mechanism in relation to toxic compounds present in the soil.

The principal objective of *in vitro* dermal bioaccessibility and permeation tests is to collect data on the bioaccessible fraction of contaminants in skin surface film liquids (SSFL), as well as on their rates of penetration through the skin, to predict the systemic risk of dermally absorbed contaminants. In order to incorporate these values into human health risk assessments, reliable *in vitro* methods for the evaluation of bioaccessibility and penetration parameters relevant to exposure conditions are needed.

The stratum corneum (SC) is the outer layer of the skin. It is covered by a liquid layer composed mainly of sweat, sebum, cellular debris, and minor amounts of intracellular lipids. It has been demonstrated that many chemicals can permeate these layers. This permeation could pose human health risks in the form of chemical exposure (WHO, 2006). However, it is not well understood how the penetration of some chemicals is related to toxicity.

Due to the complexity and variability of human skin surface film liquids (SSFLs), their artificial surrogates often lack some or many of their constituents. Although comprehensive compositions are available, they can be difficult and expensive to prepare. Studies in the dissolution of metals have used different formulations of artificial sweat and sebum, making inter-study comparisons difficult. Therefore, performance evaluations of SSFL formulations in bioaccessibility and permeation tests are missing.

This study evaluates the use of *in vitro* methodologies to assess the dermal bioaccessibility and permeation of commonly found metal(loid)s present in soils. The performance of several compositions of artificial SSFLs is studied, and its influence on the bioaccessibility and permeation of metal(loid)s is addressed. The primary materials studied are certified reference soils and soils contaminated with chromated copper arsenate (CCA). The parameters found using the *in vitro* protocols are used in multi-pathway risk characterization, including oral and inhalation bioaccessibility.

1.1 State of knowledge and research needs

Traditionally, because the total metal(loid) content is considered, risk assessment of contaminated soils mostly overpredicts the dose of contaminant absorbed by humans. Moreover, the permeation coefficient used to calculate dermal risk comes from generic values. The absorbed dose can be predicted more accurately if the bioavailable fraction of the contaminant is considered instead.

Additionally, the attention paid to the dermal pathway in exposure studies of metal(loid)s from contaminated soils has been underwhelming. Most of the published research has focused on the intake of contaminants through the ingestion and inhalation routes. Regardless of this historical shortcoming, the dermal route should be studied because the risk associated with this pathway can be significant in specific scenarios. However, there is no consensus in the *in vitro* methods used to determine the bioaccessible fraction and dermal permeation rates of contaminants. Moreover, the

SSFL composition is a critical factor in the bioaccessibility and permeation of metal(loid)s since it can affect its behaviour. Therefore, characterization of SSFL regarding its performance for *in vitro* testing to assess dermal exposure is needed.

1.2 Objectives and methodology

1. Compare dermal bioaccessibility of metals from reference soils using five artificial SSFL compositions (EN 1811, NIHS 96-10, synthetic sweat described by Van Lierde et al. (2005), synthetic sebum described by Wertz (2008), and synthetic sebum described by Stefaniak et al. (2010)):
 - a. Characterize soil materials (pH, CEC, TOC) to be used in bioaccessibility tests.
 - b. Implement and perform metal bioaccessibility tests of CCA-contaminated and reference soils using SSFL compositions.
 - c. Compare bioaccessibility values of metal(loid)s, discuss the influence of soil properties and SSFLs properties in the bioaccessibility results.
2. Assess the influence of SSFL compositions (sweat as a donor solution, sebum to coat the membrane) in the permeation parameters of metal(loid)s from soils:
 - a. Implement and perform permeation tests of metal(loid)s from geological materials using two artificial SSFLs as the donor solution following the methodology described by Franz (1975) and the synthetic membrane Strat-M.
 - b. Calculate the parameters to describe the diffusion of metals through Strat-M (K_p , Lag time, Flux). Identify and compare the influence of SSFL composition in penetration tests of metals.
 - c. Determine the influence of sebum in the permeation of metals through the membrane.
3. Using chemical equilibrium modelling (Visual Minteq V3.1), perform a speciation analysis to the soluble fraction of metal(loid)s in two synthetic sweat compositions.
 - a. Determine the influence of SSFL properties in the speciation of metal(loid)s following bioaccessibility tests.
 - b. Determine the influence of speciation of metal(loid)s in their permeation through the Strat-M membrane.

4. Assess the human health risk associated with exposure to CCA-contaminated soil samples using a multi-pathway approach including bioaccessibility fractions and compare it with the risk obtained using the total metal(loid) content approach:
 - a. Perform *in vitro* dermal bioaccessibility and use previously published oral and inhalation bioaccessibility data to characterize the metal(loid)s dose for two exposure scenarios to CCA-contaminated soils.
 - b. Characterize the non-carcinogenic and carcinogenic human health risks associated with exposure to CCA-contaminated soils using a multi-pathway exposure considering oral, inhalation, and dermal bioaccessibility.
 - c. Compare the non-carcinogenic and carcinogenic risks obtained using bioaccessibility fractions and the total metal(loid) content approach.

1.3 Research hypotheses

1. Dermal bioaccessibility of soil-bound metal(loid)s is dependent on the properties of the SSFL solution and the geochemical properties (pH, CEC, TOC) of the soil.
Null hypothesis: The dermal bioaccessibility of soil-bound metal(loid)s is not dependent on the properties of the SSFL solution and the geochemical properties (pH, CEC, TOC) of the soil.
2. The permeation of the bioaccessible fraction of metal(loid)s present in SSFL through the synthetic skin membrane (Strat-M) is dependent on the SSFL physicochemical properties.
Null hypothesis: The permeation of the bioaccessible fraction of metal(loid)s present in SSFL through the synthetic skin membrane (Strat-M) is not dependent on the SSFL physicochemical properties.
3. The use of bioaccessibility fractions to calculate risk via a multi-pathway exposure from toxic metal(loid)s in CCA-contaminated soils results in acceptable risk, while using total concentration in soils produces unacceptable risk.
Null hypothesis: There is no significant difference in the risk associated with toxic metal(loid)s in CCA-contaminated soils via a multi-pathway exposure between considering bioaccessibility fractions and total concentration in soils that produces unacceptable risk.

1.4 Originality

This thesis is original on four different levels: (1) for the first time, the performance of five SSFLs, including sebum, for *in vitro* dermal bioaccessibility tests of soil-bound metal(loid)s has been compared; (2) the influence of synthetic sweat formulation on K_p evaluation to assess the dermal permeation through synthetic skin of metal(loid)s from contaminated soils is also reported for the first time; (3) speciation determination of the bioaccessible fraction of metal(loid)s from CCA-soils in synthetic sweat compositions using chemical equilibrium modelling (Visual Minteq); additionally, (4) the study breaks new ground by performing a risk characterization considering a multi-pathway approach (oral, inhalation, and dermal), including bioaccessibility data from actual *in vitro* testing for each pathway and comparing the risk with the one produced by total metal(loid) content.

1.5 Plan of dissertation

This thesis has nine chapters. Chapters 3, 4, 5, and 6 are scientific articles already published or submitted for potential publication in peer-reviewed journals.

Chapter 2 presents a review of the literature on the following topics: 1) metal(loid)s in soil, emphasizing CCA-contaminated soils, their anthropogenic origin, and their risk to human health; 2) exposure assessment methodology and the main steps for evaluating risk following exposure to contaminated soil; 3) description of SSFLs and human skin, including synthetic human skin surrogates used in this thesis; and 4) the description of the Franz diffusion cell methodology, the main principles that explain diffusion through a membrane, and the calculation methods of diffusion of metal(loid)s through the membrane.

Chapter 3 (Article #1) compares the performance of five SSFLs (three sweat and two sebum formulations) for *in vitro* bioaccessibility tests of metals present in two certified soils. The main objective of this chapter is to improve existing dermal *in vitro* bioaccessibility tests by providing a deeper understanding of the influence of the SSFL in the tests, including sebum. This chapter deals with all components of objective 1.

Chapter 4 (Article #2) includes a permeability study of the bioaccessible fraction of metal(loid)s from four soil samples using the Franz cell methodology, two sweat formulations as the donor solution, and the synthetic membrane Strat-M as a human skin surrogate. The diffusion parameters

were calculated for the metals that permeated the membrane. Additionally, the influence of sebum-coated membranes in the permeation of metals is investigated. This chapter deals with objectives 1a, 1b, 2a, 2b, and 2d.

Chapter 5 (Article #3) presents the speciation of potentially toxic metal(loid)s in CCA-contaminated soils following in vitro dermal bioaccessibility, and permeation of metal(loid)s through synthetic skin. Additionally, to broaden the understanding of the permeation results presented in Chapter 4, this study increased the number of soils subjected to bioaccessibility and permeation tests. To grasp the influence of SSFL properties in bioaccessibility and permeation of metal(loid)s, chemical equilibrium modelling (Visual Minteq) was used to perform a speciation analysis of the soluble fraction of metal(loid)s in two synthetic sweat compositions following bioaccessibility tests with CCA-contaminated soils as well as a certified reference soil. This chapter deals with objectives 2b, 2c, 2d, and all components of objective 3.

Chapter 6 (Article #4) incorporates oral, inhalation, and dermal bioaccessibility into human health risk characterizations of CCA-contaminated soils. The study in this chapter presents a risk characterization for two situations: a residential exposure scenario, which represents a child's exposure during a two-hour play period, and an industrial exposure scenario, which represents an adult worker's exposure during an eight-hour shift. The risk is calculated and compared with, and without, bioaccessibility values. To achieve this, characterizations of non-carcinogenic and carcinogenic human health risks of metal(loid)s found in CCA-contaminated soils using a multi-pathway exposure considering oral, inhalation, and dermal bioaccessibility are presented. This chapter deals with objectives 4a and 4b.

Chapter 7 presents the main contributions of the whole document and a general discussion, followed by Chapter 8, which is a general conclusion also containing recommendations for future work. Chapter 9 contains the bibliography used.

CHAPTER 2 LITERATURE REVIEW

2.1 Exposure of metal(loid)s from contaminated soil

The interaction of soil with metal(loid)s is complex and depends on soil texture, organic matter content, pH, cation exchange capacity, and metal oxidation state (Abdel-Rahman et al., 2006). Retention and release of metal(loid)s from soils depend on these parameters since they can be correlated with different components of soils in different ways such as ion exchange, adsorption, precipitation, or complexation (Balasoiu et al., 2001). The principal routes humans are exposed to metal(loid)s from the soil are ingestion, inhalation, and dermal contact (Abdel-Rahman et al., 2006). Since skin constitutes more than 10% of body weight and covers 1.8 m² (Stefaniak et al., 2006) of the body surface, it has the potential to absorb considerable amounts of metal(loid)s into the body during different activities, mainly children in contact with soil while playing, and adults during occupational activities (Abdel-Rahman et al., 2006; USEPA, 2004). Compared to the other pathways, less attention by the scientific community has been paid to dermal exposure. However, dermal contact can contribute to total exposure (Abdel-Rahman et al., 2006; Leal et al., 2018). Additionally, a contaminant that cannot penetrate the skin may still cause a local toxic effect on the skin without penetrating the skin and causing a systemic effect (Abdel-Rahman et al., 2006). For this reason, it is relevant to assess the dermal absorption of metal in the soil in order to assess its potential risk.

In recent years, environmental contaminants have been taking much importance by the scientific community (Adriano et al., 2004). Perhaps metals are the most critical group of contaminants since from the 11 most common contaminants at the U.S National priority list sites, 6 are metal(loid)s (As, Pb, Cr, Cd, Ni, and Zn) (USEPA, 1995).

2.2 Metal(loid)s in chromated copper arsenate (CCA) contaminated soils

Chromated copper arsenate (CCA) is a pesticide widely used as a wood preservative in North America and other parts of the world (Townsend et al., 2003). It has a broad array of applications like marine pilings, fences, utility poles, decks, and playgrounds (Solo-Gabriele et al., 1998). Arsenic, Cr, and Cu are known as environmental toxins, and since they are present in CCA in high concentrations, treated wood with CCA and soil in contact with this wood raises safety concerns (Townsend et al., 2003; Usman et al., 2012). Human exposure pathways include contact with soil

contaminated by wood (Fields, 2001; Townsend et al., 2003). Disposal of wood treated with CCA may also result in contamination of soils with these metals (Tolaymat et al., 2000).

There are reports of soils contaminated with As, Cr, and Cu at locations where wood treated with CCA is installed and in CCA treatment plants. The process to treat wood with CCA as a preservative involves impregnating wood with CCA solution using pressurized cylinders, and contamination may come from spills of the raw chemical, improper disposal of the chemical sludges and dripping of CCA from wood after treatment (Townsend et al., 2003). A study performed by Balasoiu et al. (2001) concluded that soil contamination with CCA needed to go through a corrective process. CCA contains high concentrations of As, Cr, and Cu. For this reason, poor management of the material results in high contamination levels that may pose a risk to human health (Townsend et al., 2003).

The CCA treatment process includes a fixation reaction where the metal compounds bind with the wood. This reaction is dictated by the reduction of hexavalent Cr to trivalent Cr (Cooper et al., 1997; Townsend et al., 2003). However, the metals leach over time to the surroundings when in contact with water (Hingston et al., 2001). Since CCA treated wood is used mainly outdoors where the wood is in contact with the elements and when rainfall comes in contact with the treated wood structures, As, Cr and, Cu may dissolve into the water and travel through the soil (Townsend et al., 2003). Arsenic represents a higher risk than Cr and Cu when found in soil at high concentrations because it is more toxic at lower concentrations than Cr and Cu (Townsend et al., 2003).

CCA-treated wood has been banned or restricted in many countries. For instance, the European union regulates the product under the Biocidal Products Regulation (BPR) since 2013 and can only be placed on the market if it has been authorized by the European Chemicals Agency (ECHA, 2021). In the United States, the use of CCA as a wood preservative has been regulated by the Environmental Protection Agency (EPA) since 1978. In 2003, the EPA revised the CCA registration to include restrictions on the use of CCA-treated wood in certain applications where there may be a risk of human exposure to the arsenic in the wood (USEPA, 2016).

Specifically, the EPA prohibited the use of CCA-treated wood in residential and consumer applications such as playground equipment, decks, and picnic tables. The agency also required that CCA-treated wood used in other applications be labeled with a warning statement indicating that

the wood contains a chemical that can cause cancer and that precautions should be taken when handling and disposing of the wood.

In 2007, the EPA further restricted the use of CCA-treated wood in non-residential applications, such as marine structures, to reduce the risk of environmental contamination.

In addition to the EPA regulations, several U.S. states have enacted their own restrictions on the use of CCA-treated wood. For example, California and New York have banned the use of CCA-treated wood in playground equipment, and other states have placed restrictions on the disposal of CCA-treated wood.

In Canada, the use of CCA-treated wood is regulated by Health Canada under the Pest Control Products Act. The act requires that all pesticide products, including wood preservatives, be registered by Health Canada before they can be sold or used in Canada. The registration process includes a thorough review of the product's safety and efficacy (Health Canada, 2018).

In 2018, Health Canada conducted a second evaluation of CCA-treated wood and concluded that it could be used safely, provided that appropriate precautions were taken. The agency recommended several changes to the conditions of use, including improved labeling and instructions for use, and the establishment of buffer zones around treated wood structures to minimize exposure to humans and wildlife (Health Canada, 2018).

CCA is no longer approved for use in residential and consumer applications in Canada since 2004, due to concerns about the health and environmental risks associated with the use of arsenic.

Nevertheless, many existing structures and former CCA treatment plants are already contaminated and leaching CCA into the soils (Usman et al., 2012). There are three main CCA formulations (Type A, Type B, and Type C). In North America, type C is almost exclusively the only one utilized for wood treatment (Balasoïu et al., 2001). The three formulations include CuO, CrO₃, and As₂O₅ in different proportions. Type A: 18.1%, 65.5%, and 16.4%, Type B: 19.6%, 35.4%, and 45.1%. and Type C: 18.5%, 47.5%, and 34.0% (Cooper et al., 1997). These three metals(loid)s were selected to produce CCA because of their properties. Cu is an excellent fungicide, As an insecticide, and Cr is an efficient binding agent to cellulose by complexation (Chirenje et al., 2003)

2.3 Bioavailability and bioaccessibility of contaminated soils

In recent times, researchers and risk assessors identified the need to measure the bioavailability of contaminants present in the soil to determine with greater accuracy the potential risk associated with humans following exposure.

Only a fraction of the bioaccessible contaminant present in the soil will reach the systemic circulation and be considered bioavailable (Guney et al., 2016; Ruby et al., 1996; Ruby et al., 1999). For this reason, risk assessments that take into account the bioavailable concentrations would yield a more representative result of the exposure since overestimation would be prevented (Rodriguez et al., 1999).

Bioavailability is defined as "The extent to which a chemical can be absorbed by a living organism" (Kelley et al., 2002). Oral bioaccessibility is defined as the fraction that is soluble in the gastrointestinal environment and is available for absorption (Ruby et al., 1999) and related to soil, as the fraction of a contaminant that is released from a soil matrix in the human gastrointestinal system and is available for absorption (Nathanail & McCaffrey, 2003). This definition is analogous to the inhalation pathway. Regarding the dermal pathway, bioaccessibility can be defined as the fraction of a contaminant dissolved in SSFL and is available for penetration through the stratum corneum to the underlying active epidermis (Hillwalker & Anderson, 2014; Marin Villegas et al., 2019; Stefaniak et al., 2014).

Usually, *in vivo* bioavailability of a contaminant present in the soil is difficult to measure in the laboratory. For this reason, *in vitro* bioaccessibility of the contaminant in the soil is used as a surrogate. Although bioavailability can be determined by *in vivo* testing using animals, this practice is time-consuming, expensive and raises ethical considerations inherent in animal testing. However, *in vitro* bioaccessibility tests can be produced at a shorter time, lower cost and without raising ethical considerations inherent to working with animals (Guney & Zagury, 2014; Marin Villegas et al., 2019).

2.3.1 Contaminants in soils

Because of its uses, soils may contain potentially toxic contaminants originated from anthropogenic activities. Some common metal(loid) contaminants, their most usual sources and exposure hazards are summarized in Table 2.1.

Table 2.1. Common inorganic contaminants in soils, their sources and major health hazards.

(ATSDR, 1994; ATSDR, 1995; ATSDR 2007a; ATSDR 2007b; USEPA 2005; USEPA 2011; USEPA, 2014; WHO, 2010)

Contaminant	Source/ activity	Major health hazards
Arsenic (As)	Pesticides, lumber treatment facilities, CCA-treated wood	Cancer in the liver, bladder, skin, and lungs. Decreased production of white and red cells, neurotoxicity, cardiovascular disease.
Chromium (Cr)	Lumber treatment facilities, CCA-treated wood, cement producing plants, leather tanneries	Cancer in liver and kidney, immune and neurological effects.
Copper (Cu)	Lumber treatment facilities, CCA-treated wood, contaminated manure	Liver and kidney damage, gastrointestinal distress.
Nickel (Ni)	electroplating, electroforming, Nickel-cadmium batteries, electronic equipment, cast coins, and Ni alloys r recycling and Ni waste disposal.	teratogenic effects and has carcinogenic potential, decrease Mg, Mn, and Zn levels in different tissues
Lead (Pb)	Leaded paint, leaded gasoline, mining, waste, leather tanning, landfill activities, pesticides, coal-burning	Damage to the nervous system, liver, and kidneys increases blood pressure, anemia, and the risk of miscarriage. In children, behavioural problems, hearing loss, brain damage.
Zinc (Zn)	Mining, use of zing in brass, and bronze, casting metal, rubbers, and paints	Hematology, kidney, pancreas, and gastrointestinal tract damage.

2.3.1.1 Properties of soil influencing metal bioaccessibility

The soil's physical and chemical interactions can dictate metal solubility, migration, and transportation to different environmental components. These interactions depend on the speciation of the metal and the soil characteristics such as organic matter content, pH, particle size and clay content, and cation exchange capacity. Therefore, the bioaccessibility of metals in soils has a high dependency on soil characteristics (Dube et al., 2001; Thornton et al., 1998)

2.3.1.2 Cation exchange capacity (CEC)

Cation Exchange Capacity (CEC) is a helpful technique to evaluate the propensity of soil to capture cations by ion exchange. Conversely, anion exchange capacity (AEC) is used to evaluate the tendency of soil to capture anions. Since the current project deals with the release of metal(loid)s from the soil, it is important to understand the CEC from the soils subject to analysis.

Soil CEC depends on its soil composition, the content of clay minerals and organic matter (Kaiser et al., 2008). Exchangeable cations in the soil can be found on the surface of minerals within their crystalline lattice or bound to organic matter (Chapman, 1965). Minerals in soil capture the ions with their surfaces, and for this reason, clays that have a large specific surface retain the cations well. On the other hand, organic matter has many functional groups on its surface and a complex molecular structure that allows it to retain cations. Solids are created on their surface because of imperfections on the surface of their crystalline lattice or isomorphic substitutions inside it and by the ionization of surface functional groups as a function of pH (Stumm et al., 2012).

2.3.1.3 pH

Soil pH in soils is influenced by land use and ecosystem practices and is one of the main factors of the availability, solubility and bioaccessibility of metal(loid)s (Alloway, 2013; Fomunyam, 2018; Blake et al., 2002; Dube et al., 2001). Variation of pH in soils has significant consequences on the composition of metals. In general terms, an increase of pH may lead to a more negative surface charge and electrical potential on the adsorption sites of Fe and Mn oxides, silicate clays and humus, which may increase the adsorption strength of soil solids for metals. Therefore, a decrease in solubility (Alloway, 2013; Fomunyam, 2018; Blake et al., 2002; Dube et al., 2001). When pH is on the neutral or basic side, complexation is common, and mobilization will be reduced. Alternatively, at low acidic soils conditions, metal partitioning, dissolution, mobility, and

bioavailability will have different values (Alloway, 2013; Fomunyam, 2018; Blake et al., 2002; Dube et al., 2001).

2.3.1.4 Organic matter

Organic matter in soils can be found in the top layer as decomposing vegetal material decays (Fomunyam, 2018). Although organic matter in soils is a relatively small fraction compared to other materials such as clay, the organic fraction has an important role in metal binding (Zimdahl et al., 1977). Soil organic matter in soils is composed of humic and non-humic substances. Humic substances are humic and fulvic acids and humins. It corresponds to the organic matter fraction that has been extensively decomposed and resistant to further alteration (Foth, 1978; Thornton et al., 1998). Most of the organic matter in soils can be associated with inorganic colloids by adsorption of clays (Zimdahl et al., 1977). Therefore, the metals can be retained by organic matter by complexation and adsorption (Thornton et al., 1998).

Organic matter has negatively charged groups responsible for metal binding, and these groups increase during the humification of organic matter (Eriksson, 1989).

Some authors suggested that there is a strong link between soil organic matter content and fixation of metals in the soil in the solid phase (Zimdahl et al., 1977), whereas some other authors found no association (Arienzo et al., 1994; Harter, 1979). This contradiction may be because of the propensity of metals to bind with organic compounds in both solid and solution phases of soil (Thornton et al., 1998). The effect of organic matter on metal mobility has also been studied. The residence time of Pb in soils with a high organic matter content has been calculated as thousands of years (Manceau et al., 1996). This high residence time could be produced by the slow decomposition rates of organic matter (Dörr et al., 1991). A study by Miller et al. (1994) reported an association between the movement of Pb in the soil profile and organic matter of soils. A fraction of the solid phase of organic matter in soils is mobile, and some Pb could have been transported through soils bonded to the organic matter (Thornton et al., 1998).

2.3.1.5 Particle size

Rather than particle size, the effect of soil particle size is best stated in terms of clay, silt and sand fractions (Thornton et al., 1998). The adsorption order for Pb and other metals is clay > silt > sand (Andersson, 1979). Eriksson (1989) reported that Cd was more soluble and bioavailable in soils

with a higher content of sand rather than clay. In another research, extractability of Pb, Zn Cd and Cu by ammonium acetate was higher in soils with a high content of sand and lower loam content (Scokart et al., 1983). In conclusion, there is no general rule regarding how particle size affects interactions with metals, as metals from different groups have different properties and will interact in different ways with the soil fractions.

2.3.2 Speciation of metal(loid)s in soils

Speciation is a term used to specify the physical form of an element, oxidation state, stoichiometry, coordination, and physical state. These properties directly influence an element's behaviour and dictate its toxicity (Reeder et al., 2006). Some metals are essential for life, like Zn or Fe, but can lead to toxicity in excess. Metals like Cd or Pb have no proven beneficial function and could generate health problems even in low levels of exposure (Reed, 1969). Metal speciation is an important factor when assessing toxicity for an array of metals. Inorganic dissolved mercury and methyl mercury are both toxic. However, the properties of methyl mercury make it much more relevant in toxicology since it can pose a higher health risk (Reeder et al., 2006). Another relevant case of speciation in toxicology is Cr. Its hexavalent form is water-soluble, therefore mobile and available for organisms. Hexavalent Cr (Cr VI) is also a known carcinogen (Leal et al., 2018; Reeder et al., 2006). On the other hand, trivalent Cr (Cr III) is insoluble and tends to form hydroxide solids and is considered an essential metal in low concentrations (Langard, 1982; Reeder et al., 2006; Van Lierde et al., 2005).

In real environmental scenarios, it is common to find multiple species of metal coexisting because the different species share some properties, such as the oxidation state. Furthermore, sometimes it can be challenging to determine the species among these (Reeder et al., 2006).

Reactivity and solubility are the main aspects that define speciation control of metals because toxicity is closely related to these two parameters. During an exposure event, the speciation of the metal influences the absorption across the body membrane, and the biological fluids can affect speciation (Reeder et al., 2006). Another point worth considering regarding speciation is that each species might exhibit a different behavior which makes generalizations about mobilization, stability or reactivity of the metal difficult (Reeder et al., 2006).

2.4 Health risk assessment

2.4.1 Exposure assessment

USEPA (2004) defines exposure assessment evaluates the type and magnitude of exposure to a potentially toxic chemical. It considers the source of the chemical released to the environment, how the chemical is transported, and the routes by which humans are exposed. Exposure assessments require using quantitative parameters such as permeability coefficients, soil absorption factors, body surface area exposed, and soil adherence factors. Regarding the dermal pathway, USEPA provides methodologies to perform exposure assessment for two media, water, and soil.

According to the Guidance on Risk Characterization for Risk Managers and Risk Assessors (USEPA, 1992), the most sensitive parameters should be very well-identified. The sensitivity of a parameter refers to its impact on the exposure estimate, and it correlates with the degree of variability of the parameter value (USEPA, 2004). When a parameter has a high degree of variability, values are more likely to significantly impact the range of risk estimates than values with lower variability. Generally, exposure duration, exposure frequency, contact rate, and soil adherence factor are the parameters likely to be more sensitive in dermal exposure assessments.

2.4.2 Estimation of dermal exposure to chemicals in water

USEPA (2004) assumed that skin comprises two layers: the stratum corneum and the viable epidermis. The absorption of substances from water through the skin is described as a function of the thickness of the stratum corneum (l_{sc}) and the duration of the event (t_{event}) (USEPA, 2004). The mass balance equation follows Fick's second law and has concentration as a function of time and distance. The solution of the partial differential equation from Fick's law is approximated by Equation 2.7, which describes the absorption process when a chemical is in the stratum corneum and Equation 2.8, which describes the absorption as a function of t_{event} (USEPA, 2004). An essential assumption by the model is that absorption continues after the exposure ended so that the final absorbed dose DA_{event} can be estimated as a total dose dissolved in the skin at the end of the exposure.

DA_{event} (mg/cm²-event) can be calculated with Equation 2.1 for inorganic compounds.

$$DA_{event} = K_p * C_w * t_{event} \quad (2.1)$$

Where,

DA_{event} (mg/cm²-event) is the absorbed dose per event, K_p is the dermal permeability coefficient of a compound in water (cm/hr), C_w is the chemical concentration in water (mg/cm³) and t_{event} the event duration (hr/event) (USEPA, 2004).

Using the DA_{event} is possible to calculate the Dermal Absorbed Dose (DAD). In the case of the methodology of water (water approach) , DAD can be calculated with Equation 2.2.

$$DAD = \frac{DA_{event} * EV * ED * EF * SA}{BW * AT} \quad (2.2)$$

Where,

DAD is the dermally absorbed dose (mg/kg-day), DA_{event} (mg/cm²-event) is the absorbed dose per event, SA (cm²) the skin surface area available for contact, EV (events/day) is the event frequency, EF is the exposure frequency (days/year), ED (years) exposure duration, BW (kg) body weight and, AT (days) the averaging time.

Permeability coefficients (K_p) also contribute to uncertainty in assessing dermal exposure by the water approach. This uncertainty was identified to be associated with two major factors; the Flynn database, upon which the predictive of the permeability coefficients are derived and includes *in vitro* data from over 90 compounds, and the use of obtained permeability coefficient from *in vitro* studies to estimate *in vivo* dermal exposure at Superfund sites (USEPA, 2004).

2.4.3 Estimation of dermal exposures to chemicals in soil

To calculate the DAD, the standard equation for dermal contact with chemicals in soil is the same equation utilized to calculate DAD in the case of water (Equation 2.1). To calculate DA_{event} , in soils, Equation 2.3 is recommended by USEPA (2004).

$$DA_{event} = C_{soil} * CF * AF * ABS_d \quad (2.3)$$

Where,

DA_{event} (mg/cm²-event) is the absorbed dose per event, C_{soil} (mg/kg) the concentration of the chemical in soil, CF (10⁻⁶ kg/mg) is a conversion factor, AF (mg/cm²-event) the adherence factor, and ABS_d dermal absorption fraction.

The skin surface area (SA) utilized to calculate the DAD refers to the amount of skin exposed to the soil. This amount of skin depends on the exposure scenario. SA estimates used the 50th percentile to correlate with average body weights for different scenarios and pathways (USEPA, 2004). USEPA provides values for SA for different scenarios. The adult resident is assumed to be wearing a short sleeves shirt, shorts, pants and shoes. Skin exposed comes from the head, hands, forearms and lower legs. USEPA (2004) recommends a SA for adult residents of 5700 cm². For the adult in a commercial or industrial environment, the assumptions consider a person wearing long pants instead, so the SA recommended by USEPA is 3300cm². In the case of a child from 1 to 6 years old, they are considered to wear a short-sleeved shirt, short pants, and no shoes. Skin exposure is limited to the head, hands, forearms, lower legs and feet. The recommended SA for child residents is 2800cm². When SA has been selected, site-specific conditions must be properly evaluated. In the case of colder climates, SA can be weighted for different seasons.

The adherence factor (AF) describes the amount of soil that adheres to the skin per unit of surface area (Leal et al., 2018). However, soil properties influence adherence, soil adherence varies across different parts of the body, and soil adherence varies with activity (Holmes et al., 1996). USEPA recommends considering the activity and uses the body part weighted equations (Equation 2.4) to calculate AF. Considering that soil adherence depends on the body part, an overall body part weighted AF must be calculated. The clothing scenario will determine which part of the body AF is used for the calculations. The weighted AF are used with the relative absorption, exposure frequency and duration, exposed surface area, body weight, and averaging time to estimate the DAD (USEPA, 2004)

$$\text{Weighted AF} = \frac{AF_1SA_1 + AF_2SA_2 + \dots + AF_iSA_i}{SA_1 + SA_2 + \dots + SA_i} \quad (2.4)$$

Where,

AF (mg/cm²-event) is the adherence factor of soil to skin, AF_i (mg/cm²-event) the overall adherence factor of soil to skin, and SA_i the skin surface area available for contact for body part 'i.' Values of SA and AF can be found in the "Risk Assessment for Superfund part E, appendix C."

ABS_d is measured experimentally, and Risk Assessment for Superfund part E provides values for some organic compounds and some metals. It is recommended to use an ABS_d of 0.001 when the exact value is unknown (Leal et al., 2018; USEPA, 2004).

Spalt et al. (2009) reviewed the dermal absorption of environmental contaminants from soil. This study highlighted that the protocol for soil presented in the guidance described by USEPA (2004) depends on results from the few empirical investigations of dermal absorption of soil that has been published and does not provide recommendations for all types of contaminants. Spalt et al. (2009) also mentioned in their review that the protocol for water utilizes a more robust model based on regressions derived from in vitro experiments with 90 different compounds and a standard methodology.

For the soil, fewer investigations of dermal absorption have been conducted for chemicals than those from the water. In addition to this, some of the methodologies used in these studies have flaws (Spalt et al., 2009). Because of these issues, comparison across soil studies has been difficult.

Leal et al. (2018) modified the water approach proposed in USEPA (2004) to calculate the DAD. In this study, the chemical concentration in water, was replaced by concentration of metals in sweat. However, the generic values of K_p , pertinent for water were used instead of K_p specific for the synthetic sweat used. This study concluded that the water approach might be useful to calculate DA_{event} since it provides a more realistic result than the soil approach, since the interaction of soil and sweat is simulated.

2.4.4 Toxicity assessment

Direct contact with contaminants can result in direct toxicity at the contact site or systemic toxicity by percutaneous absorption. Toxicity factors ideally should consider the pathway effects and provide dosimetry information and dose-response relationships for systemic effects (USEPA, 2004).

USEPA methodologies lack dermal toxicity factors, but there is an oral to dermal extrapolation for systemic effects. This methodology considers that oral reference doses (RfDs) are expressed as mass of substance administered per unit of time and body weight. In contrast, exposure estimates for the dermal pathway are expressed in absorbed dose (USEPA, 2004). The extrapolation

methodology uses dose-response relationships from oral administration studies and adjusts for absorption effects to represent toxicity factors in terms of dose absorbed (USEPA, 2004)

USEPA provides recommended values for gastrointestinal absorption values (ABS_{GI}). When compounds have no gastrointestinal absorption values, assuming 100% (worst case scenario) is recommended. This assumption contributes to an overestimation of the risk for compounds that are poorly absorbed. The overestimation is inversely proportional to gastrointestinal absorption. This methodology for the adjustment can be used for the assessment using both the soil and water approach.

The reference dose based on absorbed dose is expressed by Equation 2.5, where RfD_{ABS} (mg/kg-day) is the chemical-specific absorbed reference dose, RfD_o (mg/kg-day) the chemical-specific oral reference dose, and ABS_{GI} the compound-specific fraction of contaminant absorbed in the gastrointestinal tract from toxicity studies (USEPA, 2004).

$$RfD_{ABS} = RfD_o * ABS_{GI} \quad (2.5)$$

2.4.5 Risk characterization

Once the dermal absorbed dose (DAD) and the absorbed reference dose (RfD_{ABS}) are calculated using the methodology suggested by USEPA, the hazard index can be calculated using Equation 2.6 (USEPA, 2004).

$$Dermal\ hazard\ quotient = \frac{DAD}{RfD_{ABS}} \quad (2.6)$$

The methodology to estimate exposure and risks from dermal contact is not exempted from uncertainty from various sources. Exposure parameters with a variable distribution are likely to have a higher impact on risk assessment outcomes than those with lower variability. This depends on the receptor scenario and contaminant evaluated (USEPA, 2004). For the soil approach case, the adherence factor and the concentration of a contaminant in the soil are sensitive variables regardless of the receptor. For the water approach, K_p and the concentration of the contaminant in water are sensitive variables.

The bioavailability of chemicals in water depends on the chemical's ionization state (USEPA, 2004); when a chemical is in a non-ionized state, it is more bioavailable than in an ionized form. This affects the accuracy of the estimation of the DA_{event} since the calculation of it is not the sum

of the DA_{event} for the different species of the chemical but instead is based on the C_w derived directly from laboratory analysis and represented the total concentration of ionized and not ionized species and does not provide necessary information to calculate DA_{event} separately for different species (USEPA, 2004).

2.4.6 Bioavailability in health risk assessment

Health risk assessment consists in mainly four steps: (1) hazard identification, (2) dose-response assessment, (3) exposure assessment, and (4) risk characterization and is related to the evaluation of the likelihood of adverse human health effects caused by contaminants present in the environment (Chen, 1994).

Exposure assessment includes the characterization of the exposure settings, identification of exposure pathways and quantification of the exposure (USEPA, 1992). Exposure and dose are the main parameters to consider for toxic responses. Dose-response relationships are the core of health risk assessment, and it mainly relies on the accurate estimation of the exposure and effective dose (Chen, 1994). Exposure is the total concentration of a contaminant at the exposure point over time (Lioy et al., 1990). Dose refers to the total mass that crosses the skin layer and is absorbed or deposited into the body. It will eventually reach the target sites over a certain period (Lioy et al., 1990). Another essential term that must be well defined is the biologically effective dose, which is defined as the delivered mass of an active toxic species at the site of action and includes the pharmacokinetic fraction, bioavailability factor and contact rate (Lioy et al., 1990).

The biologically effective dose is an important parameter when the dose-response relationship is being evaluated. It can be estimated by biological monitoring (*in vivo*) or mathematical modelling based on physicochemical properties of contaminants and physiological parameters (Chen, 1994). However, the biologically effective dose is not commonly used for quantitative risk assessment because of its practicality since it is difficult to measure in the human body and animals, and databases for this dose to be used in mathematical modelling are not always available for most of the environmental contaminants. In addition to that, it cannot be traced directly to determine the source of environmental contaminants or to identify the exposure pathways (Chen, 1994).

Alternatively, the internal dose has been used to determine the dose-response relationship since the internal dose is related to the occurrence of toxic effects for many contaminants (Chen, 1994).

Quantification of internal dose involves calculating the average daily internal dose, which is the internal dose per unit body weight over an average time. The bioavailability factor has been used to evaluate the internal dose and the average daily internal dose in risk assessment. The internal dose can be defined using the average concentration of an environmental contaminant, the contact rate and exposure duration.

For the dermal pathway, contaminants soluble in water in contact with the skin can potentially penetrate the skin barrier and reach the systemic circulation. In this case, the internal dose is dependent on the concentration of contaminants in water or SSFL.

2.5 Skin surface film liquids (SSFL)

Chemicals in contact with human skin surface liquids (sweat and sebum) could cause adverse health effects such as irritation, dermatitis or find their way to the bloodstream and contribute to systemic intake (Stefaniak et al., 2006). Dissolution of metal(loid)s in direct contact with human skin can be estimated using artificial SSFL, and *in vitro* methods can be used to estimate *in vivo* measurements. Researchers used many different artificial SSFL formulations for different applications. From these formulations, only a few included sebum constituents. To reproduce dissolution of metals correctly using *in vitro* modelling, the appropriate surrogate for SSFL is required (Wertz, 2008).

Constituents of artificial SSFL play an essential role in the dissolution of metal(loid)s, highlighting the importance of a proper characterization of the *in vitro* test system. The SSFL should accurately match the properties of SSFL *in vivo* (Flint, 1998). A better understanding of metal(loid) bioaccessibility would improve the data to perform a risk assessment and the decision-making process from adverse health effects caused by dermal contact of metal(loid)s (Stefaniak et al., 2006).

Human skin surface film liquids are a mix of 50% sweat and 50% sebum. Sweat is secreted by glands consisting of two parts: duct and coil. Sweat secreted is the product of the active secretory function of epithelial cells that line the coiled portion of the gland (Agache et al., 2004).

Sebum is secreted by pilosebaceous units, consisting of a sebaceous gland connected to a hair follicle by a sebaceous duct (Agache et al., 2004). Sebaceous glands are present in all the skin in different concentrations except in the palms of hands and soles of feet (Zouboulis, 2004). The hair

follicles secrete sebum produced by the sebaceous glands onto the surface of the skin and then is mixed in the skin with epidermally derived lipids forming the sebum film (Agache et al., 2004; Stefaniak et al., 2006)

2.5.1 Sweat

The composition of sweat is highly variable depending upon body weight, age, diet, season, acclimation degree, activity level, gender, race and sampling technique (Stefaniak et al., 2006). Sweat is constituted by a variable number of electrolytes, ions, organic acids, carbohydrates, amino acids, nitrogenous substances, and vitamins. Sweat is > 99.0% water and 1% solids and has a specific gravity of 1.001 (Agache et al., 2004).

Stefaniak et al. (2006) studied the reported compositions of human sweat and compared them with the different formulations of synthetic sweat used in different fields such as pharmaceutical and risk assessment. Stefaniak et al. (2006) concluded that many of the formulations utilized to mimic human sweat lack many of the main constituents commonly found in human sweat or have the constituents in a different proportion to what can be found in reality.

The primary concentrations of electrolytes in human sweat are sodium, chloride, and in lower concentrations, calcium, potassium, magnesium, phosphate, and hydrogen carbonate (Chopra et al., 1940). However, the concentration of electrolytes has a high variability of several orders of magnitude (Robinson et al., 1954). Human sweat contains a highly variable of ions which includes sulphate, sulphur in a higher concentration and fluoride (F), phosphorus (P), bromide (Br), cadmium (Cd), copper (Cu), iodine (I), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni), and zinc (Zn) in lower but quantifiable concentrations (Chopra et al., 1940; Robinson et al., 1954; Stefaniak et al., 2006).

Regarding organic acids and carbohydrates, lactic acid and pyruvic are the most common in sweat in concentrations averaging 0.28% (Robinson et al., 1954). There are 16 reported amino acids in human sweat: alanine, arginine, aspartic acid, citrulline, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, ornithine, phenylalanine, threonine, tryptophan, tyrosine and valine (Stefaniak et al., 2006). Ammonia, uric acid, creatinine, and creatine are the nitrogenous substances that can be found in human sweat (Chopra et al., 1940; Stefaniak et al., 2006).

Measured values of pH from sweat have a median of 5.3 (Stefaniak et al., 2006). However, different studies reported dramatic differences, from a very acidic pH of 2.1 (Haudrechy et al., 1993) to an alkaline of 8.2 (Altman et al., 1974). It has been demonstrated that sweat pH can vary during a sweating period and change by body region (Robinson et al., 1954).

2.5.2 Sebum

Human sebum is a non-polar lipid substance composed of squalene, wax esters, triglycerides, free fatty acids, and cholesterol esters (Abrams et al., 1993). At human skin temperature, sebum has a specific gravity of 0.92 and behaves both as a solid and a liquid (Abrams et al., 1993; Rothman, 1954). Sebum forms a thin film of a thickness of $<0.05\ \mu\text{m}$ in poor sebum areas and $>4\ \mu\text{m}$ in rich sebum areas such as in the face (Sheu et al., 1999). Sebum secretion varies among the body parts and climatic season and age (Agache et al., 2004; Youn et al., 2005). The composition of sebum may vary between people but is constant among each person (Agache et al., 2004; Rothman, 1954).

Stefaniak et al. (2006) reviewed the reported constituents of human sebum and the formulations of synthetic sebum used in research until 2006. There is high variability in the proportions of the sebum constituents between studies. Stefaniak et al. (2006) concluded that a comprehensive synthetic sebum model needs to represent the human sebum adequately and include all main constituents at the proper proportions.

Squalene is one of the principal components of sebum, with a median of 10.6 % (Abrams et al., 1993; Agache et al., 2004; Stefaniak et al., 2006). Wax esters constitute around 25% of human sebum and are long-chain fatty alcohols of sebaceous wax-like carbons from the carboxyl group (Spector, 1956; Stefaniak et al., 2006). Triglycerides are neutral fats secreted by hair follicles to the skin's surface (Rothman, 1954). In sebum, both saturated and unsaturated triglycerides in a ratio of 1:2. Lipases can hydrolyze triglycerides to form diglycerides and free fatty acids (Motwani et al., 2001). Reports of Triglycerides found in sebum showed a median of 32.5%, ranging from 14.8% to 44% of total sebum content (Stefaniak et al., 2006). Free fatty acids are formed on the skin surface by bacterial enzymatic hydrolysis of triglycerides (Agache et al., 2004; Rothman, 1954; Stefaniak et al., 2006). It is believed that these acids are responsible for the low natural pH of the skin (Stefaniak et al., 2006). The free fatty acids in sebum are typically formed by 16 carbon chains and can either be saturated or unsaturated. Around 21 different lipids form 87.55% of fatty acids in the skin; the other 12.45% is composed of around 200 different fatty acids. The more common are

palmitic acid, stearic, oleic, and linoleic (Stefaniak et al., 2006). The mean reported percentage of free fatty acids in human sebum is 28.3% ranging from 2.3% to 38.3% (Reed, 1969). In a lower proportion, cholesterol esters are found in human sebum. They are produced by skin cell degeneration, sebaceous gland production and esterification of cholesterol to cholesterol-esters by skin bacteria (Downing et al., 1987). The median reported value for cholesterol esters is 2% (Agache et al., 2004).

2.5.3 Synthetic sweat formulations

Due to the complexity of human SSFL, artificial compositions often lack many of their constituents. However, Harvey et al. (2010) developed a comprehensive model of synthetic sweat that matches human composition as it includes a complex formulation. However, preparing this artificial sweat requires time and costs not associated with simpler formulations containing only the main constituents of sweat. Numerous studies used different formulations of artificial sweat and sebum. The most commonly used is the formulation specified in the European Standard EN 1811. This formulation is inexpensive and relatively easy to prepare. However, it has its limitations as it lacks amino acids, which could be essential in the dissolution of some metals (Brown et al., 1982) and other constituents.

Midander et al. (2016) compared metal release in two different sweat compositions; the EN1811 and the comprehensive formulation described by Harvey et al. (2010). This study concluded that the metal release from a white gold alloy and some hard metal materials were within the same range after immersion in the two different artificial sweat solutions. So, in this particular case, the simpler and more cost-effective test using EN 1811 synthetic sweat provides results that satisfactorily indicate the metal release in sweat (Midander et al., 2016). However, this study was limited to specific metals and materials. There is still a need to understand how the differences in SSFL composition can influence the behaviour of soil-bound metal(loid)s.

2.6 Dermal permeation

2.6.1 The skin as a permeate layer

The skin is the largest organ in the human body and functions as a protective barrier from the external environment. It is also crucial for physiologic processes like allergic immune responses, vitamin D production and regulation of temperature (Beriro et al., 2016; Jung, 2000). The skin is

composed of two main layers, (1) dermis, which acts as the inner layer, and (2) epidermis, the external layer (Jung, 2000; Tortora et al., 1988). Because the skin is composed of proteins and lipids, it has both hydrophilic and lipophilic properties. Due to this particularity, the skin functions as a selective barrier to exogenous to protect the body or act as a media to absorb substances to the metabolism (Jung, 2000).

The dermis is constituted by fibroblasts which produce structural proteins, collagen, and elastin. Collagen provides support, and elastin is responsible for the skin's resilient and flexible properties (Jung, 2000). There is also a network of blood vessels in the dermis, which can mobilize compounds from the dermis other organs inside the body. The blood circulation in the dermis also has a thermal regulatory function for the entire body (Jung, 2000). Other cells found in the dermis are the Mast cells, responsible for histamine release as an allergic response (Jung, 2000).

The epidermis is a stratified layer constituted by skin cells and keratinocytes. The principal layers of the epidermis are the stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum (Jung, 2000). The outer layer, the stratum corneum (SC), is often studied as a separate layer from the epidermis due to its importance and unique properties. Other cells in the epidermis include Langerhans cells which are essential for immune response, and Merkel cells, involved in sensory perception (Jung, 2000).

The SC typically is 10-20 μm thick and is formed by 15 to 25 layers of keratinocytes. The keratinocytes are often replaced with new cells through continuous regeneration coming from the basal layer (Jung, 2000). Cells in the SC are typically composed of 70% keratin and 20% lipids.

There is a wide array of compounds in contact with the skin for various reasons. Some of this may be benign, or others could cause skin irritation, allergies or contribute to the body burden of the compound. It is critical to perform studies to help understand the fate of different compounds and chemicals in contact with skin. Absorption studies can help understand the potential changes in distribution and localization and the modification of molecules leading to toxicity (Jung, 2000). The skin performs a biotransformation role of endogenous and exogenous compounds (Jung, 2000; Krishna et al., 1994). It has been demonstrated that the skin performs metabolic functions to toxic molecules (Merk et al., 1996).

2.6.2 Dermal absorption

The absorption of substances through skin happens by dissolution and molecular diffusion through the skin in a protein-lipid interaction (Beriro et al., 2016; Michaels et al., 1975). There are three dermal absorption pathways: (1) intracellular bulk pathways, (2) Intercellular bulk pathways, and (3) Shunt pathways (Beriro et al., 2016; Price et al., 2008). The most important route among the three pathways of dermal absorption is the intercellular pathway. The lipids facilitate this pathway in the stratum corneum (Beriro et al., 2016; Michaels et al., 1975).

Percutaneous absorption can be defined as the process where a chemical penetrates the skin to reach the systemic blood supply. This definition differs from dermal absorption, which includes the chemical remaining in the skin depot that is not considered bioavailable systematically for the duration of the exposure period. However, the depot may become bioavailable at extended exposure periods (Moody et al., 2007).

Two factors determine the dermal bioavailability of a contaminant in soils: (1) transfer of the contaminant from the vehicle and (2) the capacity for penetration of the contaminant through the skin (Pugh et al., 2008). Both processes need to occur for contaminants to be absorbed into the skin and be available for systemic circulation. However, just the presence of the contaminant in the skin can create localized effects (Beriro et al., 2016). Because the sole presence of a contaminant can cause localized effects, bioavailability in dermal exposure should be defined as a substance “absorbed into any part of the epidermis” rather than only the fraction available to systemic absorption (Beriro et al., 2016).

Depending on the dosage and the physio-chemical properties of the substance being absorbed by the skin, exposure may be under equilibrium or non-equilibrium conditions (Beriro et al., 2016). There is a maximum concentration of a substance that the skin can absorb. This is relative to time and is referred to as penetration rate or flux (J) ($\text{g}/\text{cm}^2\text{h}$) (Beriro et al., 2016; Pugh et al., 2008). This flux happens in a theoretically unlimited supply of the substance, referred to as the infinite dose. If the amount of substance available limits the exposure scenario, the exposure is instead referred to as the finite dose (Beriro et al., 2016). In order to calculate kinetic parameters such as flux (J), the permeability coefficient (K_p) and diffusion coefficient (D), the infinite dose approach is preferred. For chronic exposure to soil, the finite dose should be used instead (Beriro et al., 2016;

Jeffries et al., 2009). After a lag phase (T_{lag}), steady-state flux is reached (J_{ss}). The lag face is graphically measurable as the intercept in x.

Furthermore, the infinite dose across the skin or a permeable membrane can be expressed mathematically by Fick's First Law of diffusion (Equation 2.7 and Equation 2.8), where J is the flux, dc is the concentration gradient, and dx is the distance travelled and D the diffusion coefficient. J_{ss} is the steady-state flux per unit area, K_p is the permeability coefficient in distance and time for a given compound in soil, and C_o is the concentration of the compound in soil. K_p is also known as K_s /soils or skin/soil partition coefficient (Beriro et al., 2016).

$$J = -D \frac{dc}{dx} \quad (2.7)$$

$$J_{ss} = K_p * C_o \quad (2.8)$$

The finite dose can be expressed mathematically by Fick's Second law of diffusion (Equation 2.9), where Q is the amount of compound absorbed, J_s the flux through the epidermis, A the area of application, lag is the time between application and absorption, and T is the exposure time. (Beriro et al., 2016; Crank, 1975).

$$Q = J_s A (T - lag) \quad (2.9)$$

Properties such as molecular weight, solubility, charge, and hydrogen bonding affect the dermal bioavailability of contaminants (Brain et al., 2008). The solubility of a chemical is determined by its partition coefficient. This is the proportion of the substance dissolved in an immiscible liquid mixture, usually octanol and water. The log of the concentration ratio in the two liquids can be used to calculate the partition coefficient $\log K_{ow}$. Chemicals with this coefficient between 1 and 3 are considered to penetrate skin and entry system circulation. Higher values will remain in the lipids of the SC, and lower values will fail to penetrate the SC (Beriro et al., 2016; Brain et al., 2008).

2.6.3 Franz diffusion cell system

The Franz diffusion cell method (Franz, 1975) has been used since 1975 to assess skin permeation. This method was initially developed to be used by the pharmaceutical and cosmetics industry to determine penetration rates and absorbed fractions of transdermal medications (Franken et al., 2015). However, the Franz cell has been adapted to be used in other fields such as occupational toxicology. It helped quantify the absorption of metals and other toxins through the skin. Generally,

the apparatus consists of two compartments (donor and receptor) with a permeable membrane clamped in between. Areas exposed to the membrane in the Franz cell may vary from 0.55 to 10 cm² (Franken et al., 2015). The receptor solution temperature typically ranged between 32 and 37 °C, with 32 °C corresponding to the skin's surface and 37 °C to body temperature beneath the skin (Franz, 1975). During the experiment, the receptor solution can be tested at different times to measure the content of the studied substance. Once the assay reaches completion, the membrane can be digested to measure the content of the substance studied that was accumulated. Figure 2.1 shows a typical experimental setup of a Franz diffusion cell. The receptor is recommended to be filled with a solution that contains salt concentrations similar to what is found in blood and a pH of 7.35 (Larese Filon et al., 2013). For studies related to toxicity and exposure, it is recommended to use synthetic sweat as the donor with pH ranging between 4.5 and 6.5 (Filon et al., 2009; Larese et al., 2007). The diffusion assay using a Franz cell can produce results that can be reported as flux or permeability coefficient; lag time; total cumulative diffused; or percentage diffused and the total mass retained in membranes or percentage retained (Franken et al., 2015)

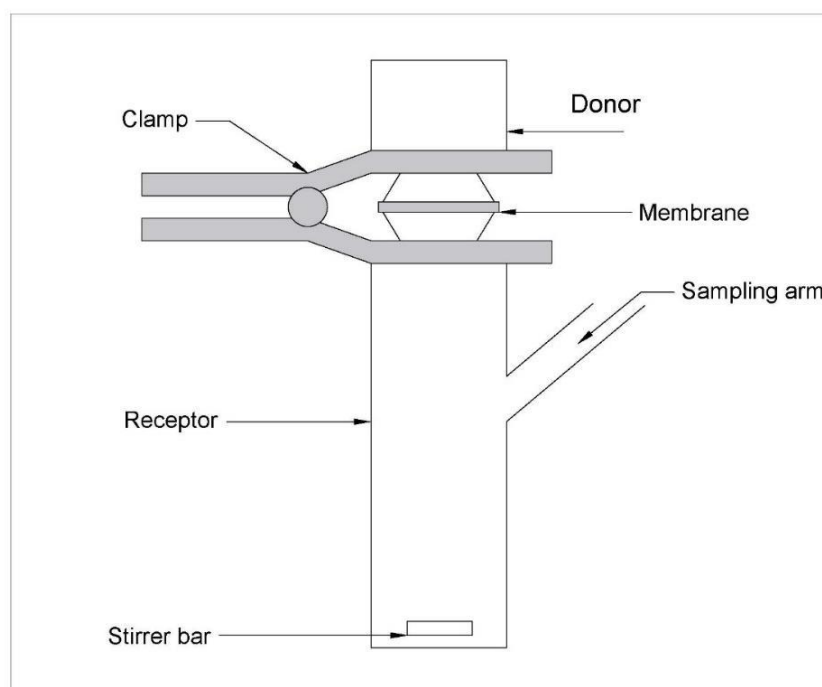


Figure 2.1 Schematic of Franz cell

2.6.3.1 Membrane properties for permeation *in vitro* studies

Over the years, different membranes have been used for diffusion studies in pharmacology and toxicology. According to the OECD and USEPA guidelines, human skin is the “gold standard” (Beriro et al., 2016). However, human skin is not always available and could present a high variability (Haq et al., 2018). Many studies used animal skin such as pig, rat and guinea pig as a human skin surrogate (Moody et al., 1995). There is an increasing interest in skin substitutes instead of *ex vivo* animal skin. There is an emerging trend of synthetic skin products commercially available and evaluated by the scientific community (Netzlaff et al., 2005; Schäfer-Korting et al., 2008). In recent years, due to ethical considerations, the *in vitro* approach is preferred by many scientists over *in vivo*, especially using synthetic membranes. Furthermore, animal and human skin have limitations such as its associated cost and availability in addition to the high variability inherent to biological membranes (CV=72%) that makes the design, statistical significance, and ability to compare results complicated (Barbero et al., 2009; A Haq et al., 2018).

2.6.3.2 Synthetic membranes for permeation studies

It has been demonstrated that *in vitro* experiments can closely predict *in vivo* absorption rates (Sartorelli et al., 2000). However, human skin is not always available and can have high variability due to skin thickness variations from donors, skin states, storage conditions, hair density, and donor age (Mathes et al., 2014). Animal skin shows a low correlation with human skin in permeation studies and could be considered ethically problematic. Synthetic membranes have been studied mainly in pharmacology to simulate penetration *in vivo* due to their advantages, like controlled thickness, preparation time, cost, and storage (Haq et al., 2018).

Haq et al. (2018) compared the permeability of two synthetic membranes (Cellulose Acetate and Start-M) and human skin. In this study, a static vertical Franz diffusion cell with a donor area of 0.64 cm² and a receptor volume of 5 mL was used. Skin from the leg of three different donors was employed to compare permeation with the two synthetic membranes. The receptor fluid used was Phosphate-buffered-Saline (PBS) with a pH of 7.4 maintained at 37 °C. At the beginning of the experiment, 100 µL of saturated solutions of Diclofenac sodium, hydrocortisone and Caffeine were added to the donor. 500 µL were sampled from the receptor every hour for 8 hours for further analysis.

Cumulative permeation of caffeine from caffeine patch and solution are shown in Figure 2.2 and Figure 2.3. A significant variation was found between permeability from skin samples and CA membranes, while the variation from Strat-M was low. The permeation rate of caffeine for the membranes tested by Haq et al. (2018) was CA > Strat-M > Human skin.

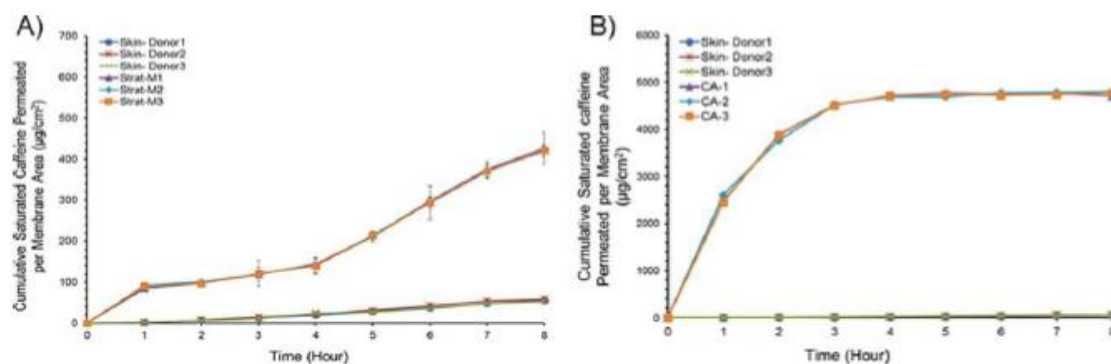


Figure 2.2 Comparison of 8-h permeation of saturated caffeine in PG through human cadaver skin samples and synthetic membranes. A) Strat-M and human skin; B) CA and human skin. (Haq et al., 2018)

Artificial membranes used to model skin permeation should mimic the stratum corneum as close as possible (Haq et al., 2018). Even though CA membranes have been used to model skin diffusion properties for some time (Montenegro et al., 2006), the pore size of CA is larger than human skin pores causing a higher permeation. However, Strat-M comprises two layers impregnated with synthetic lipids producing results closer to natural human skin (Haq et al., 2018). Strat-M does not just correlate better with human skin than CA. However, it also has insignificant permeation variability between replicates, therefore suited to be used in early-stage diffusion studies for topical and transdermal modelling (Haq et al., 2018).

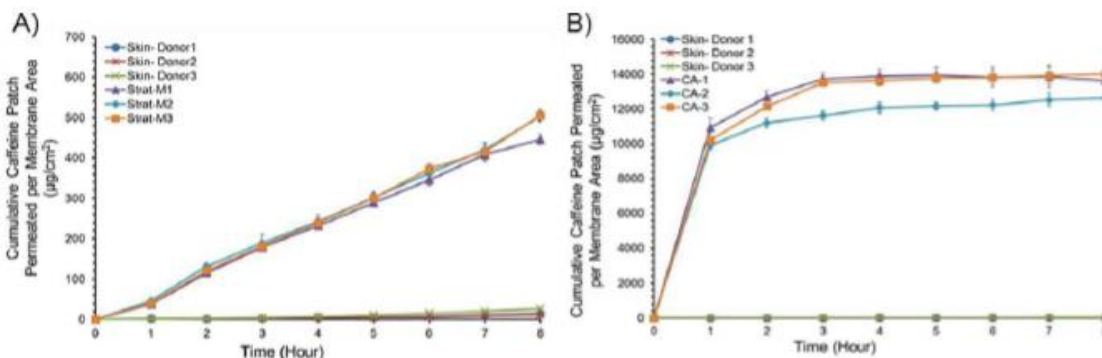


Figure 2.3 Comparison of 8-h permeation of caffeine patch through human cadaver skin samples and synthetic membranes. A) Strat-M and human skin; B) CA and human skin (Haq et al., 2018)

In another study from the same group, the correlation between human skin and the synthetic membrane Strat-M was investigated. Haq et al. (2018) used the set-up previously described, and formulations containing 1% nicotine and different diffusion enhancers were prepared.

Strat-M showed a high correlation flux to human skin for nicotine with an $R^2=0.99$. Figure 2.4 shows the correlation.

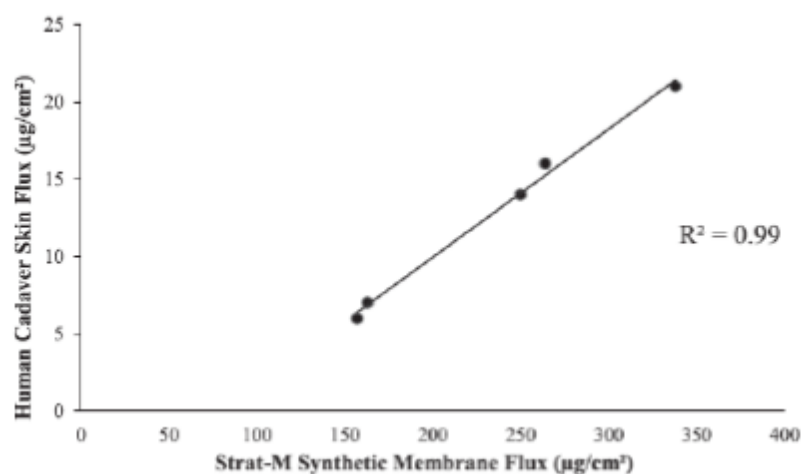


Figure 2.4 Correlation of flux between Strat-M synthetic membrane and human (Haq et al., 2018)

In another study, Joshi et al. (2012) compared the synthetic membrane Strat-M treated with a mixture of synthetic lipids to mimic the lipids in human skin for transdermal diffusion with human cadaver skin and animal and different drugs.

Joshi et al. (2012) used a vertical Franz cell to conduct the study. A saturated solution with active pharmacological substances (Aspirin, nicotine, and hydrocortisone) was added in the donor

compartment. The receptor chamber was maintained at 37 °C and filled with PBS at a pH of 7.4. The permeation experiment lasted 24 hours. Five hundred microliters samples from the donor were collected at 0, 1, 2, 3, 4, 5, 6, 8, 10, 24 and 26 hours. Every time a sample was made. The volume was replaced in the receptor.

It was reported by Joshi et al. (2012) that in the cases where the membrane Strat-M was treated with synthetic lipids. It showed a closer correlation to human skin than the untreated membrane. Figure 2.5 shows the diffusion of the drugs tested through Strat-M and various animal and human cadaver skin models. Results by Joshi et al. (2012) expose the difficulties of correlating animal studies with diffusion in human skin. In most cases, the correlation between the treated synthetic membrane Strat-M and human skin is better than the animal models. This study also reported the high lot-to-lot reproducibility of shelf life as opposed to human skin (Joshi et al., 2012). It was concluded that the synthetic membrane could be used instead of human or animal skin for *in vitro* percutaneous studies. It provides meaningful and reproducible data about permeation characteristics of a wide arrange of compounds.

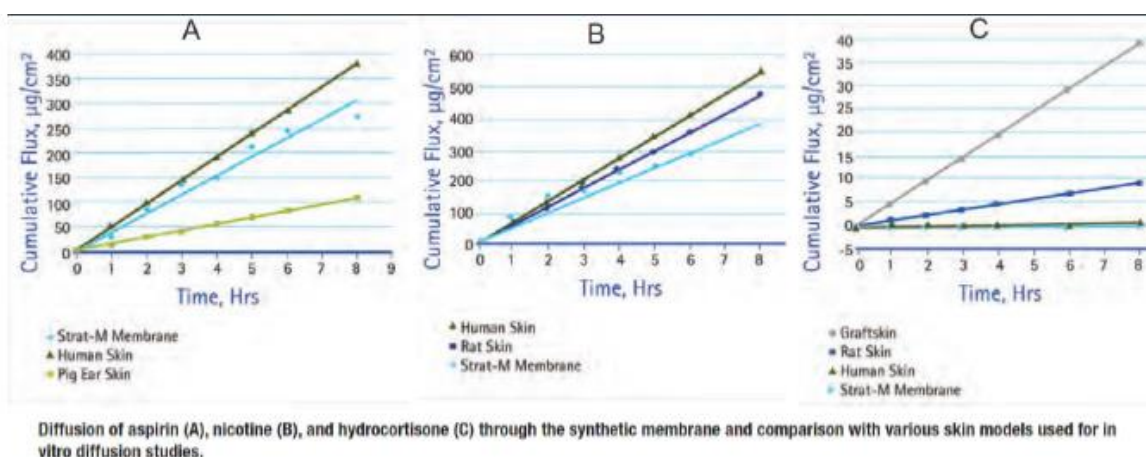


Figure 2.5 Diffusion of aspirin (A), nicotine (B), and hydrocortisone (C) through the synthetic membrane and comparison with various skin models used for in vitro diffusion studies (Joshi et al., 2012)

To conclude, even though synthetic membranes have been successfully tested to model skin permeation, the studies are specific for certain substances. Further research regarding metal(loid)s from geological materials is missing.

2.6.3.3 Donor phase for permeation *in vitro* studies

The exposure of contaminants in toxicology studies is usually performed using diffusion Franz vertical cells. The substances studied can be applied pure or using the same vehicle used in industrial exposure to reproduce a real-life scenario. In percutaneous tests, the surface of the skin or membrane is typically open to the environment, and thin layers of material can be applied alone or in vehicles relevant to *in vivo* exposure, like synthetic sweat. The Franz cell method permits liquid, solid or gel as donor phase (Sartorelli et al., 2000).

It has become clear by pharmaceutical research that the flux of a given molecule across the skin barrier is a function of two effects: Thermodynamic activity of the molecule in the donor phase and alteration of the barrier properties of the skin by the vehicle (Kadir et al., 1988; Sartorelli et al., 2000). For this reason, the donor phase design must consider the exposure scenario that the assay is representing.

The parameters that must be controlled of the substance used in the donor phase include the concentration of chemical studied, vehicle composition and concentrations (synthetic sweat), time of exposure, the amount applied (finite dose or infinite dose), and diffusion area (Sartorelli et al., 2000).

For percutaneous metal(loid) absorption, synthetic sweat should be used as the vehicle in the donor phase since it has been proved that the properties of sweat can influence the bioaccessibility of metal(loid)s (Larese et al., 2007; Leal et al., 2018; Stefaniak et al., 2011). Previous studies also used water, emulsions, and ointments as vehicles in the donor compartment (Franken et al., 2015).

2.6.3.4 Receptor fluids for permeation *in vitro* studies

The receptor fluid represents one of the significant decisions in designing *in vitro* percutaneous penetration systems (Sartorelli et al., 2000). The fluid of choice will collect the material absorbed through the membrane, and it should represent the *in vivo* blood circulation and give an absorption profile (Sartorelli et al., 2000).

The receptor fluid is under the membrane at a fixed volume. It must be replaced with time to define an absorption profile. It is critical to ensure that the receptor fluids do not get saturated because they have to act as a sink for the material absorbed (Sartorelli et al., 2000). The receptor fluid should be in constant stirring to ensure the mixing of the chemical. A principal requirement of the

receptor fluid is to ensure the penetrant must be sufficiently soluble in the fluid, so diffusion is not limited (Larese et al., 2007; Sartorelli et al., 2000).

The receptor fluid must be picked about the function of the diffusion system and the membrane used. When human or animal skin is used, the receptor fluid must ensure the viability of the skin (Franken et al., 2015). The receptor compartment temperature typically ranged between 32 °C and 37 °C, corresponding to skin temperature and body temperature respectively, pH is usually around 7.35 and NaCl concentration of 0.9%, corresponding to blood levels (Larese et al., 2007).

Different receptor fluids have been used for percutaneous penetration of metal studies: water, NaCl solutions, physiological solutions, and buffered saline solutions (Franken et al., 2015). Additives such as surfactants, antifungals, and antibiotics are usually added when working with human or animal skin to ensure the viability of the biological tissue (Sartorelli et al., 2000).

Larese et al. (2007) performed *in vitro* absorption studies using full-thickness skin. They reported slow permeation rates of Ni powder through the skin and Ni ions released from synthetic sweat oxidation. Larese Filon et al. (2013) also concluded in the following studies that Ni has significantly higher permeation through damaged skin than intact skin.

Moody et al. (2009) studied dermal permeation of Ni-63 in soil and without soil using human skin from breast and reported high absorption product to a high percentage of Ni retained by the skin. The results by Moody et al. (2009) differ from the results from Hostýnek (2003). Hostýnek (2003) reported a much lower permeation and higher retention in the skin. Moody et al. (2009) suggested that the difference in results is due to the appendageal shunt route as human breast skin used by Moody et al. (2009) has fewer hair follicles (A. Franken et al., 2015).

2.6.3.5 Evaluating permeability coefficient k_p from *in vitro* assays

By using *in vitro* permeation methodologies, permeability coefficients can be measured directly (EPA, 1992). The permeability coefficient K_p characterizes the barrier property of a permeable membrane. Nevertheless, membranes and particularly skin, are very complex. However, permeability coefficient values across the membrane can be accurately measured using Fick's law and *in vitro* diffusion studies. As previously exposed, the membrane (human skin, animal skin, synthetic skin or other) is “sandwiched” between the donor and receptor containers, and both are filled with media (donor and receptor). Since the membrane separates the donor and receptor, the

solute mobilizes by diffusion from the higher concentration medium (the donor) to the lower concentration medium (the receptor). Then, it is possible to determine the permeability coefficient of a solute; the concentrations in donor and receptor are measured as a function of time (EPA, 1992).

Some assumptions must be made in order to use Fick's first law. The central assumption is that the flux across the membrane reaches a steady-state quickly (Beriro et al., 2016; EPA, 1992). A steady-state is reached when the concentration in both donor and receptor are constant with time, and flux across a membrane is constant, so physically, this means that the volumes of the donor and receptor are much bigger than the volume of the membrane (EPA, 1992). Although the concentrations are time-dependent in the donor and receptor, Fick's first law of diffusion describes the resulting steady-state flux across the membrane.

If the mass balance in donor and receptor are considered:

$$V_{donor} \frac{dC_{donor}}{dt} = -A J_{ss} \quad (2.10)$$

$$V_{receptor} \frac{dC_{receptor}}{dt} = A J_{ss} \quad (2.11)$$

Where,

A is the surface area of the membrane. Since $V_{donor} = V_{receptor} = V$ and C_{donor} and $C_{receptor}$ can be measured in function of time, the permeability coefficient K_p can be calculated with Equation 2.12:

$$K_p = \frac{(slope) \left(\frac{V}{A}\right)}{C_{donor} - C_{receptor}} \quad (2.12)$$

Where,

the slope is the absolute value of $dC_{receptor}/dt$, the gradient of the linear part of the plot of C_{donor} or $C_{receptor}$ versus time (EPA, 1992).

Also, combining Equations 2.12 and 2.13, and using the initial condition in $t=0$, $C_{donor} - C_{receptor} = C_{donor}^0 - C_{receptor}^0$, it can be expressed as:

$$K_p = \frac{V}{2At} \ln \left(\frac{C_{donor}^0 - C_{receptor}^0}{C_{donor} - C_{receptor}} \right) \quad (2.13)$$

C_{donor} or $C_{receptor}$ have measured concentrations at time t. Many researchers have used this approach using equation 2.12 to estimate K_p (EPA, 1992).

Flick's first law analysis assumes that the membrane behaves homogeneously with an average value for the diffusion coefficient, partition coefficient, and diffusion path length, and metabolism does not occur (EPA, 1992). Therefore, the measured permeability coefficient measured in skin contains many assumptions that are intrinsic to Fick's first law. As previously exposed, the skin is a very complex organ, and percutaneous absorption *in vivo* contains many more considerations often neglected by *in vitro* systems (EPA, 1992).

The nature of the chemical to which the skin is exposed and their interaction may largely determine the suitability of the assumptions to calculate the permeation coefficient. However, Fick's first law can be used to provide reasonable estimates of permeability coefficients under steady-state diffusion. However, results should be carefully evaluated and compared, understanding the context of the exposure modelled and adequately defining the conditions of the assay (EPA, 1992).

2.6.3.6 Estimation of absorbed dose

As mentioned in the previous section, K_p describes the barrier properties of a membrane, and, to be calculated, steady-state experimental conditions are necessary. However, in real-life exposure scenarios, time is required after initial contact with the skin to achieve a steady-state condition. The initial unsteady state period is a function of the lag time. Figure 2.6 shows the cumulative amount of chemical flux out of a membrane as a function of time.

For risk assessment purposes, all the systemically amount of chemical that becomes available over the exposure time should be considered as studies indicate that this value is better predicted by the amount of contaminant which has entered the skin than by the amount which has been absorbed by the skin and entered the bloodstream during the exposure period (EPA, 1992).

During the lag phase, the contaminant entering the skin can be greater than the amount exiting the skin, causing accumulation in the skin layers. Steady-state starts once the concentration gradient across the barrier is constant and the rate of the contaminant entering and exiting the skin is the same (EPA, 1992). For this reason, a conservative estimate of the total exposure should consider the amount of contaminant crossing the skin and not the amount that reaches the body for systemic accumulation. Chemicals absorbed into the Stratum corneum will remain there until they flux into the deeper tissue layers unless there is a loss of contaminant by metabolism, binding, evaporation, or desquamation (EPA, 1992).

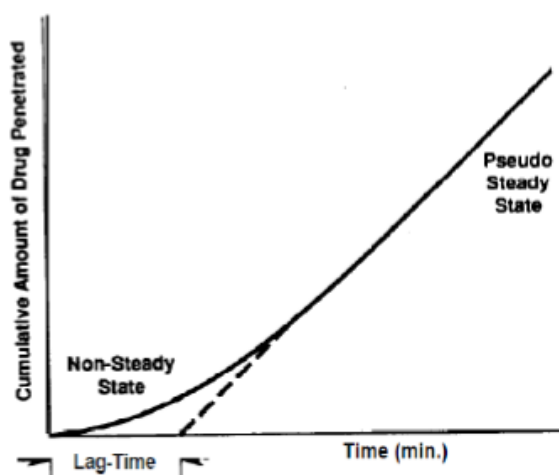


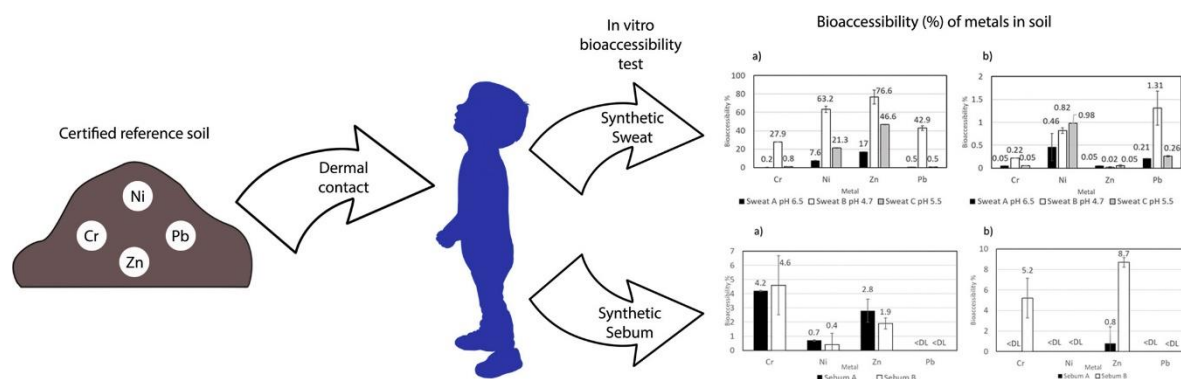
Figure 2.6 Cumulative amount of chemical fluxing out of the stratum corneum as a function of time (EPA, 1992)

CHAPTER 3 ARTICLE 1 - COMPARISON OF FIVE ARTIFICIAL SKIN SURFACE FILM LIQUIDS FOR ASSESSING DERMAL BIOACCESSIBILITY OF METALS IN CERTIFIED REFERENCE SOILS

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ABSTRACT



Dermal exposure to metals has previously received less attention than oral/inhalation exposure. Nonetheless, human health risk is significant for certain contaminants and exposure scenarios. The present study aims to (1) characterize two certified reference soils (SQC001, BGS 102); and (2) assess Cr, Ni, Pb, and Zn dermal bioaccessibility via in vitro assays using three synthetic sweat formulations (EN 1811, pH 6.5 (sweat A), NIHS 96-10, pH 4.7 (sweat B), and a more complex pH 5.5 formulation containing amino acids (sweat C)) and two sebum formulations. Metals bioaccessibility in sweat followed sweat B > sweat C > sweat A, attributed to sweat B lower pH. Dermal bioaccessibility in both sebum formulations was lower than 1% for Ni and Pb and below 9% for Cr and Zn, possibly due to low affinity of metals for non-polar lipids. It must be noted that bioaccessible Zn in BGS 102 was higher when extracted with synthetic sebum compared to any of the synthetic sweat formulations. Metal bioaccessibility in sweat was considerably higher for SQC001 (up to 76.6% for Zn using sweat B) than for BGS 102 (ranging between 0.02 and 1.3% for all elements and all sweat formulations), attributed to higher pH, higher organic carbon, and higher cation exchange capacity of reference soil BGS 102. Sebum formulations spiked with metals generally entailed low metal recovery (except for Zn), which may explain overall low

bioaccessibility values for sebum. Sebum and sweat formulation, and soil properties seem to control in vitro dermal bioaccessibility of metals.

3.1 Introduction

Humans can be exposed to contaminants in various environmental media including soils via dermal pathway. Nevertheless, less attention has been paid to the dermal pathway compared to other exposure routes in studies dealing with contaminated soils. Moreover, the majority of scientific studies on contaminant bioavailability have focused on oral bioaccessibility of contaminants (ingestion pathway), more recently followed by in vitro test development and application for assessing inhalation bioavailability (e.g. Caboche et al. (2011), Boisa et al. (2014), Guney et al. (2017)) and dermal bioavailability (e.g., Stefaniak et al. (2014), Beriro et al. (2016), Leal et al. (2018)). The dermal route must be studied since the risk associated with this route can be significant under certain scenarios.

The stratum corneum is the outer layer of human skin. It is covered by a liquid layer composed mainly of sweat, sebum, cellular debris, and minor amounts of intracellular lipids. It has been demonstrated that many chemicals can permeate its layers. This permeation could represent a human health risk in terms of chemical exposure (WHO, 2006), but it is not well understood how the penetration of some chemicals can cause toxicity. Dermal toxicity studies conducted to date are often insufficient for risk assessment due to some limitations of studies (IOMC, 2011) such as depot of contaminant inside the skin (Moody et al., 2009) and influence of sweat composition in the oxidation and valence of chemicals (Franken et al., 2015).

Human sweat is composed of electrolytes, ionic constituents, organic acids, carbohydrates, amino acids, nitrogenous substances, vitamins and other constituents in highly variable amounts (Stefaniak and Harvey, 2006). Eccrine sweat is produced by healthy humans and has a thermoregulatory function, and sebum is secreted through sebaceous glands and forms a layer of 0.5 to 44.0 μm thickness depending on the area in the body (Stefaniak and Harvey, 2006). The main function of the sebum layer is to protect the skin of drying out (Pawar et al., 2017). Sebum is composed of squalene, wax esters, triglycerides, free fatty acids, and a small amount of cholesterol. The sebum composition is constant for each individual but may vary among them (Stefaniak and Harvey, 2006).

Due to the complexity of human skin surface film liquids (SSFLs), their artificial compositions often lack some or many of the constituents. Harvey et al. (2010) developed a comprehensive model of synthetic sweat which matches human composition as it includes a complex formulation. However, its preparation requires considerable time and costs not associated with simpler formulations containing only the main constituents of sweat (Harvey et al., 2010). Numerous studies used different formulations of artificial sweat and sebum. The most commonly used one is the formulation specified in the European Standard EN 1811 (CEN, 2015). This formulation is low cost and is relatively easy to prepare, however, it has its limitations as it lacks amino acids, which may be important in the dissolution of some metals (Brown et al., 1982). There is a need to find a cost-effective synthetic surrogate for SSFL simple enough to make it accessible and affordable for testing metal bioaccessibility in soils. At the same time, the SSFL must be complex enough to include the major components of real human sweat.

Among the metals commonly found in contaminated sites, chromium (Cr), nickel (Ni), lead (Pb), and zinc (Zn) warrant special consideration in studies of dermal exposure because of their harmful properties as skin sensitizers and their other toxic effects including carcinogenicity (Guy, 1999). As reported by Franken et al. (2015) and Van Lierde et al. (2005), Cr (VI) diffuses through skin while Cr (III) does not due to the pH of sweat. Under normal physiological conditions, the mean pH of human sweat is pH 5.3 (Stefaniak and Harvey, 2006). At this pH, Cr is in the trivalent state, therefore not permeable through the skin. However, the pH of sweat is variable and in cases of more acidic sweat, Cr can be oxidized and form more soluble ions which can permeate the skin (Franken et al., 2015). Larese et al. (2007) reported that sweat with an acidic pH of 4.5 can oxidize Cr, so it can penetrate through the skin. Ni has been reported to be absorbed through the skin and potentially produce allergic contact dermatitis (Larese et al., 2007). Concerns have been raised regarding Ni sensitization from frequent exposure associated with the use of inexpensive metal jewelry (Liden and Carter, 2001). Other exposure scenarios include exposure to soil contaminated following Ni refining (Birmingham and McLaughlin, 2006). Pb is a highly toxic metal and absorption through the skin can greatly contribute to the total body burden which may be already high from other sources. It can also remain unnoticed since it is not easily detectable in blood tests (Franken et al., 2015). It is suggested by some studies that it can penetrate the human body through sweat glands and hair follicles (Guy, 1999). Regarding Zn, current data is not adequate to establish clear relationships between environmental exposure and health problems (Nriagu, 2007). However,

exposure to high Zn concentrations may cause acute effects and is highly dependent of the point of contact with the skin and chronic effects are associated with exposures to low doses over a longer period (Nriagu, 2007). There is a limited number of studies regarding this topic and the mechanism is not clearly defined (Plum et al., 2010).

Bioaccessibility can be used as an estimation of bioavailability and, when available, in vitro bioaccessibility tests should be preferred over in vivo bioavailability tests for their cost advantage and ethical considerations (Guney and Zagury, 2014). Incorporating contaminant bioavailability and using bioaccessible concentrations instead of total concentrations in exposure assessment and risk characterization considering human exposure can provide more accurate estimates than using total concentrations. The dermal bioaccessible fraction of a substance is the amount that is dissolved in SSFL and is available for penetration through the stratum corneum to the underlying active epidermis (Hillwalker and Anderson, 2014; Stefaniak et al., 2014). This data can be used in conjunction with skin permeation data to calculate bioavailability (Stefaniak et al., 2014). Midander et al. (2016) compared metal release in two different sweat compositions; the EN 1811 (CEN, 2015) and the comprehensive, and thus more complex, formulation described by Harvey et al. (2010). This study concluded that metal release from a white gold alloy and some hard metal materials were within the same range for these two different artificial sweat solutions. The simpler and more cost-effective test using EN 1811 synthetic sweat may provide results that sufficiently indicate the degree of metal release in skin contact (Midander et al., 2016). However, this study was limited to specific metals and did not target contaminated soils, so there is still a need to understand how other relevant metals are released from soils when exposed to artificial SSFLs with different compositions. To date, the dermal bioaccessibility of metals in contaminated soils has not been reported using synthetic sebum. Pawar et al. (2017) investigated the bioaccessibility of flame retardants using a mix of artificial sweat and sebum in different proportions. This study concluded that, for the chemicals tested, the general trend showed bioaccessibility to increase in formulations of SSFL with a higher percentage of sebum (Pawar et al., 2017).

Although dermal exposure to several contaminants including some heavy metals is a serious concern, the influence of SSFL composition on in vitro dermal bioaccessibility results used in exposure assessment is yet to be established. Any study supporting or refuting the relevance of using synthetic sebum in bioaccessibility testing of heavy metals in soils have not been reported until now. As soil is a complex medium, containing not only metals but organic matter, minerals,

microorganisms, and a soil solution, it seems relevant to evaluate how sebum and SSFL composition influence the dermal bioaccessibility of heavy metals from soils. Particularly, an evaluation of test performances regarding in vitro testing of contaminated soils using different artificial SSFLs including sebum is missing. Therefore, the present study aims to (1) characterize two certified reference soils including their Cr, Ni, Zn, and Pb content; and (2) determine and compare the bioaccessibility of these metals via in vitro assays using five artificial SSFLs (specifically, three synthetic sweat and two synthetic sebum formulations). Results should be useful for the improvement of existing dermal in vitro bioaccessibility tests.

3.2 Materials and methods

3.2.1 Characterization of certified reference soils

Two reference soils have been subjected to dermal in vitro bioaccessibility tests to assess % bioaccessibility of Cr, Ni, Pb, and Zn. The first reference soil, SQC001 ($d < 425 \mu\text{m}$), is produced by Sigma-Aldrich, and the second reference soil is BGS 102 (developed by the British Geological Survey (BGS)). SQC001 is produced and certified according to ISO requirements and has been created for the purpose of testing a number of metals in soils. Due to a lack of reference materials with certified bioaccessible fractions, different reference materials have been used in in vitro studies (USEPA, 2012; Hamilton et al., 2015). The reference soil BGS 102 (ironstone soil, $d < 40 \mu\text{m}$) has been developed by the BGS as part of the UBM (Unified Bioaccessibility Method) validation procedure (Hamilton et al., 2015; Wragg, 2009).

Total metal content in soil samples was determined via acid digestion using HNO_3 , HF, and HClO_4 according to standard method 3030 (Clesceri et al., 1999). Digestates were transferred to a 100-mL volumetric flask and made up to volume with deionized water. Solutions were filtered using a $0.45 \mu\text{m}$ PVDF filter. Metal analyses were performed by atomic absorption spectrophotometry (AAS) (Perkin-Elmer A200). For Cr, Ni, Pb, and Zn, detection limits (DLs) in mg.kg^{-1} were 0.3, 0.2, 1.0, and 0.1 respectively. The pH of the soil samples was measured using a solid-to-liquid ratio 1:2 using deionized water (pH meter: Eutech pH 200 series, probe: Accumet Ag/AgCl). Total organic carbon content was analyzed using a LECO furnace. Infrared determination of CO_2 was accomplished to determine organic carbon as the difference between total and inorganic carbon.

Cation exchange capacity (CEC) was measured according to the methodology described by Chapman (1965).

3.2.2 Artificial SSFLs

Five different solutions mimicking SSFLs in human skin have been used for in vitro dermal bioaccessibility tests: three synthetic sweat formulations and two synthetic sebum formulations. Regarding synthetic sweat, the first solution (sweat A) has been prepared according to the EN 1811 standardized test, commonly used for testing for allergic response from exposure to Ni, Cr, and other metals (Hillwalker and Anderson, 2014). The second formulation (sweat B) is described in the NIHS 96-10 from Swiss Watch Industry Specification (Wainman et al., 1994). These two formulations have been recently used by Leal et al. (2018) to assess dermal bioaccessibility of metals in field-collected contaminated soils. Sweat C is described in detail by Van Lierde et al. (2005) and has been previously used by Hansen et al. (2003), Flint (1998), and Nygren and Wahlberg (1998) regarding dermatitis caused by metals and allergy caused by Cr exposure. These formulations were selected because of their differences in composition, complexity, and pH (formulations presented in Table 3.1). To prepare the synthetic sweat, reactants were added to 150 mL of deionized water and stirred using a magnetic stirrer. Then pH was adjusted by using NaOH and lastly, volume was completed with deionized water. The measured median pH of human sweat is 5.5 but can be highly variable (Stefaniak and Harvey, 2006). All three SSFLs contain NaCl (the most common electrolyte in human sweat), lactic acid (the most abundant organic acid in human sweat), and urea (Stefaniak and Harvey, 2006). While sweat A and sweat B lack amino acids, sweat C includes amino acids reportedly found in human sweat. Amino acids may act as inhibitors in the dissolution of metals as they may contribute to complexation (Flint, 1998).

Table 3.1 Composition of three synthetic sweat formulations used in in vitro tests.

Chemical, % (w/w)	Sweat A ^a (pH = 6.5)	Sweat B ^b (pH = 4.7)	Sweat C ^c (pH = 5.5)
Deionized water	99.3	94.0	98.8
Sodium chloride	0.5	2.0	0.5
Lactic acid	0.1	1.5	0.1

Chemical, % (w/w)	Sweat A ^a (pH = 6.5)	Sweat B ^b (pH = 4.7)	Sweat C ^c (pH = 5.5)
Urea	0.1	0.5	0.1
Acetic acid	–	0.25	–
Ammonium chloride	–	1.75	–
Threonine	–	–	0.1
Methionine	–	–	0.1
Serine	–	–	0.1
Alanine	–	–	0.1
Glycine	–	–	0.1

^a According to standard EN 1811 (CEN, 2015), ^b According to standard NIHS 96–10 (Wainman et al., 1994), ^c According to Van Lierde et al. (2005).

A representative artificial synthetic sebum should have the appropriate types and proportions of lipids that are relatable with human sebum and be well characterized to maximize its utility (Harvey et al., 2010). Metal bioaccessibility were also determined in two formulations of synthetic sebum. The first (Sebum A) is composed of seven lipids that can be found in similar proportions in human sebum. It includes at least one constituent for each sebum lipid type: squalene, lanolin as a source for wax esters (Blanc et al., 1989; Rissmann et al., 2008), triglycerides (saturated and unsaturated), free fatty acids (saturated and unsaturated), and free cholesterol (Stefaniak et al., 2010). The second formulation (sebum B), introduced by Wertz (2009), is less complex than Sebum A. It includes jojoba oil as wax ester and cholesterol esters are absent from the formulation. Table 3.2 presents the synthetic sebum formulations. To prepare each synthetic sebum, reactants were weighted in a 100 mL beaker on an analytical balance (AY120, Shimadzu) and heated at 60 °C in a hot bath and intermittently stirred with a magnetic stirrer until all the solids dissolved. The mixture was then kept in an incubator at 36 °C until used. There were no indications of separation of lipids in sebum.

Table 3.2 Composition of two synthetic sebum formulations used in in vitro tests.

Constituent	Saturation	Chemical	Sebum A ^a (% w/w)	Sebum B ^b (% w/w)
Squalene		Squalene	10.49	12.4
		Lanolin	24.75	–
Wax esters		Jojoba oil	–	25.0
		Tristearin	21.79	–
Triglycerides	Unsaturated	Triolein	10.90	44.6
	Saturated	Palmitic acid	14.05	–
Free fatty acids	Unsaturated	Oleic acid	14.05	17.0
Free Cholesterol		Cholesterol	3.97	–
Vitamin		Vitamin E	–	1.0

^a Modified from Stefaniak et al. (2010), ^b According to Wertz (2009).

3.2.3 In vitro bioaccessibility tests

In addition to the artificial SSFL that should be properly selected, other test parameters are also important to be considered. These include the ratio of extractant volume-to-soil mass, and the extraction duration (Horowitz and Finley, 1994). The sweat-to-soil ratio should represent the ratios in scenarios that are likely to happen in real life exposure (Leal et al., 2018). Smaller particle sizes (<425 µm) are more likely to adhere to the skin (Horowitz and Finley, 1994). Accordingly, the fine particle fraction of soil samples (<425 µm) should be used for the bioaccessibility tests (Leal et al., 2018). The in vitro tests were conducted in duplicates and a procedure blank was included in each experimental batch. The tests were performed for each soil using five artificial SSFLs freshly prepared the day of the bioaccessibility test. To initiate the test, 20 mL of synthetic sweat was added to 2 g of soil sample (weighted to 3 digits), or 5 mL of synthetic sebum was added to 0.5 g of soil in 50 mL Teflon tubes. The tubes with PTFE caps containing the mixture were then placed in an orbital shaker (51704, Cole-Palmer) at 100 rpm inside a laboratory incubator (Isotemp Incubator,

Fisher Scientific) at 36 °C, the median skin temperature for humans (Stefaniak et al., 2014). The test lasted 2 h, representing the scenario for children's exposure during play (USEPA, 2011). After completion of the test, the tubes were centrifuged at 10,000 ×g for 10 min. The supernatant was collected using automatic pipettes into a Luer-Lok syringe (60 mL) fitted with a 0.45 µm PVDF filter and was then transferred into a 50-mL centrifuge tube to which 0.5 mL of HNO₃ (70% v/v) for sweat (0.125 mL for sebum) was added for sample preservation. Samples were stored at 4 °C until analysis. Sweat samples were analyzed via AAS to determine Cr, Ni, Pb and Zn concentrations whereas samples of sebum were first digested using HNO₃ (70% v/v) and H₂O₂ (30%) at 90 °C. After digestion, the metals analysis was conducted via ICP-AES (Vista, Varian Inc.) For each contaminant, bioaccessibility percentage (%_{bio}) was determined as follows:

$$\%_{\text{bio}} = \frac{C_{\text{bio}}}{C_{\text{total}}} \times 100 \quad (3.1)$$

where C_{bio} is the bioaccessible concentration of metal (mg.kg⁻¹) that passed through a 0.45 µm filter and C_{total} is the total concentration of metal in the reference soil sample (mg.kg⁻¹,

Table 3.3).

Table 3.3 Total concentrations of Cr, Ni, Pb, and Zn (mg.kg⁻¹ ± relative standard deviation in %), pH, total organic carbon (TOC, w/w %), and cation exchange capacity (CEC, meq.100 g⁻¹) of certified reference soils tested.

Parameter	SQC001	BGS 102
Cr concentration	201 ± 2.5	183 ± 0.0
Ni concentration	331 ± 4.9	97.0 ± 2.9
Pb concentration	317 ± 1.4	96.0 ± 2.9
Zn concentration	645 ± 6.7	211 ± 5.4
pH	6.08 ± 0.1	7.06 ± 0.8
TOC	0.02 ± 0.0	1.60 ± 1.8

Parameter	SQC001	BGS 102
CEC	7.40 ± 2.7	34.0 ± 0.0

3.2.4 *In vitro* tests with spiking solution

To evaluate the performance of analytical procedures and to assess the recovery of metals, the bioaccessibility assays were also performed in duplicate while adding 2 mL of a spiking solution containing 10 mg/L of Cr, Ni, Zn, and Pb. This procedure was done in synthetic sweat and sebum with and without the presence of a soil sample. The spiking solution was prepared using deionized water and standard solutions for metals (Guney et al., 2017), and was then added to the synthetic sweat or sebum. This resulted in samples containing an additional 1 mg/L of Cr, Ni, Zn, and Pb. Then, the samples were processed and analyzed as described in the bioaccessibility tests section.

3.3 Results and discussion

3.3.1 Soil characterization

The total concentrations of Cr, Ni, Pb, and Zn in the reference soil samples SQC001 and BGS 102 (Table 3.2) obtained following digestion were comparable to the guidance values presented in the certificates of analysis. For SQC001, the recoveries were Cr: 83.9%, Ni:111.2%, Zn: 108.9%, Pb: 114.7%; and for BGS 102 they were Cr: 81.3%, Ni:121.2%, Zn: 108.9%, Pb: 120.3. Overall, the recovery percentages were acceptable.

Table 3.3 also shows pH, total organic carbon content and cation exchange capacity of reference soils. Both reference soils have pH values in the neutral range, SQC001 having a lower pH than BGS 102. Both TOC and CEC of SQC001 are much lower than the values measured in BGS 102. Soils with higher organic carbon content and higher CEC are expected to retain a higher amount of metals (BalasoIU et al., 2001; Sparks, 2003).

3.3.2 Metal recoveries in *in vitro* tests performed with spiking solutions

Results of dermal bioaccessibility tests performed by adding a spiking solution containing known concentrations of Cr, Ni, Pb, and Zn to synthetic sweat and sebum formulations (without soil

sample) are presented in Table 3.4. Overall, sweat compositions A, B, and C yielded satisfactory recovery percentages for all tested metals (from 70 to 106% of added metals) with sweat A (EN 1811 standard formulation) yielding the highest recovery (92–106%) for all tested metals. This implies that there was no significant precipitation, adsorption, or complexation effect that took place during the dermal bioaccessibility test using synthetic sweat in absence of soil. Similar results were reported by Leal et al. (2018) when using sweat formulations A and B. For sweat B formulation, a limited matrix effect was observed resulting in slightly lower recoveries compared to sweat A and C. This could be linked to the presence of ammonium chloride and acetic acid that is not found in other formulations. Except for Zn, results of tests using sebum as an extraction fluid were drastically different. To start, for sebum A, there was no recovery of spiked Cr or Pb (below DLs), and a low recovery of Ni (13%). In the case of Sebum B, spiked Pb was below detection, and there was a low to moderate recovery of Cr, Ni, and Zn (between 34% and 62%). These lower recoveries of Cr, Ni, and Pb in sebum compared to sweat can be attributed to the fact that sebum is composed of non-polar lipids (Abraham et al., 2005; Stefaniak and Harvey, 2006) and metals tend to have low solubility in sebum (Hemingway and Molokhia, 1987). Zn possibly yielded higher recoveries than the other metals in sebum due to its ability to form organic complexes that are more soluble in lipids (Simkiss, 2009).

Table 3.4 Recovery percentages (%) for Cr, Ni, Pb, and Zn following spiking (in absence of soil) after 2 h of in vitro dermal bioaccessibility testing using artificial sweat and sebum.

Metal	Sweat A	Sweat B	Sweat C	Sebum A	Sebum B
Cr	92	70	86	<DL	62
Ni	104	80	92	13	34
Pb	106	84	84	<DL	<DL
Zn	96	72	84	84	58

<DL: Value not calculated as sample concentration was lower than the one found in the blank., DL: Cr: 0.3 mg/kg, Ni: 0.1 mg/kg, Pb: 0.4 mg/kg, Zn: 0.1 mg/kg.

Results of bioaccessibility tests performed by adding a spiking solution with Cr, Ni, Pb, and Zn to the synthetic sweat and sebum compositions with reference soil samples are presented in Table 3.5. For tests performed using synthetic sweat formulations, a higher percentage of metal recovery was systematically found in liquid extracts in presence of SQC001 (Cr: 11%–38%, Ni: 56–70%, Pb:

72%–73%, Zn: 15%–48%) compared to BGS 102 (Cr: 0.2%–0.7%, Ni: 0.8%–2.5%, Pb: 0%–0.3%, Zn: 0.7%–1.3%). Differences in the recovery among reference soil samples can be attributed to their difference in TOC and CEC content (

Table 3.3), BGS 102 having much higher values than SQC001, which can lead to complexation and/or adsorption of spiked metals (Leal et al., 2018; Lin and Chen, 1998). Pb showed the highest recovery percentage for all sweat formulations tested in the reference soil SQC001, followed by Ni, and Zn. Cr presented the lowest recovery. For both sebum formulations, the recovery percentages of all metals were low (Table 3.5). Some of the metals present in the spiked solutions (such as Zn) could have been removed by adsorption/complexation onto reference soils since it was recovered in the absence of soil (Table 3.4). Also, the precipitation of insoluble complexes or minerals may occur as previous studies stated that metals are not very soluble in sebum (Hemingway and Molokhia, 1987; Stefaniak et al., 2011). These results indicate that sebum could indeed inhibit metals mobilization via possible formation of insoluble complexes or minerals. This raises questions on whether sebum is a significant medium for metals uptake following human contact with contaminated soils.

Table 3.5 Recovery percentages (%) for Cr, Ni, Pb, and zinc following spiking (in the presence of reference soils SQC001 and BGS 102) after 2 h of in vitro dermal bioaccessibility testing using artificial sweat and sebum.

		Recovery percentage (%)			
Liquid	Soil	Cr	Ni	Pb	Zn
Sweat A	SQC001	19	68	73	48
	BGS 102	0.2	0.8	0.3	0.9
Sweat B	SQC001	38	70	72	45
	BGS 102	0.7	1.5	0.2	1.3
Sweat C	SQC001	11	56	72	15
	BGS 102	0.3	2.5	0 ^a	0.7

		Recovery percentage (%)			
Liquid	Soil	Cr	Ni	Pb	Zn
Sebum A	SQC001	0	<0.2	0	0.8
	BGS 102	0	0	0	0
Sebum B	SQC001	5.1	<0.2	0	9.7
	BGS 102	4.2	0	0	5.8

^aValues shown as zero indicate that procedure blank concentrations were higher than tested sample concentration.

3.3.3 Dermal bioaccessibility of metals

3.3.3.1 Comparison of in vitro tests using different synthetic sweat formulations

According to the bioaccessibility (%) of metals obtained by tests performed on soils SQC001 and BGS 102 (Figure 3.1) using three formulations of synthetic sweat, sweat B generally yielded the highest bioaccessibility values, followed by sweat C and sweat A. The results can partly be explained by the lower pH (pH 4.7) of synthetic sweat B. Regardless of synthetic sweat formulation, dermal bioaccessibility of metals was much higher for SQC001 (up to: 27.9% for Cr, 63.2% for Ni, 76.6% for Zn, and 42.9% for Pb) compared to BGS 102 (all values below 2%). This dramatic difference can be attributed to the difference in TOC and CEC, which is also supported by the findings of the tests using spiking solutions. The TOC content of BGS 102 can be considered average and its CEC content high (compared to very low TOC and low CEC in SQC001). This may have resulted in much lower metal solubilization in all sweat formulations for BGS 102.

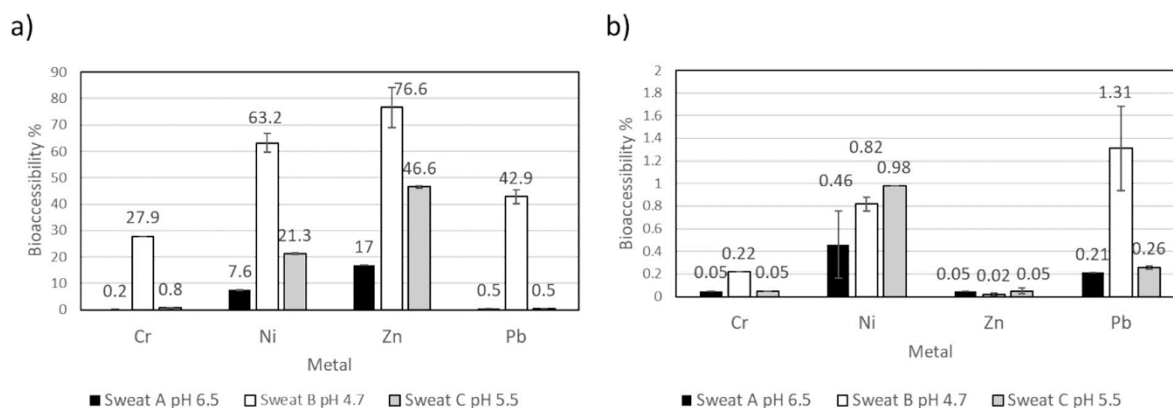


Figure 3.1 Bioaccessibility (%) of Cr, Ni, Zn, and Pb in certified reference soils using synthetic sweat formulations A, B, and C: a) SQC001, b) BGS 102. The error bars represent the % of difference between duplicates.

The bioaccessible percentage of metals in sweat A was the lowest among all the sweat compositions. In reference soil BGS 102, dermal bioaccessibility of all metals was very low (<0.5%) and in reference soil SQC001 it ranged between 0.2 and 17%. For both soils, Cr was not bioaccessible in sweat A formulation, which is consistent with previously reported results (Leal et al., 2018; Larese et al., 2007).

As previously mentioned, sweat B yielded a higher bioaccessibility percentage among the sweat formulations, possibly due to its lower pH of 4.7. It should also be noted that sweat B contains acetic acid and ammonium chloride that is not found in other sweat formulations. In reference soil SQC001 the order of dermal bioaccessibility (%) was $Zn > Ni > Pb > Cr$. The bioaccessibilities were also substantially higher for SQC001 compared to BGS 102. Bioaccessibility of Cr, Ni, Pb, and Zn in the reference soil BGS 102 have been previously studied using sweat A and sweat B by Leal et al., 2018. Bioaccessible concentrations reported were very similar to the findings of the present study. For both sweat A and sweat B the bioaccessibility of Cr, Ni, Zn, and Pb in BGS 102 was very low, <0.86% for sweat A and <4.6% for sweat B.

Sweat C led to higher bioaccessibility than sweat A for all metals in both soils used in the test. This difference may be attributed to lower pH of sweat C (5.5 vs. 6.5 for sweat A).

In general, Cr was less bioaccessible than the other metals in reference soil SQC001 (Figure 3.1a). Cr is rather insoluble especially as Cr (III) except at very high pH (Reeder et al., 2006). Ni can be oxidized and dissolved in sweat and is able to form Ni^{2+} ions in artificial sweat (Leal et al., 2018;

Flint, 1998). Ni found in soil SQC001 was bioaccessible (7.6% to 63.2%) but it was not (<1%) in reference soil BGS 102 when extracted using the same synthetic sweat formulations. As previously mentioned, this can be explained by specific soil properties of each reference soil and metal fractionation in these soils. Pb generally had a very low dermal bioaccessibility except when extracted with sweat B. Factors such as pH of soil and sweat, organic matter content and particle size affect solubilization of this metal (Reeder et al., 2006). Zn was the most bioaccessible element in reference soil SQC001 for all sweat formulations tested. However, Zn dermal bioaccessibility was very low (<0.1%) in reference soil BGS 102, indicating strong retention and/or re-adsorption onto soil sample as shown by the low recovery percentages following spiking. As a matter of fact, Ni, Pb, and Zn fractionation in reference material BGS 102 were assessed in a recent study on inhalation bioaccessibility (Gosselin and Zagury, 2019). All metals in reference soil BGS 102 were predominantly found (avg. $67 \pm 9\%$) in the most strongly retained fraction (residual fraction F5) of the sequential extraction scheme.

3.3.3.2 Comparison of in vitro tests using different synthetic sebum formulations

Overall, bioaccessibility of metals in synthetic sebum was low for both formulations tested (Figure 3.2). Pb was not bioaccessible (below detection) for both sebum compositions in agreement with spiking results which show that soluble Pb precipitates when added to sebum (Table 3.4). Bioaccessible Ni was also below detection in BGS 102 and lower than 1% in SQC 001 regardless of synthetic sebum formulation. Cr appeared to be somewhat bioaccessible when extracted with sebum from both reference soil samples (from below detection to 5.2%) and Zn dermal bioaccessibility in sebum ranged between 0.8 and 8.7% in BGS 102. It must be noted that bioaccessible Zn in BGS 102 was higher when extracted with synthetic sebum compared to any of the synthetic sweat formulations. In BGS 102, sebum B extracted more Zn and Cr than sebum A but such a pattern was not observed in reference soil SQC001, stressing again the importance of soil characteristics on metal dermal bioaccessibility. However, based on both reference soils tested, one can conclude that Ni and Pb are not bioaccessible in sebum.

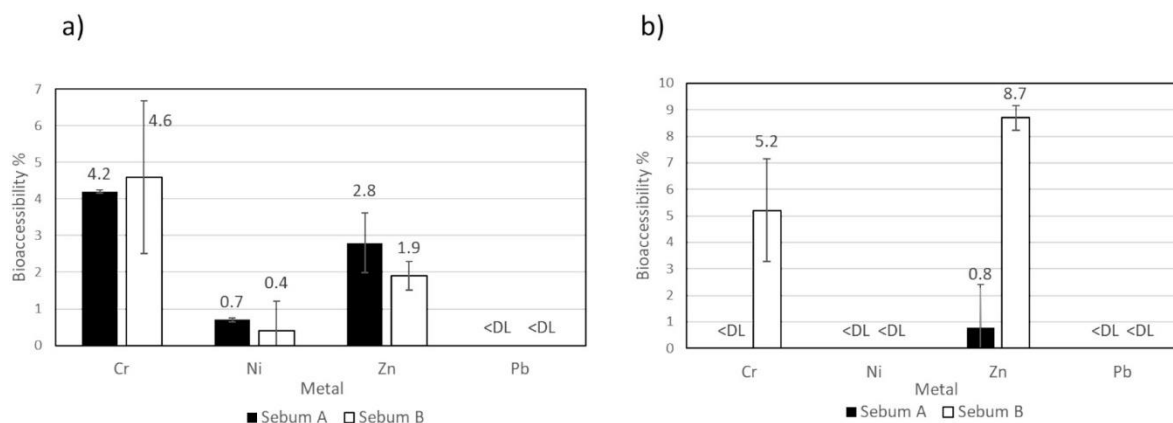


Figure 3.2 Bioaccessibility (%) of Cr, Ni, Zn, and Pb in certified reference soils using synthetic sebum formulations A and B: a) SQC001, b) BGS 102 (<DL: concentrations below detection limit). The error bars represent the % of difference between duplicates.

The complications of using sebum in dissolution studies have been previously exposed, given the difficulty of dispersing lipids in aqueous solvents during in vitro bioaccessibility tests (Hemingway and Molokhia, 1987; Stefaniak et al., 2011). Moreover, previous studies stated that sebum has a negligible influence on the dissolution of metals, therefore, sweat is the main cause that influences the rate of metal dissolution (Collins, 1957; Flint, 1998; Stefaniak et al., 2011; Van Lierde et al., 2005). However, our results show that for Zn and Cr, some sebum formulations are able to extract more metals from soils than synthetic sweat formulations.

3.4 Conclusion

All sweat formulations yielded satisfactory metal recovery percentages for all tested metals in the absence of soil. However, lower recoveries of Cr, Ni, and Pb in sebum (composed of non-polar lipids) were measured. For spiking tests performed using synthetic sweat formulations and soils, a higher percentage of metal recovery was systematically found in liquid extracts in presence of SQC001 compared to BGS 102. Differences in metal recovery among reference soil samples were attributed to their different physicochemical properties (CEC and TOC). For both sebum formulations, the recovery percentages of all metals were very low in the presence of soils indicating strong retention and/or re-adsorption onto soil sample. With regards to sweat formulations, sweat B generally yielded the highest dermal bioaccessibility values, followed by sweat C and sweat A. The results can partly be explained by the lower pH (pH 4.7) of synthetic sweat B. Regardless of synthetic sweat formulation, dermal bioaccessibility of metals was much

higher in reference soil SQC001 compared to BGS 102. This important difference can again be attributed to the difference in TOC and CEC and therefore, metal retention between the two reference soils. The pH of sweat formulation and soil properties are the most relevant parameters when dermal bioaccessibility from different synthetic sweat formulations are compared.

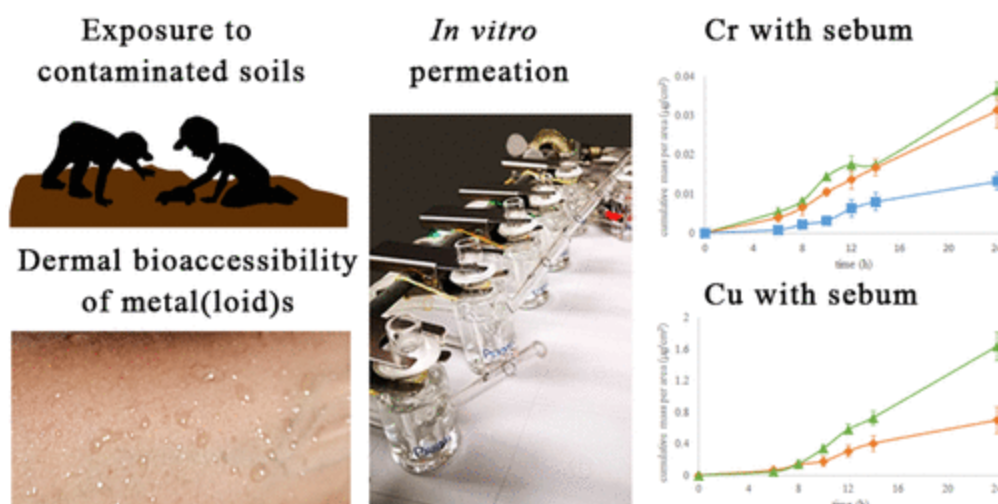
The present study is the first one to assess the effect of sebum on dermal bioaccessibility of metals from contaminated soils. Overall, bioaccessibility of metals in synthetic sebum was low for both formulations tested. However, it must be noted that bioaccessible Zn in BGS 102 was higher when extracted with synthetic sebum compared to any of the synthetic sweat formulations. Stressing again the importance of soil characteristics on metal dermal bioaccessibility. Even though, previously published studies (not using geological materials) state that sebum has a negligible influence on the dissolution of metals, our study shows that for Zn and Cr, some sebum formulations are able to extract more metals from soils than synthetic sweat formulations. These findings warrant further studies on whether sebum is a significant medium for metal solubilization following dermal contact with contaminated soils.

CHAPTER 4 ARTICLE 2 - COMPARISON OF SYNTHETIC SWEAT AND INFLUENCE OF SEBUM IN THE PERMEATION OF BIOACCESSIBLE METAL(LOID)S FROM CONTAMINATED SOILS THROUGH A SYNTHETIC SKIN MEMBRANE

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ABSTRACT



Dermal exposure to metal(loid)s from contaminated soils has received less attention than oral and inhalation exposure. Still, it can be a relevant pathway for some contaminants. Comparison of synthetic sweats (donor solutions), the influence of sebum, and the characterization of diffusion parameters through a synthetic membrane (acting as skin surrogate) in the permeation of metal(loid)s (As, Cr, Cu, Ni, Pb, and, Zn) from polluted soils is missing. The dermal bioaccessibility tests were performed using two sweat compositions (EN 1811, pH 6.5 (sweat A) and NIHS 96–10, pH 4.7 (sweat B)). Diffusion parameters of soluble metal(loid)s using the Franz cell methodology were calculated using the Strat-M membrane. The influence of synthetic sebum in the permeation of metal(loid)s was also investigated. The metal(loid) bioaccessibility percentage was higher for sweat B (pH 4.7) compared to sweat A (pH 6.5), attributed to lower pH of sweat B. Among the six elements tested, only chromium and copper permeated the membrane. Permeation coefficient (K_p) was higher for chromium in sweat A ($0.05\text{--}0.11\text{ cm h}^{-1}$) than sweat B (0.0007--

0.0037 cm h⁻¹) likely due to a higher pH and thus more permeable Cr species. The presence of sebum increased lag times for copper permeation. Additional studies regarding speciation of metal(loid)s following extractions in synthetic sweat and comparison of synthetic membrane Strat-M and human skin in the permeation of metal(loid)s are recommended.

4.1 Introduction

Humans can be exposed to toxic metal(loid)s in various environmental media, including soils, via the dermal pathway. The majority of scientific studies have focused on oral bioaccessibility of pollutants (ingestion pathway), and more recently on in vitro test development and application for assessing inhalation bioavailability (e.g. Guney et al. (2017), Gosselin and Zagury (2020), Van der Kallen et al. (2020), Kastury et al. (2020)) and in vitro dermal bioavailability (e.g., Stefaniak et al. (2014), Beriro et al. (2016), Leal et al. (2018), and Marin Villegas et al. (2019)).

It is generally acknowledged that there are very few quantitative and qualitative data on dermal exposure to toxic chemicals present in geological materials (Sartorelli et al., 2000). Additionally, some of the permeation data of chemicals through the skin were obtained using different experimental conditions in terms of concentration in the donor solution, type of membrane (animal, ex vivo), and duration, making a comparison of the results difficult (Franken et al., 2015a). Quite the reverse, there is a copious amount of data regarding the passage of drugs and cosmetics through the skin. However, very little is known about this route of entry for environmental contaminants bound to geological materials. The technology used to study the percutaneous penetration of drugs could be used to assess dermal penetration of toxic compounds as well (Sartorelli et al., 2000; Sartorelli et al., 2012).

The most common method to evaluate dermal diffusion was introduced by Franz in 1975. This method has been adapted and modified by many researchers to study the dermal pathway for drugs, cosmetics, and chemicals due to its low cost, short time, and good reproducibility (Sartorelli et al., 2000).

Franken et al., (2015a) highlighted the importance of the donor solution composition in the Franz cell methodology. The donor solution should be standardized because certain components may promote or inhibit oxidation of metal(loid)s. There is no standardized donor solution for permeation studies, and to date, a comparison of the influence of the synthetic sweat composition as donor

solution in the permeation of metal(loid)s from geological materials is missing. Recent studies reported that synthetic sweat composition strongly influences the solubility of metals from geological materials ((Leal et al., 2018) and (Marin Villegas et al., 2019)). Even though the influence of synthetic sweat formulation on the solubility of metals has been demonstrated, the impact of synthetic sweat characteristics when used as donor solution in the dermal permeation of metal(loid)s from geological materials is not clear.

Different membranes to simulate human skin have been used for dermal permeation studies using the Franz cell methodology. According to the OECD and USEPA guidelines, human skin is the “gold standard” (Beriro et al., 2016). Unfortunately, human skin is not always available, can be expensive, and is highly variable depending on age, sex, origin of the donor, and body area (Haq et al., 2018a). Because of these limitations, some studies used animal skin, such as swine, rat, or guinea pig, as human skin surrogates (Franken et al., 2015a; Moody et al., 1995). Animal skin as a surrogate for human skin also has limitations such as high variability inherent to biological membranes (coefficient of variation = 72%) rendering the experimental design, and ability to compare the results difficult (Barbero and Frasch, 2009; Haq et al., 2018a). Moreover, ethical consideration inherent to the use of biological tissues of animal origin and in vivo experiments with animals must be considered.

Artificial membranes used to model skin permeation should mimic the stratum corneum as close as possible, have low variability, and be commercially available (Haq et al., 2018b). The Strat-M (EMD Millipore, MA, USA) synthetic membrane is an ultrafiltration membrane made of polyethersulfone composed of multiple layers, including two layers impregnated with synthetic lipids and a very tight top layer, producing diffusion results similar to natural human skin (Haq et al., 2018b; Joshi et al., 2012). In diffusion studies with human cadaver skin, animal skin, and the Strat-M membrane, Joshi et al. (2012) tested the Strat-M membrane with a mixture of synthetic lipids (to mimic the lipid phase in human skin) in the permeation of nicotine and hydrocortisone. The authors reported that when the membrane Strat-M was treated with synthetic lipids, it showed a closer correlation with human skin than the untreated membrane and animal skin. They also exposed the difficulties of correlating the diffusion data between animal skin and human skin (Joshi et al., 2012). In most cases, the correlation between the treated synthetic membrane Strat-M and human skin was better. Moreover, the Strat-M membrane showed a high lot-to-lot reproducibility and a high shelf life as opposed to human and animal skin. Yet, to our knowledge, the use of the

membrane Strat-M to assess the dermal permeation of metal(loid)s from contaminated geological materials has not been reported yet.

The dermal bioaccessible fraction of a chemical is the amount that is dissolved in sweat and is available for penetration through the skin (Hillwalker and Anderson, 2014; Stefaniak et al., 2014). This concentration can be used in conjunction with the permeation data to calculate bioavailability (Stefaniak et al., 2014).

To estimate the dermal absorption of contaminants from aqueous media, the USEPA (2004) proposes the water approach methodology. It assumes contact with contaminated water and aims to calculate the dermally absorbed dose using the migration rate of a chemical through the skin. This migration is characterized by the permeability coefficient K_p (cm h^{-1}). This coefficient is available in the literature for several inorganics and originates from experimentally measured or derived values (Leal et al., 2018; USEPA, 2004). Nevertheless, published K_p values involve a high level of uncertainty (because they do not take speciation into account except for chromium) and they are available for metal(loid)s soluble in water but not soluble in sweat (USEPA, 2004). Improvement in K_p determination can reduce uncertainty in the calculation of dermal exposure, thereby refining exposure assessment. To our knowledge, the influence of synthetic sweat formulation on K_p evaluation to assess the dermal permeation of metal(loid)s from contaminated soils has not been reported to date.

Therefore, the present study aims to (1) assess and compare the dermal bioaccessibility of As, Cr, Cu, Ni, Pb, and Zn present in various geological materials via in vitro experiments using two artificial sweat formulations; (2) evaluate the diffusion parameters of the bioaccessible fraction of these metal(loid)s through artificial membrane Strat-M using the static Franz cell methodology; and (3) investigate the influence of synthetic sebum in the permeation of these metal(loid)s through artificial membrane Strat-M.

4.2 Materials and methods

4.2.1 Soil sampling and characterization

Three geological materials have been subjected to in vitro bioaccessibility tests to assess the dermal bioaccessible fraction of As, Cr, Cu, Ni, Pb, and Zn: one certified material) SQC001 lot number LRAC0025, produced by Sigma-Aldrich in accordance with ISO 17034 and ISO/IEC 17025

procedures ($d < 425 \mu\text{m}$)), and two field-collected soils (S7 and S8), sampled near chromated copper arsenate (CCA)-treated utility poles in the Montreal area (Quebec, Canada). The soils were sampled in a 20 cm radius of the poles and up to a 10 cm depth. Coarse material ($>2 \text{ cm}$) and topsoil vegetation were removed prior to sampling. The samples were collected using a plastic shovel and stored in zip-lock plastic bags. Containers and tools were washed with a phosphate-free detergent and soaked overnight in 10% (v v^{-1}) HNO_3 and rinsed with deionized water ($18.2 \text{ M}\Omega \text{ cm}$) prior to use (Gosselin and Zagury, 2020, Van der Kallen et al., 2020). Field-collected soil samples were air-dried, gently disaggregated using a mortar and dry sieved to $420 \mu\text{m}$ using a sieve shaker (Retsch AS-200). Samples were then stored at 4°C .

The total metal(loid) content in soil samples was determined via acid digestion on a hot plate using HNO_3 (70% w/w), HF (50% w/w), and HClO_4 (70% w/w) according to standard method 3030 (Clesceri et al., 1999). Digestates were transferred to 100 mL volumetric flasks and made up to volume with deionized water. Solutions were filtered ($0.45 \mu\text{m}$) with glass microfiber filters (Whatman) and stored in polypropylene centrifuge tubes with high-density polyethylene (HDPE) screw caps. Cr, Cu, Ni, Pb, and Zn concentrations were measured via atomic absorption spectroscopy (Perkin-Elmer A200). Detection limits [DLs, determined based on the signal-to-noise approach (ratio of 3:1)] in mg kg^{-1} were 0.3, 0.3, 0.2, 1, and 0.1, respectively. The arsenic content was determined via inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Varian Vista), with a DL of 0.004 mg kg^{-1} . Soil pH was measured in duplicate in a solid-to-liquid ratio of 1:2 with deionized water (pH meter: Eutech pH 200 series, probe: Accumet Ag/AgCl) according to the ASTM D4972-13 method (ASTM, 2013). The total organic carbon content with a DL of 0.1% w/w was analyzed using a LECO furnace. Infrared determination of CO_2 was carried out to determine the organic carbon content as a difference between total and inorganic carbon (Tiessen and Moir, 1993). Cation exchange capacity (CEC) was determined using the sodium acetate method with NaOAc 1N and NH_4OAc 1N (Chapman, 1965).

4.2.2 Artificial SSFLs

Three solutions have been prepared to mimic human skin surface film liquids (SSFL): two synthetic sweats [sweat A (pH = 6.5) and sweat B (pH = 4.7)] and one synthetic sebum (Table 4.1). The SSFL formulations are further described in Marin Villegas et al. (2019) and have been selected for their differences in pH and composition. Sweats A and B simulate the sweat layer on the skin,

while sebum was used to treat the synthetic membranes to mimic the hydrophobic properties of the skin, caused by the presence of lipids.

Table 4.1 Composition of artificial SSFLs

Chemical, % (w/w)	Sweat A ^a (pH = 6.5)	Sweat B ^b (pH = 4.7)	Sebum ^c
Deionized water	99.3	94	-
Sodium chloride	0.5	2	-
Lactic acid	0.1	1.5	-
Urea	0.1	0.5	-
Acetic acid	-	0.25	-
Ammonium chloride	-	1.75	-
Squalene	-	-	12.4
Jojoba oil	-	-	25
Triolein	-	-	44.6
Oleic acid	-	-	17
Vitamin E	-	-	1

^a According to standard EN 1811 (CEN, 2015), ^b According to standard NIHS 96-10 (Wainman et al., 1994), ^c According to Wertz (2009).

4.2.3 In vitro dermal bioaccessibility test

Tests were started by adding 20 mL of synthetic sweat to 2 g of the soil sample ($d < 425 \mu\text{m}$) in 50 mL polypropylene tubes with HDPE caps. Tubes containing the soil and sweat mixture were placed on an orbital shaker (Cole-Parmer 51704 Series, radius 9.5 mm) at 100 rpm inside an incubator (Isotemp, Fisher Scientific) at 36 °C, corresponding to the median skin temperature for humans (Stefaniak et al., 2014), for 2 h. The tubes were then centrifuged (Heraeus Megafuge 8, Thermo Fisher) at 10,000g for 10 min, and the supernatant was collected with 60 mL Luer-Lok syringes and filtered with a 0.45 μm polyvinylidene fluoride filter fitted to the syringe. The filtered supernatant was transferred into 50 mL polypropylene centrifuge tubes with HDPE caps and stored at 4 °C until analysis. Samples were analyzed via ICP-atomic emission spectroscopy (AES) (Vista,

Varian Inc.) to determine As, Cr, Cu, Ni, Pb, and Zn concentrations (with DLs in mg kg⁻¹ of 0.004, 0.001, 0.006, 0.001, 0.006, and 0.024, respectively). For each metal(loid), bioaccessibility percentage (%_{bio}) was determined as follows:

$$\%_{\text{bio}} = \frac{C_{\text{bio}}}{C_{\text{total}}} \times 100 \quad (4.1)$$

Where C_{bio} is the bioaccessible concentration of metal(loid) (mg kg⁻¹) and C_{total} is the total concentration of metal(loid) in the soil sample (mg kg⁻¹). A more detailed description of the dermal bioaccessibility protocol can be found elsewhere (Marin Villegas et al., 2019).

4.2.4 Permeation test

Glass-jacketed vertical Franz diffusion cells (PermeGear Inc.) with a 9 mm orifice diameter, a 5 mL receptor volume, and a 1 mL donor volume were used. The temperature of the receptor was maintained at 37 °C by circulating water from a water bath (model 2849, Thermo Fisher Scientific) to simulate temperature below the skin (Franken et al., 2015a, Franken et al., 2015b). The receptor compartment was filled with phosphate-buffered saline (PBS) solution (Fisher Scientific) at a pH of 7.4 (Haq et al., 2018a) and NaCl 8.0 g/L, KCl 0.2 g/L, Na₂HPO₄ 1.44 g/L, and KH₂PO₄ 0.24 g/L, to represent the blood salt concentration and blood pH (Franken et al., 2015a). The receptor compartment was subjected to constant stirring (300 rpm) with a magnetic stirrer (Poly 15, Variomag). Twenty-five millimeters diameter sterile Strat-M membranes (EMD Millipore) were used as a surrogate for human skin. Each membrane was mounted on the Franz diffusion cell with the shiny side in contact with the donor compartment (Haq al., 2018a). To simulate the hydrophobic character of the skin and investigate the influence of the lipid fraction of SSFL in the permeation of metal(loid)s, half of the membranes were coated with 0.1 mL of sebum.

One milliliter of the filtered supernatant collected from the bioaccessibility test (donor solution) was immediately added to the open-top donor compartment to start the permeation experiment. Tests were performed in duplicate and in the presence of procedure blanks (fresh synthetic sweat as donor solution). The receptor solution was completely removed from the receptor compartment at 6, 8, 10, 12, 14, and 24 h and placed in 15 mL polypropylene centrifuge tubes. After each sampling event, the receptor compartment was rinsed with 5 mL of fresh PBS solution using a syringe. This rinsing solution was added to the tube containing the receptor solution. After every

sampling event followed by rinsing, 5 mL of fresh PBS solution was added to the receptor compartment. The procedure blank cells received the same treatment.

The receptor and donor solution samples were kept refrigerated at 4 °C until analyzed via ICP-AES (Vista, Varian Inc.) to determine the As, Cr, Cu, Ni, Pb, and Zn concentrations.

As an additional quality control measure, a mass balance was performed for one sample per batch. At the end of the permeation test (after 24 h), the donor compartment and the membrane were rinsed four times with 1 mL of deionized water. The rinsing solution was added to the remaining donor solution for analysis. To assess the amount of metal that remained in the membrane, membranes were placed in Teflon beakers and digested on a hot plate with HNO₃, HCl, and HF for 45 min and then filtered with 0.45 µm filters (Whatman), diluted to 100 mL, and analyzed for total metal content via ICP-AES. Mass balance was calculated by comparing the metal recovered from the donor solution, membrane, and receptor solution at the end of the experiment with the initial mass of metal present in the donor solution.

4.3 Results and discussion

4.3.1 Soil characterization

The pH was neutral to slightly alkaline for CCA-contaminated soil samples S7 and S8 and acidic for reference material SQC001 (Table 4.2). A low total organic carbon content (<2.9%) was reported in all soil samples. CEC values ranged from 12.8 to 41.4 meq 100 g⁻¹. High CEC suggests that cationic metals such as Cu, Ni, and Zn could be retained by cation exchange on the soil (Gosselin and Zagury, 2020). Total metal(loid) concentrations in soil samples S7 and S8 are also shown in Table 4.2. Values in bold are exceeding the Quebec's regulatory limit for industrial land-use (C criterion) MELCC(2021). Extensive As contamination was observed for S8 (1639 mg kg⁻¹ ± 6.8%) (more than 30 times the C criterion) and S7 (311 mg kg⁻¹ ± 1.1%). A Cu content exceeding the C criterion (500 mg kg⁻¹) was also observed for S7 (824 mg kg⁻¹ ± 5.8%) and S8 (1070 mg kg⁻¹ ± 11.0%). As previously reported, Cr contamination was less problematic than As and Cu (Gosselin and Zagury 2020) but soil samples S7 and S8 still contained elevated Cr concentrations. Certified reference material SQC001 had a lower content of As, Cr, and Cu but a higher content of Pb and Zn. The measured total metal(loid) content of SQC001 was within 100 ± 10% of the certified values provided in the certificate of analysis.

Table 4.2 Total concentrations of As, Cr, Cu, Ni, Pb, and Zn (mg kg^{-1}), pH, total organic carbon (TOC, w/w %), and cation exchange capacity (CEC, $\text{meq } 100 \text{ g}^{-1}$) of soils. Precision is expressed as mean $\pm$ relative standard deviation %.

Parameter	SQC 001	S7	S8
As	173 ± 20	311 ± 1.1	1639 ± 6.8
Cr	124 ± 6.5	371 ± 3.9	582 ± 14.3
Cu	82 ± 1.9	824 ± 5.8	1070 ± 11.0
Ni	112 ± 9.8	26 ± 11.5	223 ± 11.3
Pb	263 ± 2.5	57 ± 8.8	80 ± 10.6
Zn	512 ± 4.0	261 ± 7.3	223 ± 10.8
pH	5.7 ± 0.2	7.2 ± 0.7	7.1 ± 0.5
TOC	< 0.01	1.3 ± 2.1	2.9 ± 1.5
CEC	12.8 ± 8.8	15.5 ± 16.3	41.4 ± 2.8

4.3.2 Dermal bioaccessibility of metal(loid)s

Table 4.3 shows the bioaccessibility (%) and bioaccessible concentration (mg L^{-1}) of metal(loid)s obtained following bioaccessibility tests performed on S7, S8, and SQC001 using sweat A and sweat B. In agreement with the findings of Marin Villegas et al., (2019) sweat B generally yielded the highest bioaccessibility percentage values. The difference in the bioaccessibility of metal(loid)s for the different sweat compositions can be attributed to the fact that lower pH increases the solubility, particularly for cationic metals.

Table 4.3 Bioaccessibility of As, Cr, Cu, Ni, Pb, and Zn in soils using synthetic sweat formulations A and B: (a) bioaccessibility expressed in percentage (%) and (b) bioaccessible concentration (mg L⁻¹)

(a) Bioaccessibility (%)

Donor solution	Soil	As	Cr	Cu	Ni	Pb	Zn
Sweat A (pH=6.5)	S7	1.4	0.15	0.5	< 0.4	< 0.2	0.05
	S8	0.5	0.06	0.4	< 0.4	< 0.2	0.02
	SQC001	0.06	0.13	2.1	20.4	0.4	40.3
Sweat B (pH=4.7)	S7	2.8	2	7.2	0.5	< 0.2	9
	S8	0.8	1	2.9	0.8	< 0.2	3
	SQC001	5.1	25.1	61.3	44.9	34.9	91

(b) Bioaccessible concentration (mg L⁻¹)

Donor solution	Soil	As	Cr	Cu	Ni	Pb	Zn
Sweat A (pH=6.5)	S7	0.45	0.05	0.38	< 0.01	< 0.01	0.01
	S8	0.76	0.04	0.41	< 0.01	< 0.01	0.01
	SQC001	0.01	0.02	0.17	2.29	0.09	20.6
Sweat B (pH=4.7)	S7	0.86	0.76	5.93	0.01	< 0.01	2.34
	S8	1.36	0.59	3.13	0.02	< 0.01	0.66
	SQC001	0.88	3.12	5.07	5.02	9.2	46.6

The reference material SQC001 revealed an overall higher dermal bioaccessibility percentage compared to S7 and S8, especially for Cu, Ni, Pb, and Zn. In sweat B, which yielded higher

bioaccessibility percentages than sweat A due to its more acidic pH (4.7), metal(loid) dermal bioaccessibility ranged from 5.1 to 91.0% in SQC001 but it remained lower than 7.2% in soil S9 and lower than 2.9% in soil S8 (

Table 4.3). The overall higher bioaccessibility percentage measured in the reference material can be explained by its lower pH, low total organic carbon content ($<0.1\%$ w/w), and the fact that the reference material SQC001 has not undergone a natural aging process, which reduces the bioaccessibility of metals in soils (Liang et al., 2016).

In summary, bioaccessibility percentages in terms of sweat are in the following order sweat B > sweat A and in terms of geological materials they are SQC001 > S7 > S8. In agreement with bioaccessibility percentages, bioaccessible concentrations were relatively low (below 1 mg L^{-1}) in sweat A except for Ni (2.29 mg L^{-1}) and Zn (20.6 mg L^{-1}). The highest bioaccessible concentrations were measured in SQC001 for Ni, Pb, and Zn. Even though Cu bioaccessibility percentages following extraction with the more acidic sweat B were only 7.2 and 2.9% in soils S7 and S8, respectively, high bioaccessible concentrations (5.9 and 3.1 mg L^{-1} respectively) were obtained because of the very high Cu content (largely exceeding the C criterion) measured in these field-collected soil samples (Table 4.2).

A more comprehensive analysis of the influence of soil properties and synthetic sweat formulation on bioaccessibility of metal(loid)s from soils can be found in Leal et al., (2018), and Marin Villegas et al., (2019).

4.3.3 Permeation test

Following the bioaccessibility test, the supernatant containing the bioaccessible fraction of metal(loid)s was used as the donor solution in permeation tests. The concentration of metal(loid)s in the receptor ($\mu\text{g L}^{-1}$) was converted to the total metal(loid) amount that permeated ($\mu\text{g cm}^{-2}$) and then plotted against time (Figure 4.1 and Figure 4.2). Flux ($\mu\text{g cm}^{-2} \text{ h}^{-1}$) was calculated as the slope from the steady-state region of graphs shown in Figure 4.1 and Figure 4.2 and the lag time as the intercept of the curve with the X-axis (time). The permeation coefficient K_p was calculated from the linear steady-state region of the plot by dividing the flux through the membrane ($\mu\text{g cm}^{-2} \text{ h}^{-1}$) by the concentration in the donor solution (Larese et al., 2007).

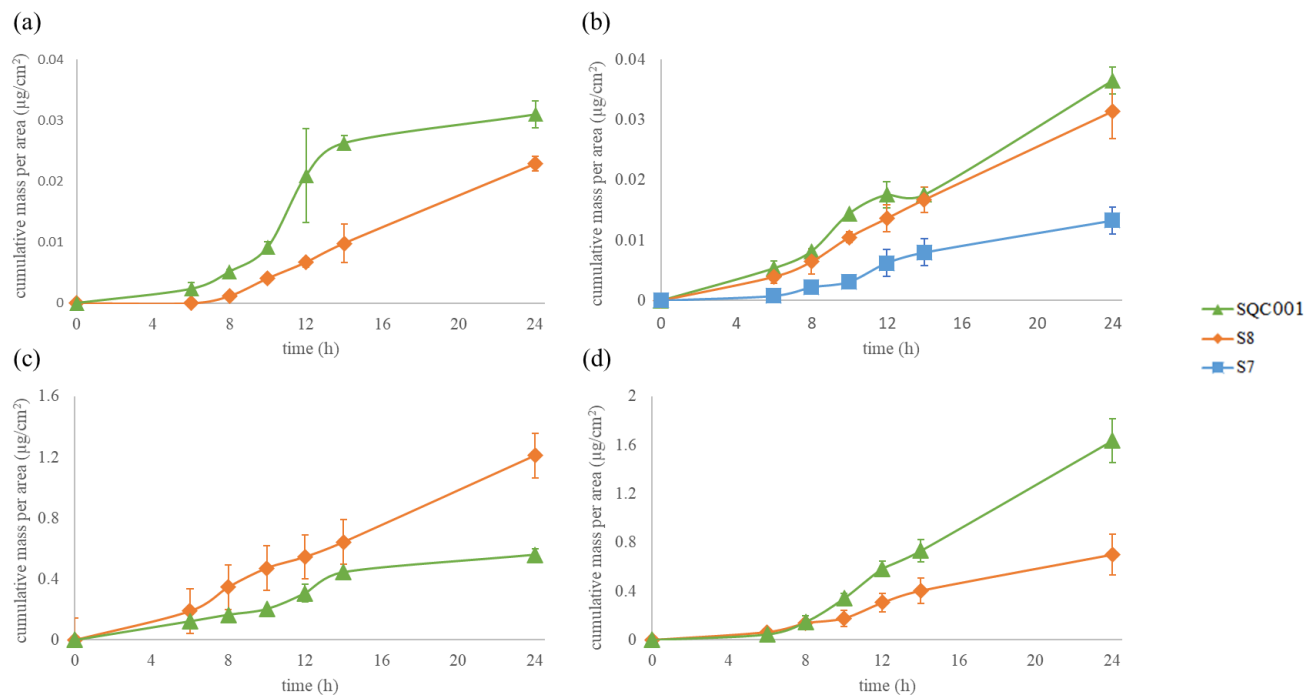


Figure 4.1 Mean cumulative mass per area and standard deviation of metal in sweat A (pH = 6.5) that permeated through Strat-M membrane for (a) chromium (not coated with sebum), (b) chromium (coated with sebum), (c) copper (not coated with sebum), and (d) copper (coated with sebum)

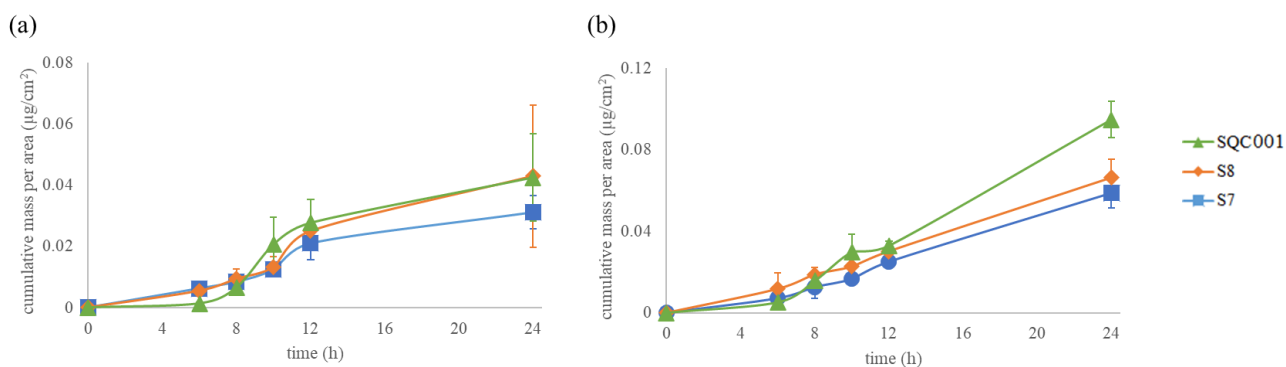


Figure 4.2 Mean cumulative mass per area and standard deviation of chromium in sweat B (pH = 4.7) that permeated through Strat-M membrane when (a) not coated with sebum and (b) coated with sebum.

Overall, mass balance recovery calculated from donor solutions, synthetic membranes, and receptor solutions yielded satisfactory percentages. The results for all analyzed samples ranged

from 58.1 to 101.3% for Cr, and from 85.3 to 128.4% for Cu (when sweat A was used as a donor solution), and from 81.9 to 115.2% for Cr, and 73.4 to 101.2% for Cu when sweat B was used as a donor solution.

4.3.3.1 Arsenic

Arsenic did not permeate the synthetic skin membrane for all sweat formulations and soil samples tested. At the pH of both sweats and under oxidizing conditions, As is expected to be found as As(V), the less mobile form of this metalloid (Dobran and Zagury, 2006; McLean and Bledsoe, 1992). Experimental data indicate that penetration of metal(loid)s through the skin is significantly dependent on ion mobility and charge (Hostýnek et al., 1993).

4.3.3.2 Chromium

Figure 4.1 and Figure 4.2 show that Cr permeated through the membrane for both synthetic sweat formulations and all geological materials (except for soil S7 in sweat A, in the absence of sebum). The results (

Table 4.4) indicate that K_p is higher for sweat A than sweat B for the same geological material. This can be explained by the Cr(III) and Cr(VI) species in each sweat formulation. In the more alkaline sweat A, and under oxidizing conditions (donor compartment open to air), a higher amount of Cr is expected to be found as Cr(VI) when compared to the more acidic sweat B. Cr(VI) has a higher potential of permeation than Cr(III) and a higher associated K_p (Gammelgaard et al., 1992, Van Lierde et al., 2005, Franken et al., 2015a), which explains the higher flux and K_p of Cr in sweat A.

Table 4.4 Flux ($\text{g cm}^{-2} \text{ h}^{-1}$), lag time (h), and permeation coefficient (cm h^{-1}) for (a) Cr and (b) Cu (data expressed as mean $\pm$ standard deviation).

(a)

	Donor solution	Flux ($\text{g cm}^{-2} \text{ h}^{-1}$)		Lag Time (h)		K_p (cm h^{-1})	
Sweat A	S7	-		-		-	
(pH=6.5)	S8	1.4E-03	$\pm$ 4.0E-05	6.7	$\pm$ 0.3	3.8E-02	$\pm$ 1.1E-03
	SQC001	1.7E-03	$\pm$ 4.1E-04	2.8	$\pm$ 0.6	1.1E-01	$\pm$ 2.6E-02

	S7 Sebum	7.1E-04	± 6.5E-05	4.4	± 0.1	1.3E-01	± 1.2E-02
	S8 Sebum	1.5E-03	± 3.4E-05	5.5	± 0.5	4.7E-02	± 1.0E-03
	SQC001 Sebum	1.7E-03	± 1.1E-04	2.5	± 0.2	1.1E-01	± 7.2E-03
Sweat B (pH=4.7)	S7	1.4E-03	± 2.4E-04	0.6	± 0.3	1.5E-03	± 2.6E-04
	S8	2.1E-03	± 2.6E-04	2.8	± 0.4	2.6E-03	± 3.3E-04
	SQC001	2.2E-03	± 5.0E-04	2.9	± 0.7	7.3E-04	± 1.7E-04
	S7 Sebum	2.9E-03	± 2.9E-03	3.7	± 0.1	3.2E-03	± 3.2E-03
	S8 Sebum	3.0E-03	± 6.3E-05	1.9	± 0.1	3.7E-03	± 7.8E-05
	SQC001 Sebum	4.9E-03	± 2.0E-04	4.7	± 0.3	1.9E-03	± 7.7E-05

(b)

	Donor solution	Flux ($\text{g cm}^{-2} \text{h}^{-1}$)		Lag Time (h)		K_p (cm h^{-1})	
Sweat A	S7	-		-		-	
(pH=6.5)	S8	5.5E-02	± 1.8E-03	0.2	± 0.4	1.3E-01	± 4.3E-03
	SQC001	2.6E-02	± 4.2E-03	0.6	± 0.6	1.5E-01	± 2.4E-02
	S7 Sebum	-		-		-	
	S8 Sebum	3.6E-02	± 2.3E-03	4.6	± 0.3	8.8E-02	± 5.5E-03
	SQC001 Sebum	9.1E-02	± 2.5E-03	5.9	± 0.3	5.2E-01	± 1.4E-02

For sweat B, flux and K_p are roughly doubled in the presence of sebum for all the geological materials. Sebum might interact with Cr species in sweat B and foment production of more penetrable compounds. In sweat A, in addition to Cr(VI), Cr(III) species that are expected to be found are mostly Cr(OH)_2^+ and neutral thus potentially more permeable $\text{Cr(OH)}_3(\text{aq})$, whereas in sweat B due to a more acidic pH, Cr(III) is expected to be the main Cr oxidation state (WHO, 2009). This hypothesis is backed by the fact that for sweat A, there is no visible influence of sebum on the permeation parameters. However, other variables such as the molecular size, chemical reactivity, and counterions could affect permeation of Cr through the skin membrane (Hostýnek et al., 1993; Hostýnek, 2003). To validate this hypothesis, it is recommended to assess chromium speciation in SSFLs.

The permeation coefficient (K_p) also varies among geological materials within the same sweat composition. This might be partially explained by the dose factor: the rate of diffusion of Cr is not proportionate to the applied concentration, and absolute absorption can reach a plateau value and then decrease with an increment in concentration (Hostýnek, 2003). This phenomenon occurs in real skin due to the buildup of a secondary diffusion barrier as a consequence of electrophilic metals [such as Cr(III)] forming stable bonds with proteins in the skin, also causing longer lag times (Hostýnek, 2003). As previously reported by Marin Villegas et al. (2019), the concentrations of metals in the donor solution following dermal bioaccessibility tests are dependent on the physicochemical properties of the geological material and extractant (synthetic sweat). There was a much higher bioaccessible concentration of Cr in sweat B than sweat A following the bioaccessibility test (

Table 4.3) producing different diffusion profiles (Figure 4.1 and Figure 4.2).

4.3.3.3 Copper

Copper extracted from samples S8 (0.41 mg L^{-1}) and SQC001 (0.17 mg L^{-1}) with sweat A permeated the synthetic skin membrane, both in the presence and absence of sebum (Figure 4.1 and

Table 4.4). It should be noted that Cu was also detected in the receptor solution following permeation with sample S7 extracted with sweat A (0.38 mg L^{-1}) in the presence of sebum at the end of the sampling period ($t = 12 \text{ h}$ and $t = 24 \text{ h}$). However, there were not enough data to build the cumulative mass per area versus time curve and calculate the permeation parameters. Copper did not permeate the membrane when extracted with sweat B even though bioaccessible concentrations (donor solution) were much higher (3.13 mg L^{-1} for S8 and 5.07 mg L^{-1} for SQC001). This suggests that Cu speciation and complexation, which depends on pH, and sweat formulation among other parameters, might influence the permeation of Cu.

The K_p values for Cu were similar for soils S8 and SQC001 in the absence of sebum. However, in the presence of sebum, the K_p value was around five times higher for SQC001. Lag times before Cu permeation were longer in the presence of sebum for S8 and SQC001, increasing from 0.2 to

4.6 h and from 0.6 to 5.9 h, respectively. This could be caused by the added layer formed by the sebum, causing the Cu flux to take a longer time to reach equilibrium.

4.3.3.4 Nickel

High bioaccessible Ni concentrations (2.29–5.02 mg L⁻¹) were obtained when extracting SQC001 with both sweat formulations but low to below detection (<0.01 mg L⁻¹) bioaccessible Ni concentrations were measured when extracting field-collected soil samples S7 and S8 (

Table 4.3). In all cases, Ni did not permeate the synthetic skin membrane. Depending on Ni activity, Ni in solutions with a pH < 8 is expected to be mostly found as Ni²⁺ (Gonsalvesh et al., 2016). Fullerton et al., (1988) reported a strong influence of the vehicle in the permeation of NiCl₂ and NiSO₄ through the skin and lag times of 50 h (our permeation test lasted 24 h). In another study, Larese et al. (2007) tested permeation through human abdominal skin of Ni powder in suspension in synthetic sweat (50 g L⁻¹) at pH 6.5 (sweat A). In the latter study, Ni slowly permeated the skin with a lag phase of 14 h and Ni was present as a free ion in the donor and in the receptor solution. Nevertheless, in the present study, the potential for very long lag times made Ni unable to penetrate the synthetic skin membrane under the experimental design used.

4.3.3.5 Lead

Lead was only present in donor solutions when SQC001 was extracted with sweat A (0.09 mg L⁻¹) and sweat B (9.20 mg L⁻¹) (

Table 4.3). However, it did not permeate the synthetic skin membrane. In real human skin, Pb is mainly absorbed through the sweat glands and hair follicles and only slightly mobile through the transepidermal route depending on its speciation (Stauber et al., 1994; Franken et al., 2015a). The Strat-M membrane is designed to specifically simulate the diffusion pathway.

4.3.3.6 Zinc

Zinc did not permeate the synthetic skin membrane even if very high bioaccessible concentrations (

Table 4.3) were found in the donor solution for both SSFLs with SQC001. Zinc is expected to be mostly present in its charged ionic form Zn^{2+} from low to neutral pH (Powell et al., 2015). Most of the previous investigations regarding permeation of Zn were focused on ZnO from sunscreen, concluding that particles formed micron-sized aggregates reducing permeation through human skin (Franken et al., 2015a; Mavon et al., 2007; Merwe et al., 2009).

4.3.3.7 Comparison of K_p Values with Published Values

The USEPA (2004) recommends K_p values (cm h^{-1}) for some metals (Cr(III):0.001, Cr(VI):0.002, Ni:0.0002, Pb:0.0001, Zn:0.0006, and other nonspecified inorganics: 0.001). These values have been adapted from Hostýnek et al. (1998) Because of its conservative approach, USEPA (2004) listed the highest reported permeability coefficient. Nonetheless, other studies reported higher permeability coefficients than the ones recommended by USEPA (2004). Examples of these are Larese Filon et al., (2008) who reported a K_p value for Cr of 0.0124, and Fullerton et al., (1988) who reported K_p values for Ni of 0.0015 for epidermis and 0.23 for the dermis.

The K_p values from our experiments using the Strat-M synthetic membrane when metal(loid)s extracted in sweat A were used in the donor solution were higher than the ones summarized by USEPA (2004) However, the values were in the same order of magnitude or slightly higher for Cr when sweat B was used in the donor solution. Moreover, in the present study, longer lag times and differences in the K_p were found in the presence of sebum. The percutaneous data obtained using a synthetic skin membrane in this study gives valuable insights regarding the influence of the donor solution pH and the presence of sebum.

4.4 Future research and study limitations

The dermal bioaccessibility of all studied metal(loid)s from geological materials was higher at lower pH. Nevertheless, only Cr and Cu could permeate the Strat-M membrane following the bioaccessibility test in synthetic sweat, and Cr and Cu seem to have greater potential for diffusion through human skin surrogate (Strat-M membrane) at higher pH. For this reason, the characteristics of the donor solution (synthetic sweat formulation) are critical for both bioaccessibility and permeation of metal(loid)s. Further studies are needed to assess metal(loid) speciation in various synthetic SSFLs to help explain differences in bioaccessible metal(loid)s' ability to permeate the

skin. Moreover, the present study findings also warrant additional studies on the influence of sebum in the permeation of metal(loid)s from various soil types through human skin surrogates.

The diffusion of metal(loid)s through a barrier is a complex phenomenon because several factors are interrelated, such as pH, oxidation state, the presence of counter ions, dose, and solubility. For risk assessment, environmental agencies like USEPA often suggest standardized or generic values for K_p . However, K_p values are not only metal-specific but appear to be site-specific and depend on several variables that must be considered for a more accurate estimation of risk related to dermal exposure.

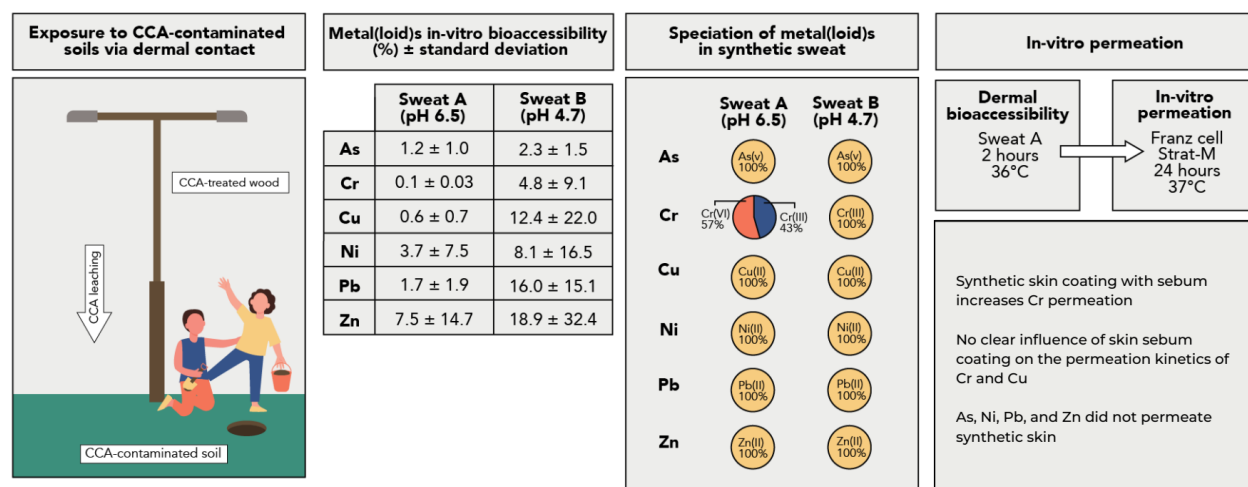
The results obtained in this study showed that the Strat-M membrane is suited for early stages of dermal permeation studies of metal(loid)s from contaminated geological materials. Synthetic membranes are commonly available, produce less variability, and are significantly less expensive than human skin. However, the Strat-M membrane only models the diffusion of chemicals and is not suitable to model other dermal pathways such as appendages (through sweat glands and hair follicles). An additional shortcoming of the Strat-M membrane is that it does probably not allow us to simulate reservoirs of metal(oids) in the stratum corneum and other layers of the skin. Further research to compare the diffusion results with real human skin is, therefore, necessary to reduce uncertainty in the calculation of dermal exposure to metal(loid)s present in contaminated soils.

CHAPTER 5 ARTICLE 3 - METAL(LOID) SPECIATION IN DERMAL BIOACCESSIBILITY EXTRACTS FROM CONTAMINATED SOILS AND PERMEATION THROUGH SYNTHETIC SKIN

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ABSTRACT



Dermal exposure to metal(loid)s from contaminated soils can contribute to health risk. Metal(loid) speciation will influence their bioaccessibility in sweat and subsequent permeation across the skin. Therefore, the speciation of the bioaccessible fraction of metal(loid)s in two synthetic sweat formulations (sweat A (pH 6.5) and B (pH 4.7)) was assessed using chemical equilibrium modelling (Visual MINTEQ). Permeation through synthetic skin and the influence of sebum in the permeation of As, Cr, Cu, Ni, Pb, and Zn were also investigated using Franz cells. Following dermal bioaccessibility tests for five Chromated Copper Arsenate (CCA)-contaminated soils and one certified soil (SQC001), mean metal(loid) bioaccessibility (%) was higher in sweat B (2.33 - 18.8) compared to sweat A (0.12-7.53). Arsenic was almost entirely found as As(V) in both sweats. In sweat A, comparable concentrations of Cr(III) and Cr(VI) were found whereas in sweat B, Cr was primarily present as Cr(III). Copper was primarily found as Cu²⁺. Bioaccessible Cr extracted from nearly all soils permeated through the Strat-M membrane when it was coated with sebum. The Cr permeation coefficient (K_p) ranged between 0.004 and 0.13 cm/h and the K_p for Cu was higher (0.024 to 0.52 cm/h). As, Ni, Pb, and Zn did not permeate the synthetic skin.

5.1 Introduction

Chromium Copper Arsenate (CCA) is an inorganic waterborne wood preservative used for over 60 years worldwide (Dobran & Zagury, 2006; Pouschat & Zagury, 2008). Arsenic (As) and copper (Cu) function as insecticide and fungicide, while chromium (Cr) fix the other two components to the wood (Chirenje et al., 2003). Due to leaching, soils can be heavily contaminated with As, Cr, and Cu in the surroundings of structures containing CCA-treated wood such as utility poles, and fences (Chirenje et al., 2003; Pouschat & Zagury, 2008; Zagury et al., 2003, 2008). Since many soils adjacent to CCA-treated wood poles are located near roads, the presence of metals such as Zn and Pb can also be expected (Guney et al., 2010). Arsenic and Cr(VI) have been linked with carcinogenic effects and are listed as priority contaminants whereas copper in high doses can be toxic to human health (ATSDR, 2019). Lead is also a soil contaminant that can cause various adverse health effects to the nervous, reproductive and immunological systems (Ara & Usmani, 2015).

Exposure to contaminants present in soils can occur via ingestion, inhalation, and dermal pathways. The latter received less attention than the other two. Limited quantitative and qualitative data on dermal exposure to potentially toxic chemicals in soils is currently available (Marin Villegas & Zagury, 2021; Sartorelli et al., 2000). Nevertheless, dermal exposure might significantly contribute to the total body burden of metal(oids) in humans (Marin Villegas & Zagury, 2023).

The toxicity and mobility of contaminants are strongly influenced by their oxidation state and valence (Avasarala, 2021). In an exposure event, metal speciation directly impacts the fate of the contaminant and its absorption across a physiological membrane (e.g., skin) (Reeder et al., 2006). Modification of a contaminant chemical form due to physicochemical characteristics of skin surface film liquids (SSFLs) may influence the absorption and toxicity of the contaminant. Furthermore, the oxidation state of a metal or its association with a specific ligand can completely change its behavior (Reeder et al., 2006; Okereafor et al., 2020). It has been reported that As(V) is the dominant arsenic form in CCA-contaminated soils (Zagury et al., 2008). Cr is initially in its hexavalent state in the CCA solution. Following chemical reactions that occur during the fixation process of CCA to the wood, Cr(VI) is reduced to Cr(III) (Cooper & Ung, 1992). However, additional reduction or oxidation can take place once the metal(loid)s are in the soil (Dobran & Zagury, 2006). A supplementary complication in predicting the behavior of metal(loid)s is that

each species present different comportment, making generalizations of the ability to penetrate the skin, bioavailability, stability, and reactivity difficult (Nikpour & Hedberg, 2021). Computational tools such as Visual MINTEQ can provide estimations of metal speciation in solutions. This software is based on chemical equilibrium models, using state of the art descriptions of complexation reactions (Gustafsson, 2019); the software has the capacity to predict metal speciation with sound accuracy, therefore providing meaningful information about the behavior of metal(loid)s in well characterized solutions (Shahid et al., 2012).

In vitro bioaccessibility as an estimation for bioavailability is a cost-effective alternative over *in vivo* bioavailability with the added value of the ethical considerations inherent of not working with biological tissues (Guney & Zagury, 2014, 2016; Marin Villegas et al., 2019). The dermal bioaccessible fraction of metal(loid)s is the quantity that is dissolved in SSFLs and is available to penetrate through the skin (Hillwalker & Anderson, 2014; Stefaniak et al., 2014; Marin Villegas et al., 2019; Marin Villegas & Zagury, 2021).

For evaluating dermal permeation, the most popular technique is the Franz cell method (Franz, 1975). This method has been adapted to study the dermal permeation of a wide array of substances and vehicles (Pulsoni et al., 2022). In this context, the Franz cell methodology, combined with *in vitro* dermal bioaccessibility, can be used to determine the permeation profile (lag time and permeability coefficient (K_p)) of the bioaccessible fraction of metal(loid)s present in contaminated soils through the skin (Marin Villegas & Zagury, 2021). Permeation of metal(loid)s through the skin is a multifactorial process that is not fully understood. The human skin presents a wide variability in its composition; hence the permeation of metal(loid)s through it is not consistent (Hostynek & Maibach, 2006). Furthermore, permeation testing with human skin includes complications associated with storage, availability, and cost. For this reason, synthetic membranes to act as human skin surrogates have been developed. The Strat-M membrane (EMD Millipore, MA, USA) is a synthetic membrane with the capacity to mimic human skin in permeation studies of different substances (Haq et al., 2018a; Joshi et al., 2012; Uchida et al., 2015) and most recently in the permeation of metal(loid)s present in geological materials (Marin Villegas & Zagury, 2021).

Different vehicles have been adapted as the donor solution of the Franz diffusion cell, such as water, acetone, or synthetic sweat, making interstudy comparisons of permeability difficult. Nevertheless, the donor solution in permeation tests is crucial since it can affect the permeant

speciation and properties of the skin barrier (Hostynek, 2003; Anselm et al., 2022). Recently, it has been shown that physicochemical properties of synthetic sweat affect the bioaccessibility and permeation of metal(loid)s (Leal et al., 2018; Marin Villegas et al., 2019; Marin Villegas & Zagury, 2021). However, it is not clear how the synthetic sweat characteristics influence speciation and subsequent permeation of the bioaccessible metal(loid) fractions.

Thus, the present study aims to (1) determine and compare the *in vitro* dermal bioaccessibility of As, Cr, Cu, Ni, Pb, and Zn in one reference soil material and five field-collected CCA-contaminated soils using two synthetic sweat formulations, (2) perform speciation analysis of the bioaccessible fraction of metal(loid)s in synthetic sweat compositions using chemical equilibrium modelling (Visual MINTEQ), (3) evaluate the diffusion parameters of the bioaccessible fraction of these metal(loid)s through the Strat-M membrane, and (4) help elucidate how speciation affects permeation of the bioaccessible metal(loid) fractions.

5.2 Materials & methods

5.2.1 Soil sampling and sample characterization

Six soils have been characterized and subjected to dermal *in vitro* bioaccessibility tests to determine the bioaccessible concentration of As, Cr, Cu, Ni, Pb, and Zn and conduct geochemical modelling to evaluate the metal speciation in the bioaccessible solution; the certified soil material SQC001 (Sigma-Aldrich, lot number LRAC0025) produced according to ISO 1704 ($d < 425 \mu\text{m}$), and five field-collected soil samples ($d < 420 \mu\text{m}$) (S2, S3, S6, S7, and S8) collected at the surface (up to 10 cm depth) in the surroundings of Chromated copper arsenate (CCA)-treated utility poles in Montréal, Canada. Coarse material ($>2 \text{ cm}$) and topsoil vegetation were removed prior to sampling. The samples were collected using a plastic shovel and stored in zip-lock plastic bags. Containers and tools were washed with a phosphate-free detergent and soaked overnight in 10% (v/v) HNO_3 and rinsed with deionized water ($18.2 \text{ M}\Omega\cdot\text{cm}$). Field-collected soil samples were air-dried, gently disaggregated using a mortar and dry sieved to $420 \mu\text{m}$ using a sieve shaker (Retsch AS-200). Only the fine fraction of soils ($< 420 \mu\text{m}$) was considered for dermal exposure because this fraction covers most of soil particles that adhere to the skin (Leal et al., 2018; USEPA, 2004). Samples were then stored at 4°C prior to use (Gosselin & Zagury, 2020; Marin Villegas & Zagury, 2021).

Total metal(loid) content in soil samples was determined by acid digestion according to standard method 3030 (Clesceri, 1999) using HNO₃ (70% w/w), HF (50% w/w), and HClO₄ (70% w/w). Procedure blanks and duplicates were included. Concentrations of As, Cr, Cu, Ni, Pb and Zn were measured via ICP-OES (Varian Vista), detection limits (DLs) in mg/kg were 0.004, 0.001, 0.006, 0.001, 0.006, and 0.024, respectively. Parameters shown in Table 5.1 for soil samples S7, S8, and SQC001 were extracted from Marin Villegas & Zagury (2021).

Soil pH was measured in duplicates according to ASTM D4972-13 (ASTM, 2013) using a 1:2 solid-to-liquid ratio with deionized water (18.2MΩ cm) (pH meter: Eutech pH 200 series, probe: Accumet Ag/AgCl). The Tiessen & Moir (1993) method was applied to determine total organic carbon (TOC) content using a LECO furnace. Cation exchange capacity (CEC) was determined with the sodium acetate method with NaOAc 1N and NH₄OAc 1N described in Chapman (1965).

5.2.2 Artificial SSFLs

Two synthetic sweat and one synthetic sebum formulations have been prepared to simulate human SSFLs. The synthetic sweats sweat A (CEN, 2015) and sweat B (Wainman et al., 1994), with pH 6.5 and 4.7, respectively, were prepared according to Marin Villegas et al. (2019) and Marin Villegas & Zagury (2021). These formulations were selected to perform *in vitro* dermal bioaccessibility extractions and geochemical speciation modelling due to their differences in composition and pH. Moreover, sebum prepared according to Wertz (2008) and Marin Villegas et al. (2019) was used to coat the Strat-M membranes to imitate the hydrophobic properties caused by the presence of lipids on the skin during the permeation test. Composition of sweat formulations A and B, and of sebum is presented in Table 5.4 of the supplementary material.

5.2.3 *In vitro* dermal bioaccessibility test

To determine the bioaccessible concentration of metal(loid)s in synthetic sweat, 2.0 g of each soil sample was added to 20 mL of synthetic sweat in a 50 mL sterile polypropylene tube with an HDPE cap. Tubes containing the soil-synthetic sweat mixture were placed on an orbital shaker (51704 Series, Cole Parmer, radius 9.5 mm) and set at 100 rpm for 2 hours. The temperature was maintained at 36°C (mean skin temperature) by placing the shaker inside an incubator (Isotemp, Fisher Scientific). The tubes were then centrifuged at 10 000 x g for 10 minutes (Heraeus Megafuge 8, Thermo Fisher), and the supernatant was collected with 60 mL Luer-Lok syringes and filtered

with a 0.45 μm PVDF filter fitted to the syringe. The supernatant was gathered in sterile 50-mL Polypropylene centrifuge tubes with HDPE caps and stored at 4°C until analysis. Tests were performed in duplicates and in the presence of procedure blanks.

Samples were analyzed to determine As, Cr, Cu, Ni, Pb, and Zn (detection limits in mg/kg of 0.004, 0.001, 0.006, 0.001, 0.006, and 0.024, respectively) via ICP-OES (Vista, Varian Inc.). For further details of the dermal bioaccessibility protocol, see Leal et al. (2018), Marin Villegas et al. (2019), and Marin Villegas & Zagury (2021).

5.2.4 Geochemical modelling

Chemical equilibrium speciation calculations were done assuming open-system conditions using the software VMINTEQ 3.1 and its associated database (Gustafsson, 2019). The VMINTEQ thermodynamic database is a revised version of the original USEPA MINTEQA2 database (Allison et al., 2011) that contains updated and expanded data from the NIST Critical Stability Constant database (ver. 7). The speciation of soluble metal(loid)s in the sweat formulations A and B obtained after extracting each soil sample in duplicates was modelled using the concentration of metal(loid)s, pH, temperature, Eh, and concentration of main anions and cations in the solution. Binding of metal(loid)s to dissolved organic matter was not considered. The mean value for each parameter was used as the input parameter for the model. For all simulations, the charge imbalance was lower than 5%, indicating a suitable quality of the sweat chemistry analyses.

Bioaccessible solution samples were used to assess oxidation-reduction potential (ORP) values as input data for VMINTEQ. ORP was measured after centrifugation and filtration using a double-junction Ag/AgCl electrode (model Cole-Parmer GH-59001-77), calibrated with ORP standard solution (Orion, Thermo Scientific), and pH meter (Eutech pH 200 series). The ORP readings were reported in millivolts (mV) with respect to the standard hydrogen electrode (Eh). Anions and cations (and associated quantification limits in mg/L) (F^- (0.1), Cl^- (0.1), NO_2^- (0.2), Br^- (0.1), NO_3^- (0.2), SO_4^{2-} (0.2), PO_4^{3-} (0.2), Li^+ (0.2), Na^+ (0.4), NH_4^+ (0.2), K^+ (0.1), Mg^{2+} (0.2), Ca^{2+} (0.2)) were measured in the solution by ionic Chromatography (ICS 5000 AS-DP DIONEX Thermo Scientific).

5.2.5 Permeation test

Although dermal bioaccessibility of metal(loid)s is expected to be higher in sweat B than in sweat A mainly due to its lower pH, permeation tests were conducted using sweat A as the donor solution in agreement with our recent findings (Marin Villegas & Zagury (2021). That study reported that permeation of Cr and Cu was higher when extracted in sweat A (pH 6.5) than sweat B (pH 4.7), possibly due to a higher presence of Cr(VI) in sweat A and less permeable Cu species in sweat B (Marin Villegas & Zagury, 2021). Glass jacketed vertical Franz diffusion cells (PermeGear Inc.) with a 9 mm orifice diameter, 5 mL receptor volume, and 1 mL donor volume were used to evaluate the permeation of the bioaccessible fractions of metal(loid)s in sweat A. The receptor temperature was maintained at 37°C to simulate the temperature below the skin (Franken et al., 2015) by circulating water from a hot bath (Model 2849, Thermo Fisher Scientific) using an electric water pump (VicTsing NP-600). For mimicking the pH and salt concentration of the bloodstream, the receptor compartment was filled with 5 mL of Phosphate-Buffered Saline (PBS) solution (Fisher Scientific) at pH 7.4 and NaCl 8.0 g/L, KCl 0.2 g/L, Na₂HPO₄ 1.44 g/L, and KH₂PO₄ 0.24 g/L (Franken et al., 2015; Haq et al., 2018a). The receptor compartment was subjected to constant stirring (300 rpm) with a magnetic stirrer (Poly 15, Variomag). Twenty-five mm OD sterile Strat-M membranes (EMD Millipore) were used as a surrogate of human skin. Membranes were placed with the shiny side upwards, in contact with the donor compartment without pre-treatment (Haq et al., 2018b). To simulate the hydrophobic properties of the skin caused by the lipid fraction of the SSFL in the permeation of metal(loid)s, half of the membranes were coated with 0.1 mL of sebum. The test started by adding 1 mL of supernatant collected from the bioaccessibility test (sweat A) in the donor compartment. Tests were performed in duplicates and with blanks consisting of fresh synthetic sweat A as donor solution. Sampling was performed at 6, 8, 10, 12, 14 and 24 h by collecting the receptor solution with a 15 mL Luer-Lok syringe. Following sampling, the receptor compartment was rinsed by adding 5 mL of fresh PBS. Samples from the receptor were placed in 15 mL polypropylene tubes. The rinsing solution was added to the receptor sample in the same tube. After sampling and rinsing, the receptor compartment was refilled with 5 mL of fresh PBS solution. Blank cells received the same treatment. Samples were kept refrigerated at 4°C until analysis of metal(loid)s via ICP-AES (Vista, Varian Inc.). To model the permeation through the synthetic membrane, the concentration of metal(loid)s in the receptor (µg/L) was converted to the total metal(loid) that permeated (µg/cm²) and plotted against time. The flux (µg/cm² h) was

calculated as the slope of the steady-state region of the graph and the lag time as the intercept with the X-axis (h). To calculate the permeability coefficient K_p (cm/h), the flux was divided by the concentration in the donor solution (Larese et al., 2007; Marin Villegas & Zagury, 2021). Permeation results for soil samples S7, S8, and SQC001 were extracted from Marin Villegas & Zagury (2021).

For assessing the metal(loid) content that potentially remained in the membrane, the Strat-M membranes were subjected to acid digestion (HNO_3 (70% v/v), HCl (50% v/v), and HF (48% v/v) in Teflon beakers. Subsequently, the digestate was filtered with 0.45 μm filters (Whatman) and diluted to 100 mL. The diluted samples were then analyzed for total metal(loid) content using ICP-AES (Marin Villegas & Zagury, 2021). Mass balance was calculated by comparing the metal recovered from donor solution, metal content in the membrane and cumulative samplings of the receptor solution with the initial mass of metal present in the donor solution.

5.3 Results and discussion

5.3.1 Soil characterization

Soil characterization and metal(loid) concentrations are shown in Table 5.1. The values showed in bold exceeded C criterion C, Quebec regulatory limit for industrial land-use MELCC (2021).

Extensive As contamination was observed for S8 ($1639 \text{ mg/kg} \pm 6.8 \%$) (more than 30 times the C criterion of 50 mg/kg) and S7 ($311 \text{ mg/kg} \pm 1.1 \%$). Levels above the C criterion (500 mg/kg) were also observed for Cu in soil samples S6, S7, and S8. Although less problematic than As and Cu (Zagury et al., 2003), elevated concentrations of Cr in soil samples S2, S7, and S8 were observed. The soil reference material SQC001 had a lower content of Cr and Cu but a higher content of Pb and Zn than the field-collected CCA-contaminated soil samples. The total metal(loid) content of SQC001 was within $100 \pm 10\%$ of the certified values.

Table 5.1 Total Concentrations of As, Cr, Cu, Ni, Pb, and Zn (mg/kg), pH, Total Organic Carbon (TOC, w/w %), and Cation Exchange Capacity (CEC, meq 100/g) of Soils. Precision is Expressed as Mean $\pm$ Relative Standard Deviation %.

Parameter	S2	S3	S6	S7*	S8*	SQC 001*
As	172 ± 1.7	131 ± 3	195 ± 2.7	311 ± 1.1	1639 ± 6.8	173 ± 20

Cr	238 ± 0.5	145 ± 1.9	154 ± 1.4	371 ± 3.9	582 ± 14.3	124 ± 6.5
Cu	281 ± 0.6	209 ± 4.1	610 ± 5.5	824 ± 5.8	1070 ± 11.0	82 ± 1.9
Ni	19 ± 20.4	11 ± 2.3	13 ± 17.9	26 ± 11.5	223 ± 11.3	112 ± 9.8
Pb	59 ± 0.7	9.0 ± 13.6	5.0 ± 15.2	57 ± 8.8	80 ± 10.6	263 ± 2.5
Zn	342 ± 0.3	102 ± 0.8	81 ± 6.4	261 ± 7.3	223 ± 10.8	512 ± 4.0
pH	7.11 ± 0.02	7.39 ± 0.02	7.88 ± 0.01	7.2 ± 0.7	7.1 ± 0.5	5.7 ± 0.2
TOC	2.8	1.7	0.8	1.3 ± 2.1	2.9 ± 1.5	< 0.01
CEC	24.8 ± 0.9	18.1 ± 1.1	23.9 ± 0.9	15.5 ± 16.3	41.4 ± 2.8	12.8 ± 8.8

* From Marin Villegas & Zagury (2021).

The pH range (7.10-7.88) across the CCA-contaminated soil samples was narrow and slightly alkaline whereas pH was acidic for the reference soil SQC001 (pH 5.72). Low to moderate TOC content (< 2.9%) was measured for all soil samples. CEC values ranged from 12.8 (SQC001) to 41.4 (S8) meq/100g.

5.3.2 Bioaccessibility of metal(loid)s

While bioaccessible concentration in solution reveals the amount of metal solubilized in synthetic sweat, bioaccessible percentages (Table 5.5) can be helpful to compare the behavior of metal(loid)s from different soil samples. Since this study focuses on the permeation of metal(loid)s in synthetic sweat solution and its speciation, bioaccessibility results are expressed in mg/L in Table 5.2. In agreement with previous studies (Leal et al., 2018; Marin Villegas et al., 2019; Marin Villegas & Zagury, 2021), sweat B generally yielded higher bioaccessible concentrations since its lower pH increases the solubility of cationic metals (Marin Villegas & Zagury, 2021). Bioaccessible concentrations were generally low (below 1 mg/L) in sweat A except for Ni (2.29 mg/L) and Zn (20.6 mg/L) in SQC001. Much higher bioaccessible concentrations of Cr, Cu, Ni, Pb and Zn in sweat B were obtained in reference soil SQC001 compared to CCA-polluted soil samples. The overall higher bioaccessible metal concentrations obtained in SQC001 can be attributed to its lower pH, low total organic carbon content (< 0.01% w/w) and the fact that it has not experienced a natural ageing process, which reduces the bioaccessibility of metals in soils (Liang et al., 2016; Marin Villegas & Zagury, 2021). For a more detailed analysis of the influence of soil properties

and synthetic sweat formulation on the bioaccessibility of metal(loid)s, see Leal et al. (2018) and Marin Villegas et al. (2019).

Table 5.2 Bioaccessible Concentration (mg/L) of As, Cr, Cu, Ni, Pb, and Zn in Soils Using Synthetic sweat Formulations A and B.

Sweat A	Soil	As	Cr	Cu	Ni	Pb	Zn
	S2	0.22	0.03	0.04	< 0.01	0.03	0.16
	S3	0.14	0.02	0.06	< 0.01	0.04	0.27
	S6	0.61	0.02	0.05	< 0.01	0.02	0.13
	S7	0.45	0.05	0.38	< 0.01	< 0.01	0.01
	S8	0.76	0.04	0.41	< 0.01	< 0.01	< 0.01
	SQC001	0.01	0.02	0.17	2.29	0.09	20.6
Sweat B	S2	0.48	0.05	0.30	0.03	0.17	0.39
	S3	0.24	0.04	0.31	< 0.01	0.30	0.61
	S6	0.14	0.04	0.33	< 0.01	0.14	0.24
	S7	0.86	0.76	5.93	0.01	< 0.01	2.34
	S8	1.36	0.59	3.13	0.02	< 0.01	0.66
	SQC001	0.88	3.12	5.07	5.02	9.2	46.6

5.4 Chemical equilibrium modeling

Chemical equilibrium modelling was conducted on bioaccessible extracts (synthetic sweat with metal(loid)s in solution) following dermal bioaccessibility test conducted for soils S2, S3, S6, S7, S8, and SQC001 (n = 6).

5.4.1 Arsenic

In both sweats, arsenic was almost entirely found as arsenate (As(V)). The mean As(V) concentration (mol/L) was $6.36\text{E-}6 \pm 4.61\text{E-}6$ in sweat A and $1.42\text{E-}5 \pm 1.13\text{E-}5$ in sweat B contrasting with much lower As (III) concentrations of $3.55\text{E-}23 \pm 1.06\text{E-}23$ in sweat A and $3.27\text{E-}19 \pm 1.68\text{E-}19$ in sweat B. The As(V) present in sweat A contained $57\% \pm 1\%$ of H_2AsO_4^- and $42\% \pm 1\%$ of HAsO_4^{2-} . In sweat B, As was primarily present as H_2AsO_4^- ($98\% \pm 1\%$) and $< 2\%$ as HAsO_4^{2-} (Figure 5.1). Arsenic speciation is key to solubility and bioaccessibility (Beak et al.,

2006). Arsenate forms insoluble precipitates with common elements in soil such as iron, aluminum, and calcium (McLean & Bledsoe, 1992), thereby entailing overall low bioaccessibility.

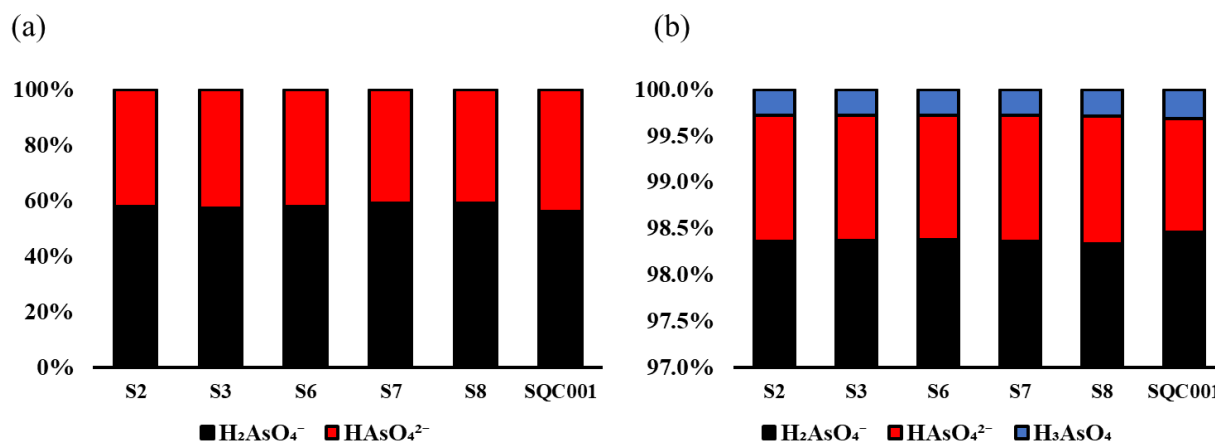


Figure 5.1 Speciation of As (V) (%): (a) in sweat A, (b) in sweat B

5.4.2 Chromium

In bioaccessible extracts obtained using sweat A, comparable concentrations of Cr(III) and Cr(VI) were found. The Cr(III) mean concentration in solution was $2.97\text{E-}7 \pm 2.21\text{E-}7$ mol/L whereas Cr(VI) mean concentration was $3.97\text{E-}7 \pm 2.69\text{E-}7$ mol/L. Cr(III) species were found in similar proportions in bioaccessible extracts from all study soils following extraction with sweat A (Figure 5.2a) with $38.2\% \pm 1.6\%$ of $\text{Cr}(\text{OH})_3$ (aq), $36.4\% \pm 1\%$ of $\text{Cr}(\text{OH})_2^+$ and $25\% \pm 1.5\%$ of CrOH^{2+} . The primary Cr(VI) compound found in the bioaccessible solutions following extraction with sweat A was CrO_4^{2-} (Figure 5.2b), ranging from 45.4% (SQC001) to 57.2% (S3), followed by HCrO_4^- , ranging from 20.5% (SQC001) to 29.2% (S7). CaCrO_4 (aq) was also present in a lower proportion for S2, S3, S6, S7, and S8 ($15\% \pm 2\%$) and a higher proportion in SQC001 (34.2%).

In the more acidic sweat B, Cr was mainly found as Cr(III), with a mean concentration of $1.79\text{E-}5 \pm 2.17\text{E-}6$ mol/L compared with $1.98\text{E-}11 \pm 7.51\text{E-}14$ mol/L of Cr(VI). For S2, S3, S6, S7, S8, a common trend was found, with CrOH^{2+} as the main Cr compound (Figure 5.2c.), with a range of 79.4% to 82.2%, followed by 14.0% to 14.5% of Cr^{3+} . In the bioaccessible (sweat B) extract of reference material SQC001, considerably different Cr(III) species were found with 67.8% of CrF_2^+ and 20.7% of CrF^{2+} (this bioaccessible extract was the only one containing fluoride above the detection limit). These fluoride containing Cr(III) species coincide with a much higher total Cr bioaccessibility measured in reference material SQC001 compared to field-collected CCA-

contaminated soils when extracted with sweat B (Table 5.2 and Table 5.5). On the other hand, Cr(VI) was mostly found as HCrO_4^- in sweat B with more than 90% in all bioaccessible extracts, followed by CrO_4^{2-} . Marin Villegas & Zagury (2021) suggested a possible higher concentration of Cr(VI) in sweat A (pH 6.5) than sweat B (pH 4.7) due to its higher pH. Indeed, speciation modelling results showed a much higher Cr(VI) concentration in sweat A ($3.97\text{E-}7 \pm 2.69\text{E-}7$ mol/L) than sweat B ($1.98\text{E-}11 \pm 7.51\text{E-}14$ mol/L).

Larese et al. (2007), Leal et al. (2018) and Marin Villegas et al. (2019) who studied the bioaccessibility of metals in synthetic sweat, all report that at a lower sweat pH, Cr bioaccessibility % increases. This being said, it has been demonstrated that an increase in sweat pH boosts the dermal permeation of Cr, due to potential oxidation of Cr(III) to Cr(VI), increasing the concentration of compounds with higher potential for permeation such as CrO_4^{2-} (Gammelgaard et al., 1992; Marin Villegas & Zagury, 2021).

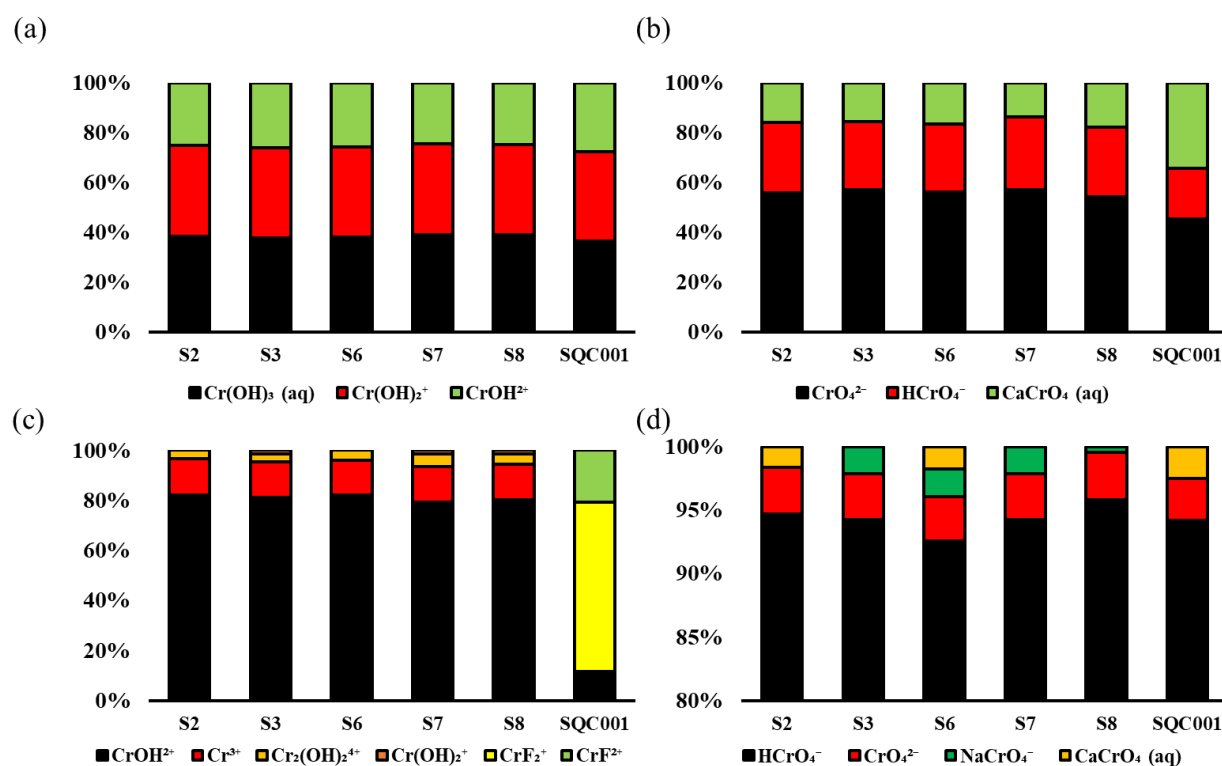


Figure 5.2. Chromium speciation (%): (a) Cr(III) in sweat A, (b) Cr(VI) in sweat A, (c) Cr(III) in sweat B, and (d) Cr(VI) in sweat B.

5.4.3 Copper

In sweat A, Cu was mainly present as Cu(II). The concentration of Cu(II) and Cu(I) in mol/L were $4.93\text{E-}6 \pm 1.18\text{E-}6$ and $4.93\text{E-}10 \pm 3.14\text{E-}10$, respectively. Most of Cu(II), was found as Cu^{2+} (Figure 5.3a) ranging from 57.4% to 69.3%, followed by CuHPO_4 (aq) (18.6% - 30.9%). CuOH^+ , CuCl^+ and CuSO_4 (aq) were also present but in much lower proportions (< 10%).

In soil extracts using sweat B, most of the Cu was also found as Cu(II). Mean concentrations (mol/L) of Cu(II) and Cu(I) were $5.28\text{E-}5 \pm 2.47\text{E-}5$ and $3.67\text{E-}8 \pm 1.80\text{E-}8$, respectively. Similarly to sweat A, the most abundant compound was Cu^{2+} with a proportion of $72.3\% \pm 19.2\%$. However, CuCl^+ at $27\% \pm 1\%$ was the next most abundant species in all soil bioaccessible extracts except again for SQC001 extract where Cu^{2+} was found in a much higher proportion (91.6%) (Figure 5.3b) and CuCl^+ at 8.15%. This much higher proportion of Cu^{2+} ion helps explain the 61.3 % bioaccessible percentage of Cu (Table 5.5) in the reference material when extracted with the more acidic sweat B compared to a significantly lower average dermal bioaccessibility of $2.6\% \pm 2.7\%$ for the five CCA-contaminated soils. Regarding Cu(I), most of it was found as CuCl_2^- in both sweats (> 90% in sweat A and > 80% in sweat B).

Although Cu permeation through human skin has been confirmed (Hostynek, 2004; Hostynek & Maibach, 2006; Pirot et al., 1996), its degree of absorption through the skin and its mechanism is uncertain due to the inconsistency in experimental conditions (Hostynek et al., 2011).

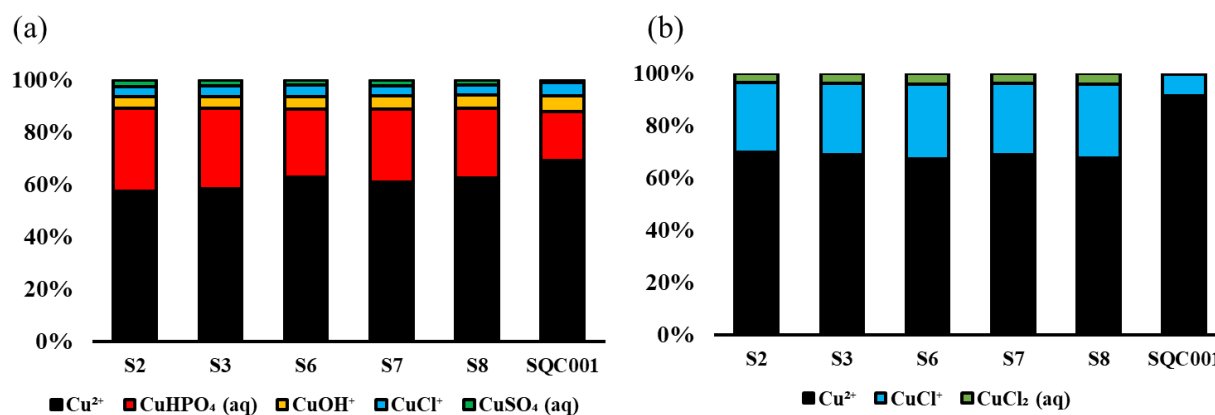


Figure 5.3 Copper speciation (%): (a) Cu(II) in sweat A (b) Cu(II) in sweat B

5.4.4 Nickel

When extracted with near neutral sweat A, Ni was not detectable in all bioaccessible extracts except for SQC001 where it was mainly present as Ni^{2+} (95.1%) (Figure 5.5). In sweat B, Ni was measured in very low concentrations (0.01-0.03 mg/L) following dermal bioaccessibility tests with soil samples S2, S7, S8, and at a higher concentration in SQC001 extract. In a similar trend to sweat A, most of Ni was Ni^{2+} (> 92.6%) in CCA-contaminated soils and 97.4% in SQC001 (Figure 5.5).

5.4.5 Lead

As shown in Table 5.2, bioaccessible Pb concentrations were rather low when soil samples were extracted with synthetic sweat A. Most of Pb in sweat A was found as PbCl^+ (50.4%-54.8%) and Pb^{2+} (37.8% - 40.0%). Other species such as $\text{PbCl}_2(\text{aq})$, $\text{PbSO}_4(\text{aq})$, and PbOH^+ were also present but in lower proportions (< 10%) (Figure 5.6).

In soil extracts obtained using sweat B, bioaccessible concentrations were higher (up to 9.2 mg/L for SQC001) except for soils S7 and S8 (< 0.01 mg/L). The majority of Pb was in the form of PbCl^+ (40.6% - 55.2%). In the SQC001 extract, Pb^{2+} was present in a high percentage (33.9%) but represented less than 6.3% of Pb species in field-collected soil extracts. $\text{PbCl}_2(\text{aq})$ represented $28.5\% \pm 0.5\%$ in the bioaccessible extracts of soils S2, S3, and S6 but only 8.5 % in reference soil SQC001 (8.5%) (Figure 5.6).

5.4.6 Zinc

Dermal bioaccessibility of Zn was particularly high in reference soil SQC001 when extracted with both synthetic sweats (40.3 % in sweat A and 91.0 % in sweat B (Table 5.5)). Zinc was primarily found as Zn^{2+} (> 80%) in sweat A (Figure 5.7). In sweat B, the most abundant species was also Zn^{2+} (44% - 48%) but followed by ZnCl^+ ($25\% \pm 1\%$) and $\text{ZnCl}_2(\text{aq})$ ($11\% \pm 1\%$) for all soils except SQC001. In SQC001 extract (sweat B), Zn^{2+} comprised more than 87 % of Zn (Figure 5.8) explaining the 91 % dermal bioaccessibility (Table 5.5).

5.5 Permeation

Since our previous work has shown that a high metal dermal bioaccessibility does not automatically imply synthetic skin permeation (Marin Villegas & Zagury, 2021), it is important to assess

permeation in addition to bioaccessibility for a more accurate input into human health risk assessment. It is also noteworthy to see how metal(loid) speciation can explain permeation of the bioaccessible fractions. Therefore, permeation through the synthetic membrane ($\mu\text{g}/\text{cm}^2$) was assessed and plotted against time (Figure 5.4). Bioaccessible As, Ni, Pb, and Zn extracted from the five field-collected CCA-contaminated soils and the reference soil did not permeate the synthetic skin membrane, corroborating results previously obtained with two field-collected soils (Marin Villegas & Zagury, 2021). Mass balance calculated from donor solution, synthetic membranes, and receptor solutions produced acceptable results. Recovery ranged from 58.1% to 118.4% for Cr and 71.3% to 128.4% for Cu for all analyzed samples. No remaining Cr and some Cu ($< 0.25 \mu\text{g}/\text{cm}^2$) was found in the membranes following digestion.

5.5.1 Chromium

Bioaccessible Cr extracted from all soil samples permeated through the synthetic skin (Table 5.3 and Figure 5.4). This was not unexpected as more than 50 % of Cr found in the bioaccessible solutions following extraction with sweat A was Cr(VI) and the primary Cr(VI) compound was CrO_4^{2-} (Figure 5.2b), a known skin penetrant (Hostynek et al., 1993). It is recognized that Cr(III) has a lower dermal permeability and therefore lower bioavailability than Cr(VI) (Franken et al., 2015; Gammelgaard et al., 1992; J. Hostynek, 2003; Van Lierde et al., 2006). Cr(III) ions are electrophilic and form stable complexes with dermal and epithelial tissues, which slow the diffusion through the skin. Chromate and dichromate (Cr(VI)), however, are negatively charged and do not tend to bind to organic substances (Hostynek, 2003)

More precisely, bioaccessible Cr extracted from 3 soil samples (S2, S8, and SQC001) permeated through the Strat-M membrane in the absence of sebum but bioaccessible Cr extracted from nearly all soil samples (S3, S6, S7, S8, and SQC001) permeated through the Strat-M membrane when it was coated with sebum (Figure 5.4). These findings confirm preliminary results reported by Marin Villegas & Zagury (2021). Sebum deposited on the synthetic skin might interact with Cr species in the bioaccessible extracts and probably foment formation of more permeable compounds. Nevertheless, there was no clear influence of sebum in the permeation kinetic parameters (Table 5.3). The mean flux ($1.4\text{E}-3$ – $1.9\text{E}-3 \mu\text{g}/\text{cm}^2 \text{ h}$), mean lag time (4.6–5.0 h) and mean K_p (0.077–0.07 cm/h) values were similar with and without sebum. However, the permeation coefficient (K_p) varied (by 2 orders of magnitude) between soil samples, possibly due to the differences in

concentration in the bioaccessible solution, and therefore variable Cr concentrations in the donor compartment of the Franz diffusion cells.

The USEPA (2004) recommends generic K_p values (cm/hr) for Cr (Cr(III): 0.001, Cr(VI): 0.002) in the same order of magnitude or lower than those found in the present study (from 0.004 to and 0.13 cm/h). These values have been adapted from Hostýnek et al. (1998) using human skin. Based on the variability between soil samples and on the observed influence of sweat formulation on Cr speciation and subsequent permeation, one should be cautious when using generic values.

Table 5.3 Flux ($\mu\text{g}/\text{cm}^2 \text{ h}$), Lag Time (h), and Permeation Coefficient (cm/h) for Cr and Cu (Data Expressed as Mean $\pm$ Standard Deviation)

		Cr			Cu		
		Flux ($\mu\text{g}/\text{cm}^2 \text{ h}$)	Lag Time (h)	K_p (cm/h)	Flux ($\mu\text{g}/\text{cm}^2 \text{ h}$)	Lag Time (h)	K_p (cm/h)
S2	No Sebum	$2.6\text{E-}03 \pm 2.2\text{E-}04$	5.6 ± 0.3	$6.3\text{E-}02 \pm 1.9\text{E-}05$	$6.8\text{E-}02 \pm 7.6\text{E-}03$	6.5 ± 0.5	$2.7\text{E-}01 \pm 3.0\text{E-}02$
	Sebum	-	-	-	$7.0\text{E-}02 \pm 6.3\text{E-}03$	6.4 ± 0.1	$2.8\text{E-}01 \pm 2.5\text{E-}02$
S3	No Sebum	-	-	-	$6.9\text{E-}03 \pm 2.6\text{E-}04$	3.2 ± 0.5	$2.4\text{E-}02 \pm 9.0\text{E-}04$
	Sebum	$1.9\text{E-}03 \pm 1.8\text{E-}04$	5.2 ± 0.3	$9.6\text{E-}02 \pm 1.6\text{E-}05$	$4.0\text{E-}02 \pm 2.6\text{E-}03$	1.5 ± 0.2	$1.4\text{E-}01 \pm 9.1\text{E-}03$
S6	No Sebum	-	-	-	$1.9\text{E-}02 \pm 1.4\text{E-}03$	6.7 ± 0.2	$6.3\text{E-}02 \pm 4.5\text{E-}03$
	Sebum	$1.2\text{E-}03 \pm 4.0\text{E-}05$	5.3 ± 0.6	$3.9\text{E-}03 \pm 1.3\text{E-}06$	-	-	-
S7*	No Sebum	-	-	-	-	-	-
	Sebum	$7.1\text{E-}04 \pm 6.5\text{E-}05$	4.4 ± 0.1	$1.3\text{E-}01 \pm 6.0\text{E-}06$	-	-	-
S8*	No Sebum	$1.4\text{E-}03 \pm 4.0\text{E-}05$	6.7 ± 0.3	$3.8\text{E-}02 \pm 1.1\text{E-}06$	$5.5\text{E-}02 \pm 1.8\text{E-}03$	0.2 ± 0.4	$1.3\text{E-}01 \pm 4.2\text{E-}03$
	Sebum	$1.5\text{E-}03 \pm 3.4\text{E-}05$	5.5 ± 0.5	$4.7\text{E-}02 \pm 7.6\text{E-}07$	$3.6\text{E-}02 \pm 2.3\text{E-}03$	4.6 ± 0.3	$8.8\text{E-}02 \pm 5.5\text{E-}03$
SQC001*	No Sebum	$1.7\text{E-}03 \pm 4.1\text{E-}04$	2.8 ± 0.6	$1.1\text{E-}01 \pm 9.9\text{E-}05$	$2.6\text{E-}02 \pm 4.2\text{E-}03$	0.6 ± 0.6	$1.5\text{E-}01 \pm 2.4\text{E-}02$
	Sebum	$1.7\text{E-}03 \pm 1.1\text{E-}04$	2.5 ± 0.2	$1.1\text{E-}01 \pm 7.5\text{E-}06$	$9.1\text{E-}02 \pm 2.5\text{E-}03$	5.9 ± 0.3	$5.2\text{E-}01 \pm 1.4\text{E-}02$
Mean	No Sebum	$1.9\text{E-}03 \pm 5.0\text{E-}04$	5.0 ± 1.6	$7.0\text{E-}02 \pm 3.0\text{E-}02$	$3.5\text{E-}02 \pm 2.3\text{E-}02$	3.5 ± 2.8	$1.3\text{E-}01 \pm 8.6\text{E-}02$
	Sebum	$1.4\text{E-}03 \pm 4.2\text{E-}04$	4.6 ± 1.1	$7.7\text{E-}02 \pm 4.6\text{E-}02$	$5.9\text{E-}02 \pm 2.0\text{E-}03$	4.6 ± 2.5	$2.6\text{E-}01 \pm 1.8\text{E-}01$

* From Marin Villegas & Zagury (2021)

5.5.2 Copper

Along with Cr, Cu was the only other trace element that permeated the membrane (Table 5.3 and Figures 5.4c and 5.4d). Copper extracted from all soil samples (S2, S3, S6, S7, S8, and SQC001) permeated the membrane (Figure 5.4) (It should be noted that Cu was also detected in the receptor solution following permeation with sample S7 extracted with Sweat A in the presence of sebum at the end of the sampling period ($t=12\text{h}$ and $t=24\text{h}$). However, there was not enough data to build the cumulative mass per area versus time curve). It should be repeated that Cu was mainly present as Cu(II) in sweat A and that Cu species were very similar in all soil extracts (Figure 5.3a). Moreover, most of Cu(II), was found as Cu^{2+} (Figure 5.3a) ranging from 57.4% to 69.3%, followed by

CuHPO_4 (aq) (18.6% - 30.9%) suggesting that some of these species permeate the Strat-M membrane. Interestingly, Marin Villegas & Zagury (2021) reported an absence of permeation through the synthetic Strat-M membrane when soil-bound Cu from S7, S8, and SQC001 was extracted with sweat B even though Cu concentration in sweat B was much higher than sweat A (Table 5.2). This suggests that Cu species present in sweat A and absent in sweat B might permeate the membrane. $\text{CuHPO}_4(\text{aq})$, CuOH^+ , and $\text{CuSO}_4(\text{aq})$ fulfill this role. Additionally, Pirot et al. (1996) and Hostynek & Maibach (2006) reported skin penetration of CuSO_4 present in emulsions and ointments at different concentrations. The presence of Cu(II) aqueous salts possibly governs the permeation of Cu through the Strat-M membrane.

Regarding sebum influence, K_p was higher when the membrane was coated with sebum than without sebum for soils S3 and SQC001. However, no clear trend was observed for other samples. Similarly, the influence of membrane coating on lag times did not follow a clear trend. For some samples, very short lag times were observed (0.2 h) whereas the lag time could reach 6.5 h for others. Overall, K_p was higher for Cu than Cr with values ranging from 0.024 to 0.52 cm/h depending on soil samples. Again, the present study values are higher than the USEPA (2004) generic K_p value for Cu of 0.001 cm/h.

Although some Cu was recovered from the membrane at the end of the experiment ($< 0.25 \mu\text{g}/\text{cm}^2$), Cu content in human skin following permeation reported by Franken et al. (2015) was much higher and ranged from 53-290 $\mu\text{g}/\text{cm}^2$. However, due to the differences in the experimental conditions, it is difficult to compare previously published values of Cu remaining in human skin with those accumulated in the Strat-M membrane. Further experiments incorporating human skin are recommended to assess the performance of the Strat-M vs human skin in the accumulation of metal(loid)s in the membrane following permeation tests.

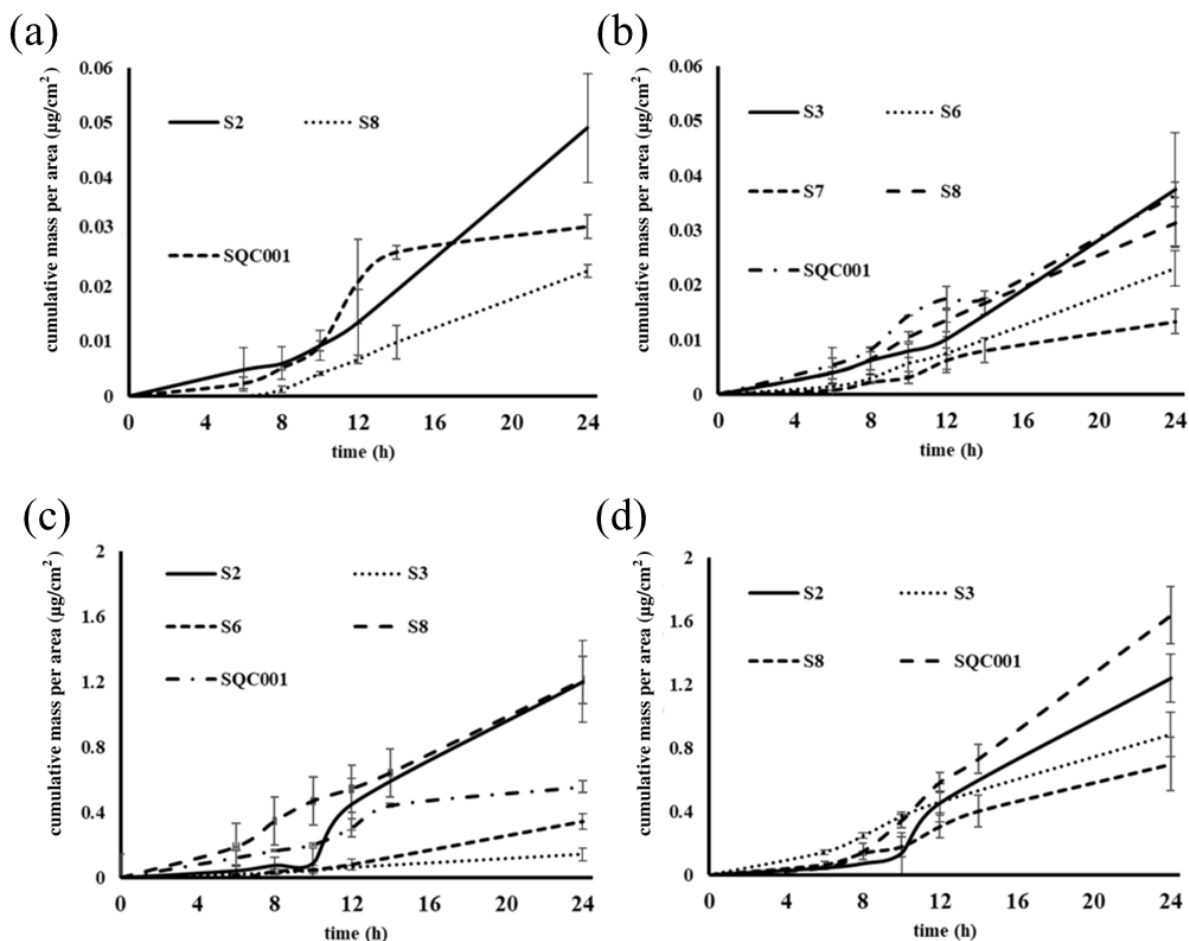


Figure 5.4 Mean Cumulative Mass per Area and Standard Deviation of Metal in sweat A (pH = 6.5) That Permeated Through the Strat-M Membrane for (a) Chromium (Not Coated with Sebum), (b) Chromium (Coated with Sebum), (c) Copper (Not Coated with Sebum), and (d) Copper (Coated With Sebum). Results of S7, S8, And SQC001 are Extracted from Marin Villegas & Zagury (2021)

5.6 Study limitations

It is essential to point out that Visual MINTEQ chemical speciation equilibria software does not provide information on reaction kinetics. Kinetics could be important, especially in chromium speciation, where even a short life of some compounds containing Cr(VI) can penetrate the membrane faster than Cr(III) (Nikpour & Hedberg, 2021). Additionally, the modelling presented in this paper did not consider dissolved organic carbon such as proteins, which might act as ligands for metal ions. Although the synthetic membrane Strat-M showed its usefulness for pilot and early-stage studies, additional testing using human skin is desirable to validate and correlate the

permeation characteristics through the Strat-M membrane. Moreover, coating the membrane with sebum seems to influence the permeation of some metal(loid)s such as Cr. However, the influence of sebum presence in the SSFL itself and the effect on bioaccessibility and permeation is yet to be established. Finally, more studies are needed to assess metal(loid) speciation (including interaction with organic sebum components) in SSFLs after extraction of contaminated soils of various origins.

5.7 Conclusions

This innovative study provides insights, for the first time, into how synthetic sweat characteristics influence the speciation and subsequent transdermal permeation of bioaccessible metal extracts from contaminated soils. Dermal bioaccessibility of metal(loid)s depends on sweat pH and formulation. Sweat B (pH 4.7) yielded the highest bioaccessibility and Sweat A (pH 6.5) the lowest. However, due to the different speciation profiles of metal(loid)s in each sweat composition, Cu could only permeate the synthetic membrane when extracted in sweat A and permeation coefficients of Cr were higher when dissolved in sweat A. This being said, higher solubility in sweat does not imply higher permeation through the skin.

Sweat composition influences the permeation of metal(loid)s through the human skin surrogate Strat-M. For instance, Cr(VI) is more pervious than Cr(III). The higher pH of sweat A (pH 6.5) promoted higher concentrations of Cr(VI) compounds (particularly CrO_4^{2-} , the better penetrant) compared to sweat B (pH 4.7). Regarding Cu, the only other metal that permeated the human skin surrogate in this study, speciation also explained the difference in permeation between sweat A and B. CuSO_4 (aq), CuHPO_4 (aq) and CuOH^+ were present in sweat A and absent in sweat B. These compounds have the potential to penetrate the skin as previously reported. Therefore, Cu was only able to permeate when present in sweat A. Vis-à-vis the other elements, although differences in speciation depending on the sweat used for the bioaccessibility test have been described, the fact that As, Ni, Pb and Zn did not permeate the membrane, makes concluding about the influence of speciation on permeation difficult.

Metal(loid)s are subject to speciation changes from their soil-bound state to their contact with SSFLs and subsequent absorption into the human body. Hence, efforts to understand the speciation of potentially toxic metal(loid)s are essential. For this reason, software that uses chemical equilibrium models to determine the speciation of potentially toxic metal(loid)s are a potent tool but analytical assessment of metal(oids) speciation in sweat is recommended to confirm results.

Unfortunately, to assess dermal exposure to potentially toxic metal(loid)s in soils, environmental agencies usually do not consider differences in oxidation state, valence, or species (except for Cr(III) and Cr(VI)). However, as shown in the present study, different metal(loid) species might produce different dermal bioavailability. Consequently, the use of generic metal(loid) K_p values might affect the reliability and accuracy of human health exposure assessments.

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5.8 Appendix A. Supplementary material

Table 5.4 Composition of artificial SSFLs formulations

Chemical, % (w/w)	Sweat A ^a (pH = 6.5)	Sweat B ^b (pH = 4.7)	Sebum ^c
Deionized water	99.3	94	-
Sodium chloride	0.5	2	-
Lactic acid	0.1	1.5	-
Urea	0.1	0.5	-
Acetic acid	-	0.25	-
Ammonium chloride	-	1.75	-
Squalene	-	-	12.4
Jobba oil	-	-	25
Triolein	-	-	44.6
Oleic acid	-	-	17
Vitamin E	-	-	1

^a According to standard EN 1811 (CEN, 2015), ^b According to standard NIHS 96-10 (Wainman et al., 1994), ^c According to Wertz (2009).

Table 5.5. Bioaccessible percentage (%) of As, Cr, Cu, Ni, Pb, and Zn in Soils Using Synthetic sweat formulations A and B

Sweat A	Soil	As	Cr	Cu	Ni	Pb	Zn
	S2	1.3	0.12	0.13	<0.4	0.5	0.5
	S3	1.1	0.16	0.3	<0.4	4.4	2.7
	S6	3.1	0.11	0.09	<0.4	4.3	1.6
	S7	1.4	0.15	0.5	<0.4	<0.2	0.1
	S8	0.5	0.06	0.4	<0.4	<0.2	<0.2
	SQC001	0.06	0.13	2.1	20.4	0.4	40.3
Sweat B	Soil	As	Cr	Cu	Ni	Pb	Zn
	S2	2.8	0.20	1.1	1.3	2.9	1.1
	S3	1.8	0.30	1.5	<0.4	31.6	6.0
	S6	0.7	0.24	0.5	<0.4	25.9	3.0
	S7	2.8	2.0	7.2	0.5	<0.2	9.0
	S8	0.8	1.0	2.9	0.8	<0.2	3.0
	SQC001	5.1	25.1	61.3	44.9	34.9	91.0

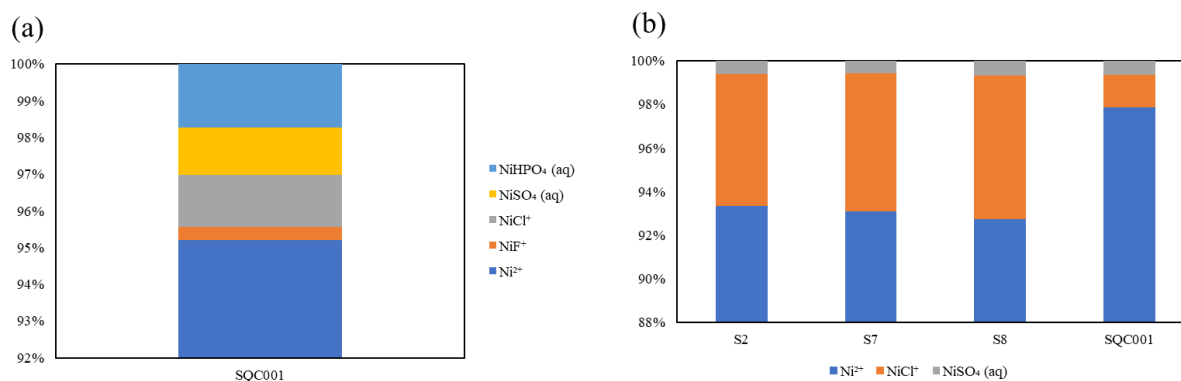


Figure 5.5 Nickel speciation (%) in: (a) Sweat A, (b) Sweat B

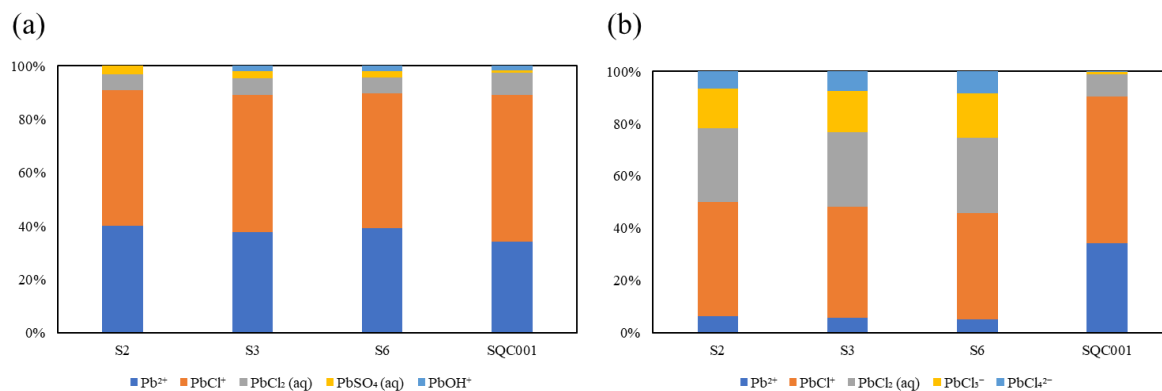


Figure 5.6 Lead speciation (%) in: (a) Sweat A, (b) Sweat B

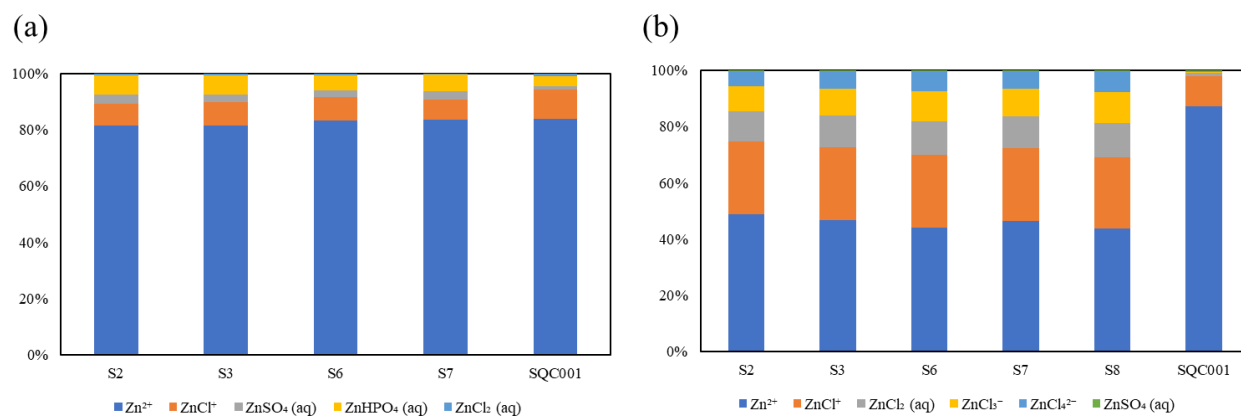


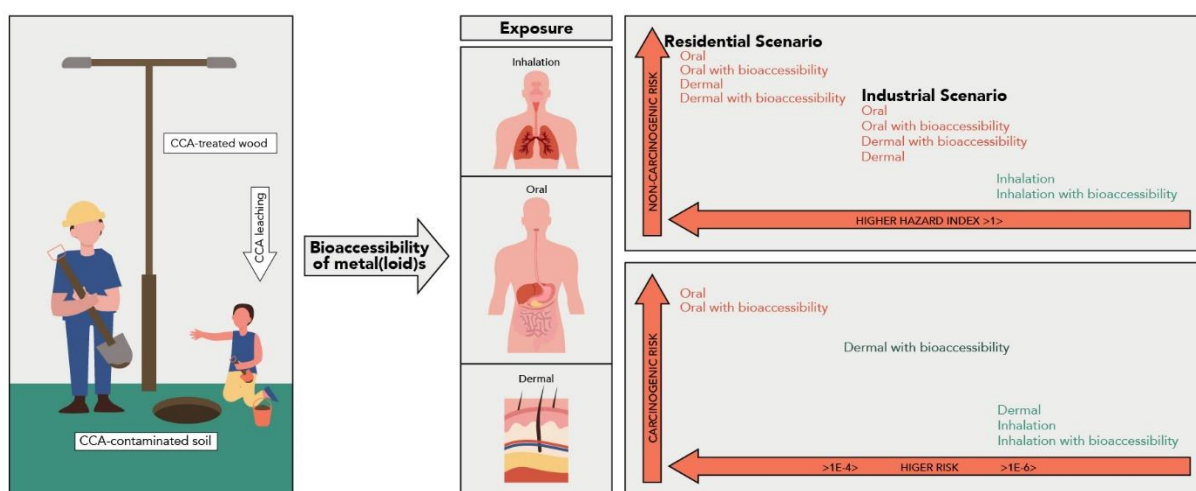
Figure 5.7 Zinc speciation (%) in: (a) Sweat A, (b) Sweat B

CHAPTER 6 ARTICLE 4 - INCORPORATING ORAL, INHALATION AND DERMAL BIOACCESSIBILITY INTO HUMAN HEALTH RISK CHARACTERIZATION FOLLOWING EXPOSURE TO CHROMATED COPPER ARSENATE (CCA)-CONTAMINATED SOILS

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ABSTRACT



Exposure to potentially toxic metal(loid)s (PTMs) in soil may happen via ingestion, inhalation, and dermal pathway. A more accurate risk characterization should consider PTM bioavailability. Using ten soil samples collected in the Montreal area (Canada) near CCA-treated utility poles, this study aims to characterize noncarcinogenic and carcinogenic human health risks associated with As, Cr, Cu, Pb, and Zn through a multipathway exposure approach. This innovative study incorporates, for the first time, the bioaccessible fraction of the metal(loid)s for three exposure routes and two different scenarios. For the residential and industrial scenarios, the oral and dermal pathways yielded a hazard index (HI) much higher than 1 with and without bioaccessibility considerations (range 1.7 – 349 without bioaccessibility and 0.8–134 with bioaccessibility), whereas the inhalation pathway caused a lower hazard ($HI < 1$). For the dermal pathway, the hazard quotient was higher when bioaccessibility of field-collected samples was considered due to inherent assumptions from

the US EPA soil approach to calculate the dermal dose. For carcinogenic risk, As and Pb were the most significant contributors to risk for the oral pathway, followed by the same elements for the dermal pathway. The overall carcinogenic risk was higher than the acceptable risk ($> 10^{-4}$) with and without bioaccessibility considerations (range $1.9\text{E-}4$ – $9.6\text{E-}3$ without bioaccessibility and $6.8\text{E-}5$ – $3.8\text{E-}3$ with bioaccessibility). Bioaccessibility tests provide a more accurate assessment of exposure to PTMs compared to total concentrations in soils.

6.1 Introduction

Contamination of soils by metal(loid)s caused by human activities is an unrelenting problem as it can pose a risk for human health. Arsenic (As), chromium (Cr), copper (Cu), lead (Pb), and zinc (Zn) have been identified among the most commonly found potentially toxic metal(loid)s in contaminated soils (van der Kallen et al., 2020; Wuana and Okieimen, 2011) and therefore they warrant particular attention.

Chromated copper arsenate (CCA) has been commonly used as a wood preservative until the 2000s (Zagury et al., 2003). This chemical preservative is sensible to weathering, leaching As, Cr, and Cu in various concentrations into adjacent soils (Zagury et al., 2003). Since many soils adjacent to CCA-treated wood poles are located near roads, the presence of Zn and Pb can be expected (Guney et al., 2010a). These metals(loid)s are relevant for risk assessment because of their potential toxicity. Arsenic and Cr (VI) are reported carcinogenic and listed as priority contaminants, and Cu can be toxic at elevated concentrations (ATSDR, 2019). Lead is also a soil contaminant that can cause various health issues such as adverse effects to the nervous and reproductive systems, hypertension, and immunological problems (Ara and Usmani, 2015; Gidlow, 2004).

In terms of risk assessment, the oxidation state of some metal(loid)s is crucial because it dictates its toxicity and mobility (Avasarala, 2021; McLean and Bledsoe, 1992). In fact, it has been shown that As(V) is the dominant As species in surface soils near CCA-treated wood poles (Zagury et al., 2008) but it is also the dominant inorganic dissolved species in gastrointestinal extracts following oral bioaccessibility tests (Girouard and Zagury, 2009; Nico et al., 2006). In CCA formulation, chromium is initially in its more toxic and soluble hexavalent form. However, chemical reactions during the fixation process of the preservative to the wood reduce Cr(VI) to Cr(III) (Cooper and Ung, 1992; van der Kallen et al., 2020) and Cr primary oxidation state in CCA-treated wood is the less toxic Cr(III) (Bull et al., 2000). In any case, reduction or oxidation may take place in-situ once

Cr and As leach into the soil depending on environmental conditions, such as pH, organic matter content, and soil composition, potentially leading to the presence of the more toxic As(III) species in soils (Balasoiu et al., 2001; Dobran and Zagury, 2006; Zagury et al., 2008).

Since people are nowadays frequently exposed to contaminated soils, increasing interest to accurately quantify the risk for human health has emerged, primarily related to children (Izquierdo et al., 2015) and workers. In past decades, soil contamination used to be evaluated by the total metal(loid)s concentration and compared with reference soil guideline values (Morton-Bermea et al., 2009; Zhang et al., 2013). It is now well known that this approach may overestimate health risks because it assumes that contaminants are 100% bioavailable. A more accurate health risk assessment for soils containing potentially toxic metal(loid)s should consider the bioaccessible fractions as an estimation for bioavailability when in vivo data is missing instead of using total metal(loid) concentration in soils (Ruby et al., 1999). Furthermore, bioaccessibility assessment should be performed for all important exposure pathways.

Exposure to hazardous contaminants in soil may happen via oral ingestion, inhalation, and dermal routes (USEPA, 1989, Madrid et al., 2007). Children are the most vulnerable demographic since they might swallow soil through mouthing contaminated hands and objects, eating contaminated food or consuming soil directly (Calabrese et al., 2021; Holmes et al., 1996). After the soil has been ingested or inhaled, only a portion of the soil-bound contaminant is bioaccessible and, therefore, available for solubilization in the gastrointestinal tract or in lung fluids. Regarding the dermal pathway, the soluble fraction of the soil-bound contaminant in skin surface film liquids (SSFL) can penetrate the skin and become available for systemic absorption (Stefaniak et al., 2014; Marin Villegas and Zagury, 2021).

Only a fraction of the bioaccessible contaminant will reach the systemic circulation and be considered bioavailable (Cave and Wragg, 2021; Guney et al., 2016; Ruby et al., 1996, 1999). For this reason, risk assessments that take into account the bioavailable concentrations would yield a more representative result of the exposure since overestimation would be prevented (van der Kallen et al., 2020; Rodriguez et al., 1999).

The bioavailability of a contaminant can be defined as "the extent to which a chemical can be absorbed by a living organism" (Cave and Wragg, 2021; Kelley et al., 2002). Moreover, oral bioaccessibility is defined as the fraction of a contaminant that is soluble in the gastrointestinal

environment and is available for absorption (Ruby et al., 1999) and related to soil, as the fraction of a contaminant that is released from a soil matrix in the human gastrointestinal system and is available for absorption (Nathanail and McCaffrey, 2003). An analogous definition can be written for the inhalation pathway replacing the gastrointestinal environment with the lung environment. Pertaining to the dermal pathway, bioaccessibility can be defined as the fraction of a contaminant that is dissolved in SSFL and is available for penetration through the stratum corneum to the underlying active epidermis (Hillwalker and Anderson, 2014; Stefaniak et al., 2014; Marin Villegas et al., 2019; Marin Villegas and Zagury, 2021).

Bioavailability can be determined by *in vivo* testing using animals such as juvenile swine or mouse, a practice that is time-consuming, expensive and raises ethical considerations. However, *in vitro* bioaccessibility tests can be used as a surrogate for bioavailability at a lower time, cost, and without raising ethical considerations (Zhao et al., 2021, Ruby et al., 1999; Kelley et al., 2002).

Although there are several published studies dealing with CCA-contaminated soils (Tardif et al., 2019; Guney et al., 2010b; Dang et al., 2003), a risk characterization considering a multi-pathway approach (oral, inhalation, and dermal), including bioaccessibility data from actual *in-vitro* testing for each pathway, is strongly lacking. First, a comparison of 10 field-collected soil samples in terms of physicochemical properties and metal(loid)s concentration (As, Cr, Cu, Pb, and Zn) is presented. Second, a risk characterization for a residential exposure scenario (which represent children's exposure during 2-hour play) and an industrial exposure scenario (which represents adult workers' exposure during an 8-hour shift) is performed with and without bioaccessibility assessment. Therefore, this study aims to characterize, for the first time, the non carcinogenic and carcinogenic human health risks of metal(loid)s present in CCA-contaminated soils using actual oral, inhalation, and dermal bioaccessibility data.

6.2 Materials & methods

6.2.1 Soil sampling

Ten soil samples (S1 to S10) have been collected at different locations near CCA-treated utility poles in the Montreal area (Quebec, Canada), at a maximum distance of 20 cm from the utility poles and at up to 10 cm from the soil surface. Coarse material (>2 cm) and surface vegetation were removed before sampling (Gosselin and Zagury, 2020; van der Kallen et al., 2020). All

implements used were washed with a phosphate-free detergent, soaked overnight in 10% (v/v) HNO_3 , and rinsed with deionized water (18.2 M Ω .cm). Soil samples were collected using a plastic shovel and stored in zip-lock plastic bags. All soil samples were air-dried at room temperature for 48 h and gently disaggregated and sieved (< 2 mm) for soil characterization (van der Kallen et al., 2020)

The soil samples were sieved into 250–90 μm and < 20 μm fractions using a sieve shaker (Retsch AS-200). For oral bioaccessibility tests, soil samples under < 250 μm are commonly used (Rodriguez et al., 1999). This soil fraction is also suitable for dermal bioaccessibility testing because it is below < 425 μm , so within the preferred range for dermal contact studies (Chaparro Leal et al., 2018). A fraction below 10 μm was primarily desired for inhalation bioaccessibility tests, but inherent limitations of dry sieving precluded it (Gosselin and Zagury, 2020; van der Kallen et al., 2020). Other authors reported similar circumstances and used PM₂₀ (< 20 μm) for inhalation bioaccessibility tests (Guney et al., 2017; Kim et al., 2014; Martin et al., 2018). Particle size distribution analysis performed by Gosselin and Zagury (2020) on the same samples showed that 7 out of 10 PM₂₀ samples had a PM₁₀ content higher than 50%.

6.2.2 Soil physico-chemical characterization

Total metal(loid) content in soil samples was measured using HNO_3 (70% w/w), HF (50% w/w), and HClO_4 (70% w/w) according to standard method 3030-I (Clesceri et al., 1999). Cr, Cu, Pb, and Zn concentrations were assessed via atomic absorption spectroscopy (AAS) (Perkin-Elmer A200). Detection limits (DLs) in mg/kg were 0.3, 0.3, 1.0, and 0.1, respectively. Arsenic content was measured via ICP-OES (Varian Vista), with a detection limit (DL) of 0.004 mg/kg. The pH of the soil samples was evaluated in duplicate in a solid-to-liquid ratio 1:2 with deionized water (pH meter: Eutech pH 200 series, probe: Accumet Ag/AgCl) following method ASTM D4972–13 (ASTM, 2013). Total organic carbon content was analyzed using a LECO furnace. Infrared determination of CO_2 was achieved to determine organic carbon content as a difference between total and inorganic carbon (Tiessen and Moir, 1993). Cation exchange capacity (CEC) was determined following the sodium acetate method (Chapman, 1965). For more details regarding methods used for soil characterization, see Gosselin and Zagury (2020), Marin Villegas and Zagury (2021), and van der Kallen et al. (2020).

6.2.3 Bioaccessibility tests

6.2.3.1 Oral bioaccessibility

The bioaccessible oral fraction (Bio_{oral}) (Equation 56.1) of a soil-bound contaminant can be calculated as the ratio of the fraction soluble in simulated gastrointestinal fluids ($C_{bio\ oral}$) (mg/kg) to the total concentration of contaminant in a soil sample (C_{total}) (mg/kg) (Guney et al., 2010b; Ruby et al., 1999).

$$Bio_{oral} = \frac{C_{bio\ oral}}{C_{total}} \quad (6.1)$$

Relative oral bioavailability (RBA) refers to the comparative bioavailability of different forms of a substance or different exposure media containing the substance (Ruby et al., 1999). RBA can be estimated using in vitro oral bioaccessibility values (Equation 6.1) when methods are validated and in vivo data is missing. In the present study, RBA values for As and Pb were calculated using the validated in vitro gastrointestinal model gastric (IVG-G) linear regressions presented in Juhasz et al. (2014) for As and published in Juhasz et al. (2013) for Pb. Although the in vitro gastrointestinal full model including the intestinal phase (IVG-GI) is more physiologically relevant than the IVG-G model, the latter was selected because the linear model has a better R^2 for As RBA prediction. Moreover, Juhasz et al. (2013) only provide regressions to calculate Pb RBA using the IVG-G model.

Oral bioaccessibility results of the gastric phase (IVG-G) were obtained from van der Kallen et al. (2020). Briefly, the IVG-G method consisted of adding 1 g of soil sample (fraction 250–90 μ m) into 150 mL of gastric solution poured in a 250 mL beaker and placed in a water bath (Thermo Scientific, model 2849) at 37 °C. The gastric solution's pH was adjusted and maintained at 1.8 using HCl at constant stirring (100 rpm). After 1 h, 20 mL of gastric solution was collected and filtered (Thermo Scientific 0.45 μ m) for Cr, Cu, Pb, and Zn content analysis via AAS. As content was determined via ICP-OES. The standard reference material SRM NIST 2710 (National Institute of Standards and Technology, Montana I powdered soil) was also subjected to oral bioaccessibility test and showed consistent results with previous studies. For more details, see van der Kallen et al. (2020).

6.2.3.2 Inhalation bioaccessibility

To mimic the fluids found in the pulmonary environment, the most widely used synthetic lung fluids (SLFs) are Gamble's solution (GS) and artificial lysosomal fluid (ALF) (Wiseman, 2015). GS is a neutral salt solution (pH=7.4) used to mimic extracellular fluid, whereas the more acidic ALF (pH=4.5) simulate conditions encountered during macrophage phagocytosis of inhaled fine particulate matter (Guney et al., 2017; Zoitos et al., 1997).

The bioaccessible fraction (Bio_{inh}) for the inhalation pathway can be defined using equation 6.2:

$$Bio_{inh} = \frac{C_{bio\ inh} * V}{C_{total} * m} \quad (6.2)$$

Where C_{total} is the concentration of the contaminant in the soil sample (mg/kg) of the appropriate soil fraction (<20 μ m, in the present study); m , the mass of soil sample (g); $C_{bio\ inh}$, the concentration of the contaminant in the lung fluid (mg/l); and V , the volume of lung fluid (mL) used in the inhalation bioaccessibility test (Guney et al., 2017).

Inhalation bioaccessibility results were obtained from Gosselin and Zagury (2020). Only the results from ALF bioaccessibility will be further considered in this paper (ALF results yielded higher, thus more conservative, bioaccessibility values). Briefly, 0.4 g of the soil sample (< 20 μ m) was added to 40 mL of pre-conditioned ALF (37 °C) in centrifuge polypropylene tubes. The tubes were then placed on a benchtop orbital shaker (Cole-Parmer 51704 Series, radius 9.5 mm) at 100 rpm inside an incubator (Fisher Scientific, Isotemp) at 37 °C for 24 h. The tubes were then centrifuged (Thermo Fisher Heraeus Megafuge 8) at 10,000 x g for 10 min, and supernatants were collected and filtered using a 0.45 μ m PVDF filter (Thermo Scientific) syringe before analysis of Cr, Cu, Pb, and Zn content via AAS. As content was determined via ICP-OES. The reference material BGS 102 (Ironstone Soil, $d < 40 \mu$ m, from British Geological Survey) was used in the inhalation bioaccessibility test, showing consistent results with previously published studies. For more details about the inhalation bioaccessibility procedure, see Gosselin and Zagury (2020) and Guney et al. (2017).

6.2.3.3 Dermal bioaccessibility

The dermal bioaccessibility fraction (Bio_{dermal}) can be calculated using equation 6.3 (Chaparro Leal et al., 2018).

$$Bio_{dermal} = \frac{C_{bio\ dermal}}{C_{total}} \quad (6.3)$$

Where $C_{bio\ dermal}$ is the bioaccessible concentration (mg/kg), and C_{total} (mg/kg) is the concentration of the contaminant in the soil sample in the relevant soil fraction (250–90 μm in the present study).

The USEPA recommends two approaches to estimate the dermal absorbed dose (DAD) depending on the contaminant matrix: (1) contact with soil and (2) contact with water. Both strategies consider total metal (loid) concentrations ((mg/kg) in the soil approach, and (mg/cm³) for the water approach).

Spalt et al. (2009) reviewed the dermal absorption of environmental contaminants from soils and sediments. They suggested that the soil approach presented in USEPA (2004) is not recommendable because the methodology is dependent on results from relatively few empirical investigations of dermal absorption of soil contaminants and does not provide recommendations for all types of compounds.

The USEPA water approach was initially designed to assume dermal contact with contaminated water. In this approach, the DAD is calculated by determining the migration rate of a chemical through the skin using the permeability coefficient (K_p) as the parameter that dictates the migration rate (USEPA, 2004). This methodology has been recently adapted by our research group for dermal contact with soils by using bioaccessible metal(loid) concentrations obtained using synthetic sweat instead of water-soluble metal(loid) concentrations (Chaparro Leal et al., 2018).

Dermal bioaccessibility tests were started by adding 20 mL of synthetic sweat NIHS-9610 at pH 4.7 (Marin Villegas et al., 2019; Marin Villegas and Zagury, 2021) to 2 g of the soil sample (250–90 μm) in 50 mL sterile polypropylene tubes with HDPE caps. Tubes containing the soil and sweat mixture were placed on an orbital shaker at 100 rpm inside an incubator at 36 °C for 2 h for the children's exposure scenario and for 8 h to represent the industrial scenario (USEPA, 2004). The tubes were then centrifuged at 10,000 x g for 10 min, and the supernatant was collected with syringes and filtered with a 0.45 μm PVDF filter fitted to the syringe. The filtered supernatant was collected into 50-mL polypropylene centrifuge tubes with HDPE caps and stored at 4 °C. Samples were analyzed via ICP-OES to determine As, Cr, Cu, Pb, and Zn, with detection limits (mg/kg) of 0.004, 0.001, 0.006, 0.006, and 0.024, respectively. The reference material SQC 001 (Sigma-

Aldrich, lot: LRAC0025, $d < 425 \mu\text{m}$) was also used during dermal bioaccessibility tests, showing consistent results with previous studies. For more details about the dermal bioaccessibility protocol, see Chaparro Leal et al. (2018), Marin Villegas et al. (2019), and Marin Villegas and Zagury (2021).

6.2.4 Risk characterization

6.2.4.1 Non-carcinogenic risk characterization

Non-carcinogenic hazards were evaluated for the three exposure pathways. Two scenarios were considered: the hazard exposure for children (residential scenario) and an industrial exposure scenario simulating a worker exposition for 8 h (industrial scenario). The CDI (mg / kg day) represents the estimated chemical daily intake for the oral route, the DAD (mg / kg day) represents the dermal absorbed dose for the dermal route and, the exposure concentration EC (mg / m³) represents the inhalation route. Equations (6.4)–(6.6) can be used to calculate CDI, DAD and EC (USEPA, 1989; USEPA, 2004; and USEPA, 2009). The descriptions of symbols and specific values are shown in Table 6.1.

$$CDI = \frac{C_{total} * IngR * EF * ED * 10^{-6}}{BW * AT} \quad (6.4)$$

$$DAD = \frac{DA_{event} * EV * ED * EF * SA}{BW * AT} \quad (6.5)$$

$$EC = \frac{C_{total} * ET * EF * ED}{PEF * ATh} \quad (6.6)$$

The CDI, DAD, and EC can be divided by the reference dose for the oral or dermal pathways (RfDo, RfDABS) and reference concentration (RfCi) for the inhalation pathway. These values are contaminant-specific and can be used to calculate the Hazard Quotient (HQ) using Equation 6.7, which measures the non-carcinogenic risk for an exposure route and a contaminant. It is assumed that all the risks associated with the metal (loid)s are additive supposing independence of action by the metal(loid)s involved. The HQ can be used to calculate the cumulative noncarcinogenic risk, expressed as the Hazard Index (HI) (Equation 6.8). The higher the value of HQ, the highest the hazard. Values of $HQ < 1$ suggest the unlikelihood of harmful health impacts.

Some parameters used to calculate dose and concentration using Equations (6.4 to 6.6) depend on specific exposure scenarios. This information is not always available, forcing the risk assessors to

use generic values. For instance, the soil-to-air particulate emission factor (PEF) represents the relationship between concentrations in soil and concentrations in the air due to particle suspension (USEPA, 2002). It is used in the calculation of the EC. The PEF depends on the inverse ratio of the air concentration to the emission flux, the fraction of vegetative cover, mean annual wind speed and direction, soil particle size, and concentration in air. This information is not always available for a specific site, so generic PEF is often used, increasing uncertainty.

Table 6.1 Parameters values for estimating human exposure to CCA-contaminated soils.

Variable	Value	Reference
C_{total} (mg / kg) concentration of metal(loid) in soil	See Table 6.3.	-
ABSd (unitless) dermal absorption factor	0.03 for As and 0.001 for other elements	USEPA, 2011 USEPA, 2004
AF (mg / cm ²) soil to skin adherence factor	0.2	USDOE, 2011 USEPA, 2004
AT (d) averaging time	2190 for Residential, 9125 for Industrial, 25550 for carcinogenic risk	USDOE, 2011
ATh (h) averaging time	52560 for Residential, 219000 for Industrial, 613200 for carcinogenic risk	USEPA, 2009
BW (kg) average body weight	15 for Residential, 70 for Industrial	USEPA, 1989
EV (event / day) event frequency	1 event per day for Residential and Industrial	USEPA, 2004 USEPA, 2011
ED (yr) exposure duration	6 for Residential, 25 for Industrial, 70 for carcinogenic risk	USDOE, 2011
EF (d / yr) exposure frequency	260 for Residential, 219 for Industrial	USDOE, 2011
ET (h / day) exposure time	2 for Residential, 8 for Industrial	USEPA 2011
IngR (mg / d) soil ingestion rate	100 for Residential, 50 for Industrial	USEPA, 2011
PEF (m ³ / kg) soil-to-air particulate emission factor	1.36E+09	USEPA, 2002

SA (cm ²)		USDOE, 2011
skin surface area available for exposure	2834 for Residential, 1575 for Industrial	
Kp (cm / h)	1E-3 for As, Cr(III), and Cu. 1E-4 for Pb,	USEPA, 2004
permeability coefficient	6E-4 for Zn	

USEPA methodologies lack dermal toxicity factors. Instead, there is an oral-to-dermal extrapolation for systemic effects. This methodology considers that oral reference doses (RfDs) are expressed as the mass of substance administered per unit of time and body weight, whereas exposure estimates for the dermal pathway are expressed in absorbed dose (USEPA, 2004). The extrapolation methodology uses dose-response relationships from oral administration studies and adjusts for absorption effects to represent toxicity factors in terms of dose absorbed.

USEPA (2004) provides recommended values for gastrointestinal absorption values (ABSGI). When compounds have no gastrointestinal absorption values, assuming 100% (worst case scenario) is recommended. This assumption contributes to an overestimation of the risk for compounds that are poorly absorbed.

The dermal reference dose based on absorbed dose is expressed by Equation 6.9, where RfD_{ABS} (mg / kg day) is the chemical-specific absorbed reference dose, RfD_o (mg / kg day) the chemical-specific oral reference dose, and ABSGI the compound-specific fraction of contaminant absorbed in the gastrointestinal tract from toxicity studies (USEPA, 2004). Values for RfD_o and ABSGI are shown in Table 6.2.

$$HQ = \frac{CDI, DAD, EC}{RfD (oral, dermal), RfCi (inhalation)} \quad (6.7)$$

$$HI = \sum HQ \quad (6.8)$$

$$RfD_{ABS} = RfD_o * ABSGI \quad (6.9)$$

Hazard characterization was performed with and without considering bioavailability/bioaccessibility values. To calculate the CDI with bioavailability considerations for As and Pb for the oral pathway, As and Pb RBAs (calculated using published linear regressions as previously explained) were multiplied by the corresponding oral CDI. For Cr, Cu, and Zn, the bioaccessible fraction was multiplied by the CDI instead. Similarly, the inhalation EC with

bioaccessibility considerations was calculated by multiplying the inhalation EC by the inhalation bioaccessible fraction for each metal(loid). For the dermal pathway, the soil approach was used to calculate the DA_{event} without actual dermal bioaccessibility data (Equation 6.10 and Table 6.1) but to incorporate dermal bioaccessibility, the water approach was used instead. In this case, DA_{event} was calculated using equation (Equation 6.11) where DA_{event} is the dermal absorbed dose per event (mg / cm² event); C_w is the bioaccessible concentration of the metal(loid) in synthetic sweat solution NIHS-9610 (mg / cm³); K_p is the dermal permeability coefficient of the metal(loid) (cm / h); and t_{event} (h) is the event duration (2 h or 8 h / event) (USEPA, 2004).

$$DA_{event} = C_{total} * CF * AF * ABS_d \quad (6.10)$$

$$DA_{event} = K_p * C_w * t_{event} \quad (6.11)$$

Once DA_{event} has been calculated using C_w obtained from bioaccessibility tests, DAD was determined (Equation 6.5) using the specific parameters (Table 6.1) for the evaluated scenario following the methodology presented by USEPA (2004).

Table 6.2 Toxicological properties of metal(loids) for health risk assessments.

Factor	RfDo	RfCi	ABS _{GI}	SFO _{oral}	IUR	SF _{ABS}
Units	(mg / kg day)	(mg / m ³)	(-)	(mg / kg d) ⁻¹	(µg / m ³) ⁻¹	(mg / kg d) ⁻¹
As	3.00E-04	1.50E-05	0.03	1.50E+00	4.30E-03	4.50E-02
Cr(III)	1.50E+00	-	0.013	-	-	-
Cr(VI)	3.00E-03	1.00E-04	0.025	5.00E-01	8.40E-02	1.30E-02
Cu	4.00E-02	6.00E-06	0.2	-	-	-
Pb	3.50E-03	3.50E-03	0.2	8.50E-03	1.20E-05	1.70E-03
Zn	3.00E-01	3.00E-01	0.2	-	-	-
References	USDOE, 2011	USEPA, 2011	USEPA, 2011	USDOE, 2011	USDOE, 2011	Calculated

Due to the differences in toxicity and mobility between Cr(III) and Cr (VI), the USEPA proposes different RfDo for Cr(III) and Cr(VI). Cr bioavailability depends on its redox state, Cr(III) showing lower oral bioavailable percentages than Cr(VI) (Kelley et al., 2002). Moreover, Stewart et al. (2003) observed that Cr(VI) bioaccessibility is strongly influenced by its reduction to Cr(III) during the gastric phase. In the presence of organic matter and under acidic conditions, Cr(VI) is reduced to Cr(III), and Cr(III) is bound to the soil-solid phase yielding low bioaccessibility (Pouschat and Zagury, 2008; Stewart et al., 2003). Therefore, the risk characterization was performed assuming

Cr(III) species are dominant in soil. This assumption is supported by the low bioaccessibility percentages (Figure 6.1) in surrogate physiological fluids. Table 6.3.

6.2.4.2 Carcinogenic risk characterization

Cancer risk was calculated for As and Pb and then added for each pathway. The dose for As and Pb was multiplied by the corresponding carcinogenic slope factor (CSF). SFO_{oral} is used for the oral route, IUR for the inhalation and SFABS for the dermal route (Table 6.2). This product expressed in Equation 6.12 is the level of excess lifetime cancer risk. Although reactions between contaminants could interact synergistically, it was assumed that all the metal(loid) risks were additive (USEPA, 1989). Carcinogenic risk is expressed as the total cancer risk (Equation 6.13). Risks lower than 10^{-6} (probability of 1 in 1,000,000 of an individual developing cancer) are considered acceptable, and cancer risk above 10^{-4} is deemed to be unacceptable (Guney et al., 2010b; USEPA, 2011).

Additionally, to assess a worst-case scenario, total cancer risk assuming chromium is present in soils as Cr(VI) was also calculated but not included in the total risk calculation.

$$Risk = CDI \text{ or } DAD \text{ or } EC * CSF \quad (6.12)$$

$$Total Risk = \sum Risks = Risk_{oral} + Risk_{inhalation} + Risk_{dermal} \quad (6.13)$$

6.3 Results and discussion

6.3.1 Soil characterization

Complete soil characterization for the study soil samples is described in details in Gosselin and Zagury (2020); and van der Kallen et al. (2020). To summarize, particle size fractions influenced the metal(loid) concentration of As, Cr, Cu, Pb, and Zn (Table 6.3) since smaller particles (< 20 μm) had higher metal(loid) concentrations (van der Kallen et al., 2020).

Overall, an extensive contamination by As was detected. From all the soil samples, only S10 presented an As concentration under Quebec regulatory limit (C Criterion) for residential use (30 mg / kg) (MDDELCC, 2021). Furthermore, very high As concentrations were detected in S2, S7, and S8 samples in both fractions. Soil sample S8 in the 250–90 μm fraction presented higher concentrations of Cr than the residential criterion (250 mg / kg) and in S7, and S8 samples,

measured concentrations in the $< 20 \mu\text{m}$ fraction were higher than the industrial land use criterion (800 mg / kg). Regarding Cu, for the soil samples S7, S8, in the 250–90 μm fraction, the concentration was higher than the criterion for industrial land use (500 mg / kg). Also, for both fractions, all soil samples except for S10 in the 250–90 μm fraction, showed Cu contents higher than the residential criteria (100 mg / kg). Not surprisingly for CCA-contaminated soils, all samples were below the criteria for residential land use for Pb and Zn content.

Table 6.3 Total As, Cr, Cu, Pb, and Zn concentrations (mean $\pm$ standard deviation ($n = 2$)) in CCA-contaminated soils in 250–90 μm , and $< 20 \mu\text{m}$ fractions.

Soil sample	As (mg/kg)		Cr (mg/kg)		Cu (mg/kg)		Pb (mg/kg)		Zn (mg/kg)	
	250-90 μm^a	$< 20 \mu\text{m}^b$	250-90 μm^a	$< 20 \mu\text{m}^b$	250-90 μm^a	$< 20 \mu\text{m}^b$	250-90 μm^a	$< 20 \mu\text{m}^b$	250-90 μm^a	$< 20 \mu\text{m}^b$
S1	130 $\pm$ 8.2	435 $\pm$ 3.2	118 $\pm$ 0.6	166 $\pm$ 1	331 $\pm$ 17	650 $\pm$ 28	49 $\pm$ 0.6	52 $\pm$ 2	107 $\pm$ 1.9	207 $\pm$ 1.5
S2	180 $\pm$ 1.1	847 $\pm$ 106	175 $\pm$ 3.5	665 $\pm$ 32	258 $\pm$ 7.8	454 $\pm$ 31	95 $\pm$ 1.7	170 $\pm$ 7.7	347 $\pm$ 1.4	832 $\pm$ 26
S3	149 $\pm$ 0.9	405 $\pm$ 6.2	174 $\pm$ 9.4	324 $\pm$ 23	272 $\pm$ 8.8	402 $\pm$ 0	40 $\pm$ 3	47 $\pm$ 0.2	125 $\pm$ 1.4	259 $\pm$ 3.4
S4	102 $\pm$ 0.4	167 $\pm$ 0.9	173 $\pm$ 2.6	228 $\pm$ 8	219 $\pm$ 1.2	188 $\pm$ 9	81 $\pm$ 1.2	89 $\pm$ 3.4	157 $\pm$ 4.5	205 $\pm$ 5
S5	135 $\pm$ 2.9	340 $\pm$ 6.5	60 $\pm$ 2.3	121 $\pm$ 0.3	180 $\pm$ 8.9	448 $\pm$ 3.7	35 $\pm$ 3.4	63 $\pm$ 2.1	37 $\pm$ 2.4	79 $\pm$ 1.1
S6	151 $\pm$ 4.7	288 $\pm$ 3	133 $\pm$ 3.0	142 $\pm$ 0.5	402 $\pm$ 1.9	517 $\pm$ 11	38 $\pm$ 0.5	49 $\pm$ 0.3	28 $\pm$ 2	160 $\pm$ 0.1
S7	164 $\pm$ 0.9	2536 $\pm$ 89	232 $\pm$ 8.5	3815 $\pm$ 403	642 $\pm$ 38	3797 $\pm$ 15	51 $\pm$ 0.5	139 $\pm$ 0.8	108 $\pm$ 4.5	1058 $\pm$ 42
S8	1397 $\pm$ 69	5857 $\pm$ 95	616 $\pm$ 14	2804 $\pm$ 94	1097 $\pm$ 32	2906 $\pm$ 158	109 $\pm$ 8.1	174 $\pm$ 2.6	140 $\pm$ 4.1	560 $\pm$ 0.2
S9	120 $\pm$ 8.1	360 $\pm$ 4.1	119 $\pm$ 3.5	169 $\pm$ 2.9	314 $\pm$ 2	398 $\pm$ 4.6	58 $\pm$ 0.4	65 $\pm$ 0.7	93 $\pm$ 1.4	131 $\pm$ 0.7
S10	28 $\pm$ 0.2	59 $\pm$ 1.4	57 $\pm$ 1.6	65 $\pm$ 2.1	62 $\pm$ 2.9	148 $\pm$ 0.4	51 $\pm$ 0.5	70 $\pm$ 0.4	69 $\pm$ 6.4	107 $\pm$ 12
Residential criterion ^c	30		250		100		500		500	
Industrial criterion ^c	50		800		500		1000		1500	

^a from Van der Kallen et al. (2020), ^b from Gosselin & Zagury (2020), ^c Regulatory criterion for soils in Quebec, Canada (MDDELCC, 2021).

The pH of the study soils was neutral to alkaline and ranged between 7.11 and 8.36 (Table 6.4). Total carbon content (TC), and total organic carbon (TOC) increased when particle size decreased. A strong correlation between CEC and TOC content was reported in these CCA-contaminated soil samples (van der Kallen et al., 2020). For a further analysis of soil properties, see Gosselin and Zagury (2020) and van der Kallen et al. (2020)

Table 6.4 Physicochemical properties (mean $\pm$ standard deviation ($n = 2$)) of different fractions of CCA-contaminated soil samples.

Soil sample	pH	TC (%)		TOC (%)		CEC (meq / 100g)
	250-90 μm^a	250-90 μm^a	$< 20 \mu\text{m}^b$	250-90 μm^a	$< 20 \mu\text{m}^b$	250-90 μm^a
S1	7.52 $\pm$ 0.02	5	9.0 $\pm$ 0.1	0.8	1.2 $\pm$ 7.1	12.2 $\pm$ 1.0
S2	7.11 $\pm$ 0.02	3.7	7.6 $\pm$ 1.4	2.8	5.1 $\pm$ 3.1	29.6 $\pm$ 0.1
S3	7.39 $\pm$ 0.02	4.2	6.7 $\pm$ 1.5	1.7	4.2 $\pm$ 1.5	20.9 $\pm$ 0.4

S4	7.91 ± 0.03	5.1	4.1 ± 0.5	3.2	2.9 ± 0.2	40.8 ± 0.9
S5	8.07 ± 0.04	3.3	8.7 ± 0.3	2.4	2.3 ± 0.3	21.7 ± 1.2
S6	7.88 ± 0.01	3.4	6.9 ± 3.0	0.8	4.0 ± 0.7	25.3 ± 0.9
S7	8.36 ± 0.04	1.1	7.8 ± 0.5	0.7	5.0 ± 2.9	9.4 ± 1.1
S8	7.60 ± 0.02	4.8	12.1 ± 5.3	3.3	8.3 ± 1.6	49.1 ± 0.5
S9	8.03 ± 0.03	5.9	9.8 ± 0.1	1.4	1.9 ± 0.7	15.8 ± 1.3
S10	8.23 ± 0.04	8.6	6.9 ± 0.0	0.4	0.6 ± 3.8	10.7 ± 1.6

^a from Van der Kallen et al. (2020), ^b from Gosselin & Zagury (2020).

6.3.2 Bioaccessibility

The bioaccessibility percentages are shown in Figure 6.1 for the oral, inhalation, and dermal pathways. The bioaccessible percentage for oral and inhalation pathways is preferred over bioaccessible concentrations because it can be directly incorporated into risk assessment. However, the bioaccessible concentration is required for the dermal pathway. It is needed as an input value for the water approach (C_w , Equation 6.11) to calculate DA_{event} . Tables 6.5 and 6.6 show dermal bioaccessible concentrations. The average oral bioaccessibility of As in CCA-contaminated soils was 32.2%, whereas for the inhalation and dermal pathways, the average bioaccessibility was 48.9% and 3.4%, respectively. Bioaccessibility percentages for chromium were 9.0%, 34.6%, and 1.2% for oral, inhalation, and dermal pathways, respectively. Copper yielded high bioaccessible percentages following oral (61.9%) and inhalation (78.4%) bioaccessibility tests. Similarly, to As and Cr, lower bioaccessibility percentages were measured for Cu dermal bioaccessibility with a mean value of 13.3%. Oral and inhalation bioaccessibility percentages for Pb showed mean values of 24.5% and 37.7%, respectively. For dermal bioaccessibility, values were much lower, with a mean of 1.7%. Zinc was relatively highly bioaccessible for the oral and inhalation pathways with a mean value of 37.8% and 54.8%, respectively. However, low Zn bioaccessibility was measured for the dermal pathway with a mean value of 6.6%. It must be reminded that low total Pb, and Zn content were measured in these CCA-contaminated soils.

Bioaccessibility percentages (Figure 6.1) arranged by exposure route shows a clear trend: Inhalation > Oral > Dermal. Also, for all pathways, bioaccessibility percentages followed the following trend: Cu > Zn > As > Pb > Cr.

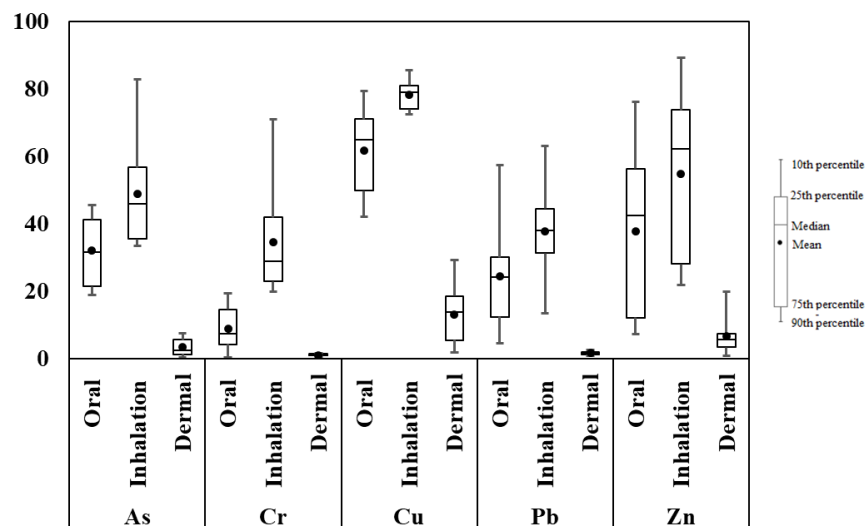


Figure 6.1 Boxplot of bioaccessibility % of metal(loid)s (n = 10). Dermal bioaccessibility % following 2 h extraction (representing children's exposure scenario) is plotted in this graph.

6.3.3 Risk characterization

6.3.3.1 Non-carcinogenic risk

When the total concentration of metal(loid)s in soils were used to calculate the hazard quotient, there was a hazard to human health for the residential and industrial scenarios associated with the soil samples since $HI > 1$ (Figure 6.2). For the residential scenario, the mean HQ values for the oral pathway were higher than for dermal and inhalation routes. The range of HI across the soils studied was 7.7 – 349, with an average of 64.5 for the residential scenario and 1.7– 73 with an average of 13.5 for the industrial scenario (see Table 6.1 for parameters used in each exposure scenario). The mean HQ for each metal(loid) considering a multipathway exposure was in decreasing order: 63.4 for As, 0.82 for Pb, 0.22 for Cu, 0.02 for Zn, and 0.01 for Cr for the residential scenario and 5.95 for As, 0.075 for Pb, 0.02 for Cu, 0.002 for Zn, and 0.001 for Cr for the industrial scenario. Although the HQ following exposure to Zn and Cr was very low for both scenarios, there was a very high risk associated to As exposure in both scenarios. There was also a significant contribution of Pb and Cu to the HI in the residential scenario.

For the residential scenario, the oral pathway contributed to 64.4% of the total HI (Figure 6.3), followed by the dermal pathway (35.6%). The inhalation route contributed $< 0.01\%$. For the industrial scenario, in a similar trend to residential, the oral pathway accounted for 62.0% of the

total HI, and the dermal pathway accounted for 38.0%. Again, the inhalation pathway contributed $< 0.01\%$. Oral, and dermal exposure routes exceeded HQs of 1, mainly due to the high concentration of As in the CCA-polluted study soils.

It is now widely accepted that metal(loid)s in contaminated soils are not fully bioaccessible, so assuming a bioaccessibility of 100% would overestimate the health hazard. Oral, inhalation and dermal bioaccessibility values were obtained, and doses and concentrations (CDI, DAD and EC) were adjusted considering bioaccessibility. To calculate the CDI with bioavailability considerations for As and Pb for the oral pathway, As and Pb RBAs (calculated using published linear regressions) were multiplied by the corresponding oral CDI. For the other elements, the bioaccessible fraction was multiplied by the CDI. HQ for the three pathways and the associated hazard (HI) was recalculated (Figure 6.2).

Interestingly, the dermal pathway contributed much more than the oral and inhalation pathways to the HI when bioaccessibility was considered (Figure 6.3). The range for the HI across the soils studied in the residential scenario was 2.5 – 134, with a mean of 34.7. The range for the HI in the industrial scenario was 0.8–44, with a mean of 12.7.

The mean HQ for each metal(loid) considering a multi-pathway exposure was in decreasing order: As 34.1, Pb 0.36, Cu 0.21, Zn 0.01, and Cr $6.9\text{E-}3$ for the residential scenario and As 9.75, Pb 0.033, Cu 0.038, Zn 0.001, and Cr $2.8\text{E-}3$ for the industrial scenario. There was a strong hazard associated with As in both scenarios and a significant contribution from Pb and Cu in the residential scenario. The dermal pathway contributed to 53.9% of the HI, followed by 46.1% for the oral pathway for the residential scenario. There was a minimal contribution to the multi-path hazard from the inhalation pathway as its contribution was $< 0.01\%$. For the industrial scenario, the dermal pathway was even a bigger contributor to the total HI, with 85.3%, followed by the oral pathway with 14.7%. Moreover, and similarly to the residential scenario, the inhalation pathway contributed less than $< 0.01\%$ (Figure 6.3). These results clearly show that the dermal exposure route should not be neglected solely based on the low bioaccessibility percentages Measured.

Figure 6.2 shows a higher hazard quotient from the dermal pathway when bioaccessibility is considered compared to the conventional method using total metal(loid) concentrations in the soil. This phenomenon can be explained by the underestimation of DA_{event} (caused by the assumed generic value for ABSd (Table 6.1)) to calculate the DAD using the soil approach. The generic

ABS_d values, which do not take into account soil physicochemical properties and metal(loid) species, correspond to bioavailable concentrations below actual bioaccessible values obtained following bioaccessibility tests (Figure 6.1). This trend was previously reported by Chaparro Leal et al. (2018).

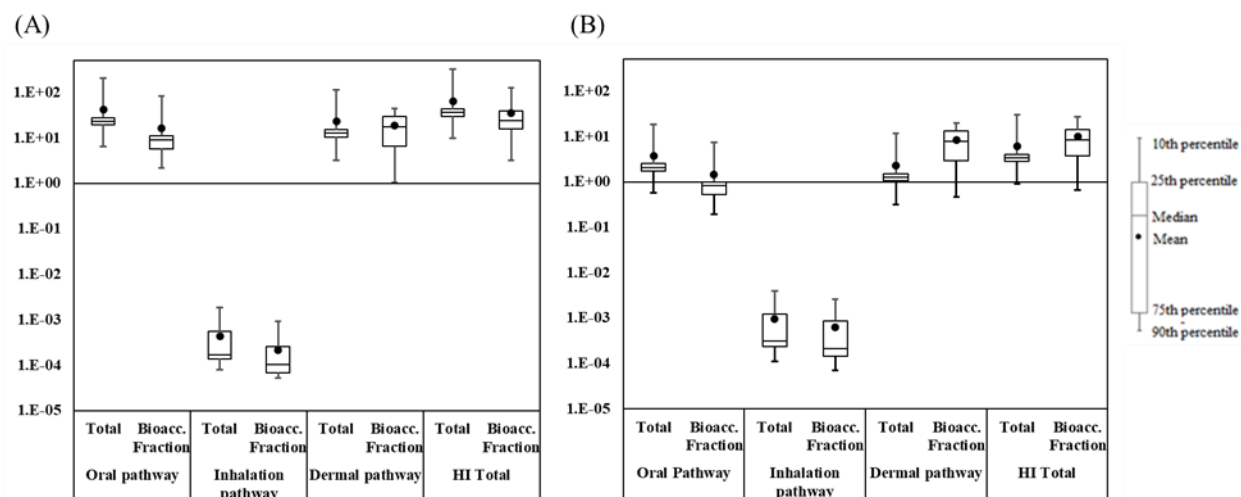


Figure 6.2 Boxplot of contribution of the different exposure pathways and combined metal(loid)s to hazard index (HI) without bioaccessibility considerations and considering bioaccessibility for (A) residential scenario, (B) industrial scenario.

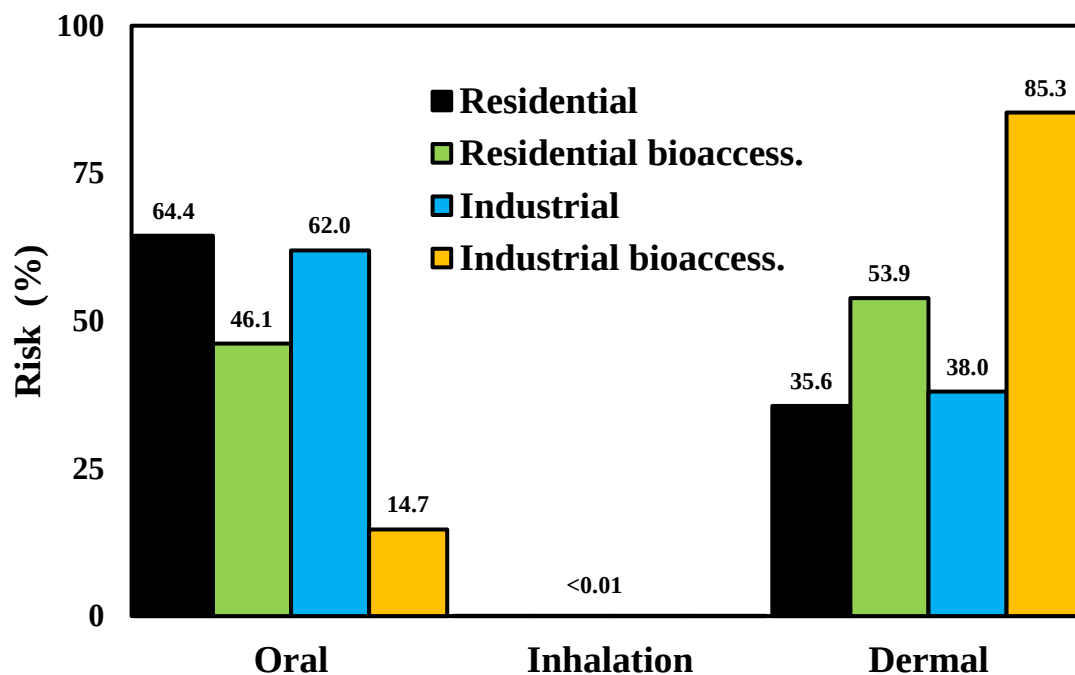


Figure 6.3 Percentage of non-carcinogenic risk contribution per pathway for residential and industrial scenarios.

6.3.3.2 Carcinogenic Risk

To evaluate the carcinogenic risk, only As and Pb were included in the analysis of the combined metal(loid) risk (Figure 6.4). The risk without bioaccessibility considerations (mean value $\pm$ standard deviation) by exposure pathway of combined metals (As and Pb) were Risk_{oral} ($1.8\text{E-}3 \pm 2.8\text{E-}03$), Risk_{inhalation} ($7.1\text{E-}14 \pm 1.1\text{E-}13$), and Risk_{dermal} ($9.3\text{E-}07 \pm 1.5\text{E-}06$). The risks with bioaccessibility considerations were Risk_{oral} ($6.8\text{E-}04 \pm 1.1\text{E-}03$), Risk_{inhalation} ($3.3\text{E-}14 \pm 5.6\text{E-}14$), and Risk_{dermal} ($2.9\text{E-}06 \pm 2.2\text{E-}06$). The cumulative multi-pathway risk range was $1.9\text{E-}04$ – $9.6\text{E-}03$ with a mean of $1.8\text{E-}03$ without bioaccessibility considerations and $6.8\text{E-}05$ – $3.8\text{E-}03$ with a mean of $6.8\text{E-}04$ when considering bioaccessibility. Additionally, the multi pathway risk per contaminant (Figure 6.5) without bioaccessibility assuming that all Cr is Cr (VI) was $9.0\text{E-}03$, $1.9\text{E-}06$, and $3.0\text{E-}03$ for As, Pb, and Cr(VI), respectively and $3.7\text{E-}04$, $1.6\text{E-}06$, and $6.1\text{E-}04$ for As, Pb, and Cr(VI), when considering bioaccessibility respectively.

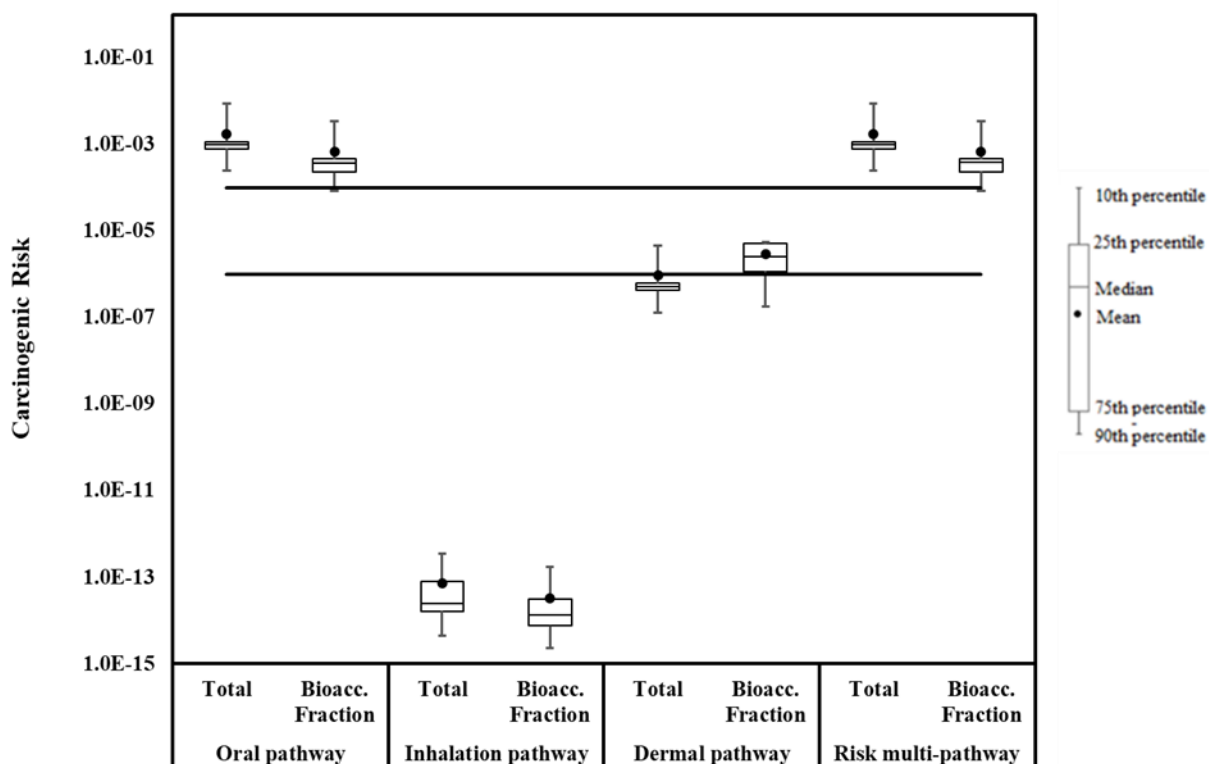


Figure 6.4 Boxplot of carcinogenic risk for combined metal(loid)s (As and Pb) for each pathway and for cumulative multi-pathway following exposure to total metal(loid) content and to bioaccessible fraction of CCA-contaminated soils.

For the oral pathway, the risk associated with As and Cr(VI) was higher than the acceptable values ($>10^{-4}$) with and without bioaccessibility considerations (Figure 6.5). For Pb, the carcinogenic risk ranged between 10^{-6} and 10^{-4} , so it is not inconsiderable but within the acceptability range with and without bioaccessibility considerations. For the inhalation pathway, the risk for As and Pb was below the significance value with and without bioaccessibility considerations for all the soil samples studied. For Cr(VI), the risk without bioaccessibility was over the significance value for soils S7 and S8 and, with bioaccessibility considerations, for soil S7 (Figure 6.5). It must be reminded that soil samples S7 and S8 had very high total Cr content in the $< 20 \mu\text{m}$ fraction (Table 6.2).

The dermal pathway did not produce average risk values $> 10^{-4}$. However, the risk from arsenic with bioaccessibility considerations was over 10^{-6} . Considering the multi-pathway risk, As and Cr(VI) pose a potentially unacceptable risk while Pb is in the acceptance threshold since its associated risk is between $1.0\text{E-}6$ and $1.0\text{E-}4$ with and without bioaccessibility considerations.

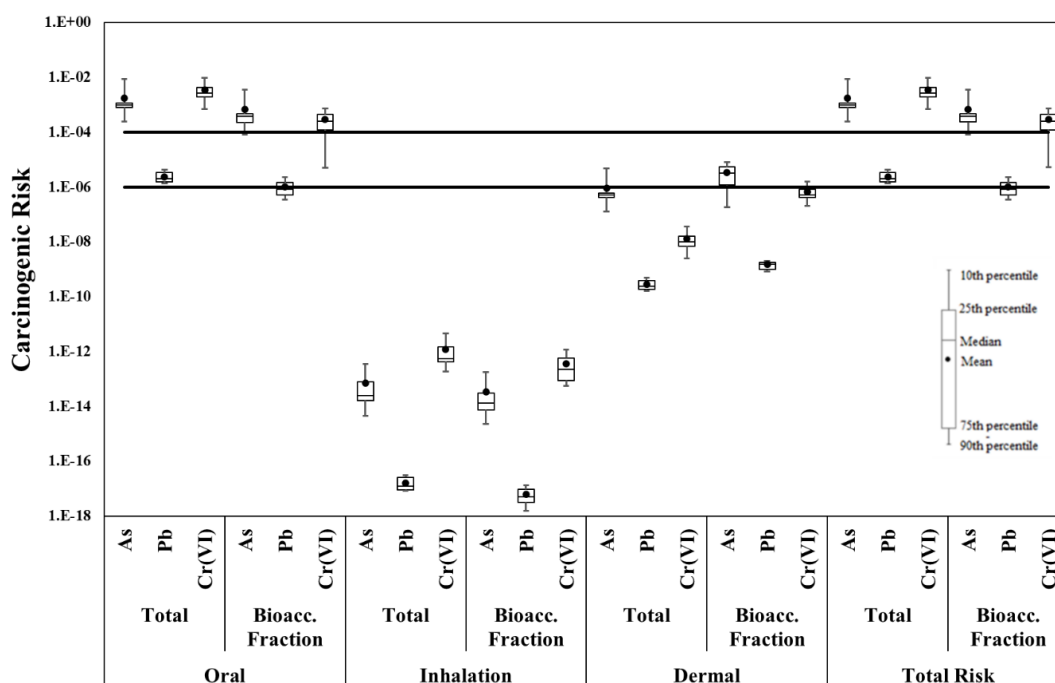


Figure 6.5 Boxplot of carcinogenic risks for As, Pb and Cr(VI) in CCA-contaminated soils, through oral, inhalation and dermal pathway for total metal(loid) content and bioaccessible fraction.

6.3.3.3 Sensitivity Analysis

A brief sensitivity analysis was performed to assess the influence of the input parameters variability in the calculation of HI for both scenarios. For each input parameter, a variability of $\pm 20\%$ was considered to build the tornado plots (Figure 6.6).

The residential and industrial scenarios follow a similar trend as ED, EF, BW, and IngR are the parameters with the highest incidence in the value of HI. Although the HI presents a considerable variation when ED, EF, BW, and IngR values are modified by $\pm 20\%$, the HI remains in the same order of magnitude. The body weight (kg) is defined by the average body weight over the exposure period. For children, the average child's body weight is used in risk assessments. Similarly, the average body weight for an age group is the average weight for that age group. For pathways where contact rate to bodyweight ratios are relatively constant over a lifetime a bodyweight of 70 kg is used and is considered constant. Similarly, a body weight of 15 kg represents 6 years old children (USEPA, 1989). There is an important bodyweight variation between populations, gender, age groups and ethnicities. For instance, the USEPA (2011) exposure handbook recommends a bodyweight for adults of 80 kg. Nevertheless, the default human body weight values recommended in the risk assessment of environmental hazards in Australia are 70 kg for adult males, 58 kg for adult females, 13.2 kg for 2-year-old children, and 64 kg for average males and females combined (Department of Health and Ageing and Health Council, 2004). Similarly, bodyweight values reported by the European Commission are 70 kg for adult males and 60 kg for adult females (EC 2003) whereas the world health organization recommends a default body weight value of 60 kg (WHO, 2010). Additionally, for risk evaluation purposes, assessors sometimes use low-end values such as the 10th or 5th percentile, as lower body weight values are more conservative for risk estimations (Guney and Zagury, 2016). Also, the use of females instead of males is sometimes recommended, when relevant (Guney and Zagury, 2016). By considering a bodyweight of 70 kg for adults and 15 kg with a variation of $\pm 20\%$, the range of body weight recommended by most health agencies is included. The significant influence of the BW value in the calculation of HI emphasizes the importance of carefully selecting input parameters in risk assessments. The IngR values used in the risk assessment for the residential and the industrial scenario (100 and 50

mg/day, respectively) correspond to the combined ingestion of soil and dust. This value is broadly accepted for risk assessment and is used by the UKEA (2009) and USEPA. Other agencies such as Health Canada (2010) use lower IngR values (80 mg/day for residential and 20 mg/day for the industrial scenario) (Guney and Zagury, 2016). Moreover, it must be mentioned that other studies have been conducted considering soil pica behavior (Rozanski et al., 2021; Calabrese et al., 1997), a disorder that refers to the consumption of higher amounts of soil. A soil ingestion rate of 1000 mg/day is usually recommended by international guidelines for pica behavior (Guney and Zagury, 2016). Bodyweight has been identified as a key parameter in the calculation of risk. Higher body weight values result in lower exposure per kg, and thus, lower risk. Similarly to body weight, the ingestion rate is also a key parameter to assess exposure to soil metal(loid)s. However, this parameter has high variability and uncertainty associated. It is usually estimated through observational studies or mass balance of trace elements. Research on soil ingestion rates for adults and children is still rather limited (Guney and Zagury, 2016).

Other relevant parameters such as exposure frequency and exposure duration are scenario specific and relatively easy to accurately calculate with low variability and uncertainty (Guney and Zagury, 2016). As expected from the low HQ yielded by the inhalation pathway, the specific parameters that dictate the inhalation exposure (PEF) have less influence in the calculation of HI. In contrast, the specific parameters used to describe oral exposure are more influential (IngR, C_{total}), followed by dermal exposure (SA) parameters.

To improve the reliability of risk assessments, attempts should be made to refine the more influential parameters by obtaining site-specific data instead of generic values.

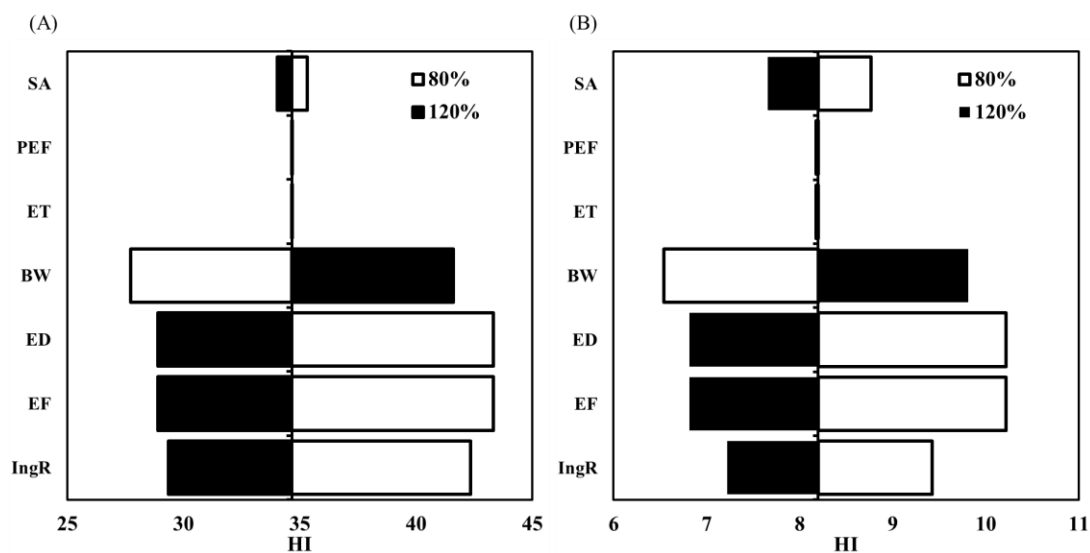


Figure 6.6 Tornado plot of the parameters to evaluate (A) HI for the residential scenario, (B) HI for the industrial scenario.

6.4 Conclusions

To characterize the risk of potentially toxic metal(loid)s following exposure to CCA-contaminated soils, *in vitro* bioaccessibility as an estimation of bioavailability gives a better approximation of the actual fraction of contaminant entering the body than the total metal(loid) content in soils. Additionally, it implicitly includes the interaction between surrogate physiological fluids and the soil, affecting contaminants' fate. However, even though *in vitro* bioaccessibility tests are helpful to estimate bioavailability, the methods should be validated against *in vivo* or *in vitro* bioavailability studies and correlation equations established to implement this approach in risk characterization. Correlation equations are available for As and Pb and were incorporated in the calculations to assess oral relative bioavailability in the present study.

The calculated non-carcinogenic hazard quotient (HQ) presented risk in residential and industrial scenarios. Arsenic was the principal concern in both cases. The oral and dermal pathways yielded high HQ with and without bioaccessibility considerations ($HQ > 1$). In contrast, the inhalation pathway seemed to have a lower non-carcinogenic hazard associated.

Due to assumptions inherent to US EPA soil approach to calculate the dermal dose (DAD), the dermal risk was higher when dermal bioaccessibility was incorporated (water approach), causing a potential underestimation of risk when the soil approach is used.

The main goal of this study was to characterize non-carcinogenic and carcinogenic human health risks of metal(loid)s present in CCA-contaminated soils using a multi-pathway exposure and considering bioaccessibility. With this objective in mind, the multi-pathway exposure yielded unacceptable carcinogenic risk since total risk $> 10^{-4}$ with and without bioaccessibility considerations. The primary source of risk was arsenic, and the main route of exposure was oral, followed by dermal. Additionally, chromium can potentially pose a carcinogenic risk to human health if present as Cr(VI) in CCA-contaminated soils. For this reason, the speciation of contaminants in soils and biological fluids is relevant and should be further investigated.

6.5 Appendix A. Supplementary material

Table 6.5. Bioaccessible concentrations (mg/L $\pm$ RSD in %) following dermal bioaccessibility tests using artificial sweat solution NIHS-9610 for 2 h

Soil	As			Cr			Cu			Pb			Zn		
S1	1.00	$\pm$	2.3	0.36	$\pm$	2.5	3.04	$\pm$	0.1	0.06	$\pm$	6.0	0.52	$\pm$	4.6
S2	0.91	$\pm$	4.3	0.32	$\pm$	0.1	0.70	$\pm$	5.1	0.11	$\pm$	4.1	2.46	$\pm$	7.6
S3	0.50	$\pm$	2.1	0.30	$\pm$	3.1	1.04	$\pm$	1.7	<0.01	$\pm$	-	0.46	$\pm$	1.3
S4	0.14	$\pm$	27.6 ²	0.15	$\pm$	6.0	0.31	$\pm$	1.6	0.11	$\pm$	57.4 ²	<0.01	$\pm$	-
S5	0.26	$\pm$	1.6	0.30	$\pm$	2.5	0.97	$\pm$	5.6	0.05	$\pm$	0.1	<0.01	$\pm$	-
S6	0.24	$\pm$	8.9	0.39	$\pm$	4.2	1.87	$\pm$	2.9	0.09	$\pm$	55.3 ²	0.25	$\pm$	1.5
S7	0.98	$\pm$	1.1	1.02	$\pm$	0.9	6.92	$\pm$	0.4	0.13	$\pm$	6.8	2.29	$\pm$	0.2
S8	1.50	$\pm$	1.5	0.95	$\pm$	3.1	3.99	$\pm$	1.1	0.09	$\pm$	- ¹	0.52	$\pm$	2.4
S9	0.66	$\pm$	2.2	0.33	$\pm$	1.7	1.65	$\pm$	0.5	0.11	$\pm$	0.7	0.28	$\pm$	2.3
S10	0.02	$\pm$	11.4	0.13	$\pm$	3.9	1.15	$\pm$	1.7	0.10	$\pm$	1.9	0.06	$\pm$	9.9
SQC	1.10	$\pm$	18.4	4.89	$\pm$	9.3	6.58	$\pm$	4.0	9.18	$\pm$	6.2	50.21	$\pm$	2.0

¹ not available as duplicate concentration was lower than quantification limits, ² RSD $> 20\%$ due to differences between low concentrations of duplicates.

Table 6.6. Bioaccessible concentrations (mg/L $\pm$ RSD in %) following dermal bioaccessibility tests using artificial sweat solution NIHS-9610 for 8 h

Soil	As		Cr		Cu		Pb		Zn	
S1	1.12	$\pm$ 2.3	0.42	$\pm$ 3.1	2.70	$\pm$ 0.1	0.05	$\pm$ 6.0	0.34	$\pm$ 4.6
S2	1.02	$\pm$ 4.3	0.36	$\pm$ 1.1	0.63	$\pm$ 5.1	0.09	$\pm$ 4.1	1.60	$\pm$ 7.6
S3	0.56	$\pm$ 2.1	0.35	$\pm$ 3.1	0.93	$\pm$ 1.7	<0.01	$\pm$ -	0.52	$\pm$ 1.3
S4	0.16	$\pm$ 27.6 ²	0.17	$\pm$ 6.0	0.28	$\pm$ 1.6	0.09	$\pm$ 57.4	<0.01	$\pm$ -
S5	0.29	$\pm$ 1.6	0.35	$\pm$ 2.5	0.87	$\pm$ 5.6	0.04	$\pm$ 0.1	<0.01	$\pm$ -
S6	0.27	$\pm$ 8.9	0.45	$\pm$ 4.2	1.66	$\pm$ 2.9	0.08	$\pm$ 55.3 ²	0.16	$\pm$ 1.5
S7	1.10	$\pm$ 1.1	1.17	$\pm$ 0.9	6.16	$\pm$ 0.4	0.11	$\pm$ 6.8	1.94	$\pm$ 0.2
S8	1.68	$\pm$ 1.5	1.09	$\pm$ 3.1	3.55	$\pm$ 1.1	0.08	$\pm$ - ¹	0.44	$\pm$ 2.4
S9	0.74	$\pm$ 2.2	0.38	$\pm$ 1.7	1.47	$\pm$ 0.5	0.09	$\pm$ 0.7	0.24	$\pm$ 3.1
S10	0.02	$\pm$ 11.4	0.15	$\pm$ 3.9	1.03	$\pm$ 1.7	0.09	$\pm$ 1.9	0.05	$\pm$ - ¹
SQC	1.04	$\pm$ 13.0	4.72	$\pm$ - ¹	6.46	$\pm$ 5.5	9.11	$\pm$ 5.7	49.86	$\pm$ 1.8

¹ not available as duplicate concentration was lower than quantification limits, ² RSD > 20% due to differences between low concentrations of duplicates.

CHAPTER 7 GENERAL DISCUSSION

7.1 Influence of soil properties in bioaccessibility

Dermal bioaccessibility of metal(loid)s greatly depends on the soil characteristics. It was previously explained in the literature review how pH, organic matter content, and CEC of soils might influence dermal bioaccessibility. Indeed, the *in vitro* bioaccessibility results presented in chapters 3 to 6 follows a clear trend. Regardless of SSFL formulation, dermal bioaccessibility was generally higher for the reference soil SQC001 than BGS 102 (article # 1) and to S1-S10 (article # 2, #3, and #4). This difference in bioaccessibility was caused by the lower TOC, CEC, and pH in SQC001. Ageing of soils might also be an essential factor for the higher bioaccessibility of metal(loid)s present in SQC001. However, even though SQC001 is a prepared material and did not withstand natural ageing processes, the ageing of soils was not measured in this study.

The soil characterization and bioaccessibility studies presented highlight the need to properly characterize the soils subjected to bioaccessibility tests since their properties directly affect bioaccessibility. These properties are relevant in the characterization of hazards related to soil exposure and should be included in decision-making processes.

7.2 Influence of SSFL in *in vitro* bioaccessibility

In this study, five artificial SSFL were used in *in vitro* bioaccessibility tests. Article #1 focused on how the properties of SSFLs influence the bioaccessibility of metals. Regarding Synthetic sweat compositions, the pH of the SSFL tested seemed to be the main parameter controlling the bioaccessibility of the measured metal(loid)s. A higher bioaccessibility percentage was generally achieved at lower pH. Although all the sweat compositions used in this study are within the normal pH human range, the bioaccessibility percentages vary among sweat formulations.

A standardized sweat composition for *in vitro* bioaccessibility studies is not available to date, making interstudy comparisons difficult. Moreover, most of the dermal bioaccessibility studies to date only used one sweat formulation. For this reason, results using more basic sweat compositions might underestimate the bioaccessibility of metal(loid)s when compared to studies that used sweat with lower pH. However, sweat pH is variable among the population and depends on many factors, so reaching a consensus on the pH of sweat is difficult. Nevertheless, in human health assessment

studies, a conservative approach is recommended, and the use of low pH sweat compositions is encouraged to measure the bioaccessible fraction of metal(loid)s.

Although it was previously reported that sebum has little influence on the bioaccessibility of metals in powders (Collins, 1957, Stefaniak et al., 2014) some soil-bound metals (Cr and Zn) were somehow bioaccessible in sebum, and, in the case of Zn, more bioaccessible in sebum than synthetic sweat (in soil BGS 102). However, there are complications inherent in testing the bioaccessibility of metal(loid)s in sebum since it is difficult to disperse lipids in aqueous solvents. However, complementary studies regarding the bioaccessibility of metal(loid)s, especially Cr and Zn in sweat and sebum mixtures are recommended.

7.3 Factors Influencing the permeation of metal(loid)s through the membrane

This study incorporated for the first time a methodology using synthetic membranes to measure the permeation parameters of the bioaccessible fraction of metal(loid)s from soils. The results obtained in articles # 2 and # 4 showed that Strat-M membrane is appropriate for pilot studies of dermal permeation of metal(loid)s present in soils. Although human skin is the golden standard, synthetic membranes are commonly available, produce less variability, and are significantly less expensive than human skin. Interestingly, even though dermal bioaccessibility is higher at lower pH for all the studied metal(loid)s, the opposite happened with the permeation. Although only Cr and Cu permeated the Strat-M membrane, Cr and Cu have greater potential for diffusion through human skin surrogates at higher pH since the most pervious species (such as CrO_4^{2-}) are present in higher concentrations at higher pH. For this reason, the characteristics of the donor solution (synthetic sweat) are critical for both bioaccessibility and permeation of metal(loid)s.

As over 50% of the Cr detected in the bioaccessible solutions extracted from sweat A (pH 6.5) was Cr(VI), with CrO_4^{2-} being the primary Cr(VI) compound known to penetrate the skin, it was expected that bioaccessible Cr would permeate through the synthetic skin. Conversely, the electrophilic nature of Cr(III) ions causes them to form stable complexes with dermal and epithelial tissues, resulting in slowed diffusion through the skin. It should be noted that the negatively charged nature of chromate and dichromate (Cr(VI)) means they do not typically bind to organic substances (Hostynek, 2003).

In sweat A, bioaccessible Cr obtained from three soil samples (S2, S8, and SQC001) was observed to permeate through the Strat-M membrane in sweat A in the absence of sebum. However, bioaccessible Cr from nearly all soil samples (S3, S6, S7, S8, and SQC001) was found to permeate through the Strat-M membrane when it was coated with sebum, suggesting that sebum deposited on the synthetic skin may interact with Cr species in the bioaccessible extracts and potentially promote the formation of more permeable compounds. Notably, there was no discernible impact of sebum on the permeation kinetic parameters.

Cu, like Cr, was observed to permeate through the Strat-M membrane. Copper was found in all soil samples and was predominantly present as Cu(II) in sweat A, with similar Cu species detected across all soil extracts. Most Cu(II) was found in the form of Cu^{+2} , followed by $\text{CuHPO}_4(\text{aq})$. Interestingly, when soil-bound Cu from S7, S8, and SQC001 was extracted with sweat B, which had a higher Cu concentration than sweat A, there was no permeation through the synthetic Strat-M membrane. This suggests that Cu species present in sweat A but not in sweat B may be responsible for membrane permeation, including $\text{CuHPO}_4(\text{aq})$, CuOH^+ , and $\text{CuSO}_4(\text{aq})$. It is possible that the presence of Cu(II) aqueous salts governs the permeation of Cu through the Strat-M membrane.

At lower pH, dermal bioaccessibility of metal(loid)s is high and permeation low, and vice versa. Conversely, if the bioaccessibility is high, the permeation rate is low. It has to be noted that more testing is necessary, taking into account soils contaminated from another sources, sweat formulations and validation with human skin.

Although generally speaking the presence of sebum caused longer lag times and produced differences in the K_p of metals through the membrane. It is inconclusive how sebum influences permeation, and more studies on the matter, including using sweat and sebum mixtures as donor solutions are suggested.

7.4 Speciation of the bioaccessible fraction of metalloids in sweat

As suggested following the permeation results presented in articles #2 and #3, the synthetic sweat properties play a crucial role in the speciation of metal(loid)s, affecting its bioaccessibility and permeation.

Particularly for Cr, the speciation drastically changed in the different sweat compositions used. Whereas in a sweat with pH of 6.5, Cr is relatively evenly distributed in terms of concentration between Cr(III) and Cr(VI) species, when the pH of sweat decreases, Cr(III) species are dominant. This finding has essential relevance from a toxicological point of view since Cr(VI) species are simultaneously more toxic and permeate the skin at a higher rate. <>

Additionally, Cu was present almost entirely as Cu(II) in both sweat compositions studied. However, although Cu(II) was dominant in both sweat compositions, there were higher concentrations of $\text{CuHPO}_4(\text{aq})$, CuOH^+ , and $\text{CuSO}_4(\text{aq})$ in sweat A. Making Cu more pervious in this sweat composition.

It is crucial to assess the speciation of metal(loid)s solubilized in sweat, and identify the compounds that are better penetrants, especially for Cr. The USEPA recommends a different K_p for Cr(III) and Cr(VI) due to its different permeation rates and toxicity. However, our results demonstrated that trivalent and hexavalent species could coexist in similar concentrations in basic synthetic sweat. This coexistence makes the use of either K_p problematic and warrants a different approach considering speciation data and concentrations of Cr(III) and Cr(VI).

7.5 Risk characterization with bioaccessibility considerations

Because metal(loid)s content in soils is not absolutely bioaccessible, assuming a bioaccessibility of 100% miscalculates the risk. This study incorporated bioaccessibility results to characterize two multi-pathway exposure scenarios (residential and industrial scenario) by adjusting the doses considering the bioaccessible fraction of metal(loid)s of the oral and inhalation pathway and bioaccessible concentration in the dermal pathway.

Regarding non-carcinogenic hazards, the oral pathway contributed the most to the HQ with and without bioaccessibility considerations for the residential scenario. The oral pathway also contributed the most to the HQ for the industrial scenario without bioaccessibility considerations. Interestingly, the dermal pathway contributed more than the oral and inhalation to the HQ for the industrial scenario with bioaccessibility considerations. The potential underestimation of risk by the dermal pathway using the soil approach (no bioaccessibility considerations) can be attributed explained by the assumed value for ABS_d, which corresponds to a bioaccessible fraction below the bioaccessible values following the bioaccessibility test.

Concerning carcinogenic hazard, As and Cr(VI) risk is higher than the acceptable values with and without considering bioaccessibility mainly due to the contribution of the oral pathway, followed by the dermal pathway. The inhalation pathway has a very low dose associated with the scenarios studied; therefore, its contribution to non-carcinogenic and carcinogenic risk is minimal compared to the oral and dermal pathways.

It is also worth mentioning that although bioaccessibility concentrations were used to calculate the dermal risk, the permeation coefficient (main parameter to calculate the dermal dose) was taken from generic values to assess risk. As previously explained, depends on a myriad of considerations and more importantly, seems to be inversely proportional to bioaccessibility. For instance, As showed the highest contribution to risk through the dermal route. However, it did not permeate the membrane in the permeation studies described in chapters 4 and 5. That been said, to properly characterize the risk associated with the dermal pathway, bioavailability tests are recommended. This way, the bioaccessibility and permeation of the contaminant can be addressed by considering the geochemical properties of the soil and the interaction with biological fluids and skin.

CHAPTER 8 CONCLUSION AND RECOMMENDATIONS

8.1 Conclusions

The main conclusion of the present work is that SSFL composition plays a critical role in the fate of potentially toxic soil-bound metal(loid)s, influencing their dermal bioaccessibility and permeation by potentially changing their speciation. Consequently, the hazard associated with dermal exposure is dependent on the SSFL characteristics.

The following specific conclusions can be extracted from this thesis:

- Dermal bioaccessibility of metal(loid)s depends on the pH of the synthetic sweat used for the bioaccessibility test. Generally, sweat B (pH 4.7) yielded the highest bioaccessibility and sweat A (pH 6.5) the lowest. Bioaccessibility was also soil dependent. Soils with lower CEC and TOC generally presented higher bioaccessibility.
- An approach including bioaccessibility should be preferred over total soil concentrations to screen potentially hazardous substances and perform risk assessments. Results have shown that total concentrations and bioaccessible concentrations of toxic elements are not equal and do not necessarily correlate. Bioaccessible concentrations better predict exposure because it avoids overestimation and include interaction with the biological fluids.
- The geochemical properties of the soil might affect the bioaccessibility and permeation parameters of metal(loid)s. Therefore, site-specific variables (pH, CEC, TOC) should be considered for a more accurate estimation of risk related to dermal exposure.
- The extent of dermal bioaccessibility of metal(loid)s is influenced by the pH and composition of sweat. However, the speciation profiles of metal(loid)s in each sweat composition were different. For instance, Cu only permeated the synthetic membrane when extracted in sweat A, and the permeation coefficients of Cr were higher when dissolved in sweat A. This being said, it is important to note that a higher solubility in sweat does not necessarily imply a higher permeation rate through the skin.
- Zinc showed important bioaccessibility in sebum-extracted samples. Because of this, further testing including mixing of sweat and sebum at physiological relevant ratios should

be performed in order to broaden the understanding of sebum-sweat interactions in the bioaccessibility and subsequent permeation of metal(loid)s.

- Efforts to comprehend the speciation changes of metal(loid)s from their soil-bound state to their absorption into the human body after coming into contact with SSFLs are crucial. It is necessary to understand the speciation of potentially toxic metal(loid)s. Chemical equilibrium models-based software is a powerful tool for determining the speciation of these metal(oids). However, it is advisable to perform an analytical evaluation of metal(loid)s speciation in sweat to verify the findings.
- Environmental agencies like the US EPA do not usually consider differences in the oxidation state, valence, or species to calculate dermal exposure to potentially toxic metal(loid)s (except for Cr(III) and Cr(VI)). Nevertheless, species containing the same metal(loid) have the potential to have drastically different bioaccessibility and permeation. Therefore, broad generalizations could affect the reliability of exposure assessments.
- For the residential scenario (children playing), most of the risk is associated with the oral pathway, followed by the dermal pathway, with and without bioaccessibility. Interestingly, there is a significant difference for the industrial scenario (worker eight-hour shift) when bioaccessibility is considered. Without bioaccessibility, the industrial scenario trend is similar to the residential scenario (oral pathway is the primary pathway of concern, followed by dermal). However, when the bioaccessible considerations are included, the risk contribution from the dermal pathway increases (from 23.8% to 92.2%). In all the scenarios studied, the inhalation pathway did not contribute to the risk (<0.001%).
- For calculating the risk associated with dermal exposure, the ABS_d assumed to calculate DA_{event} in the soil approach methodology could correspond to a bioaccessible fraction below the bioaccessible value following the bioaccessibility test. This can result in a potential underestimation of the risk leading to poor decision-making. Instead, to properly evaluate the fraction of bioaccessible metal(loid)s present in soils, the water approach methodology is recommended to calculate the DA_{event} .
- In order to achieve a comprehensive characterization of the risk associated with the dermal pathway, it is recommended to include bioavailability assessments, by measuring bioaccessibility and permeation to obtain a site-specific concentration of metal(loid)s in the

sweat (C_w) and K_p respectively. In this manner geochemical properties of the soil and the interaction with biological fluids and skin can be properly considered.

8.2 Recommendations

Based on the conclusions presented, the main recommendations can be drawn as follows:

8.2.1 Main recommendations

- Only CCA-contaminated and certified soil samples were tested. Bioaccessibility, permeation, speciation, and human health risk assessment should be investigated in a broader range of soils.
- Conclusions regarding the dermal bioaccessibility of As, Cr, Cu, Ni, Pb, and Zn from soil samples collected near wood poles treated with CCA and certified reference soils were made. However, it is essential to clarify that the bioaccessibility of metals differs from case to case. If the source of the contamination or the properties of the soil change, the behaviour of the contaminants could potentially change too.
- To decrease uncertainty in dermal risk assessments, it is recommended to measure dermal bioavailability to include site-specific C_w and K_p in risk calculations.
- The human skin surrogate Strat-M has proven helpful for early-stage permeation studies. Although correlation tools to assess the permeation of some chemicals through human skin using Strat-M already exist and are provided by the manufacturer, additional testing, including human skin, is needed to validate and correlate the permeation results provided in this study.
- Five different synthetic SSFLs have been tested, including three sweat and two sebum formulations. Experiments including additional, more comprehensive SSFLs, including sweat and sebum mixtures are recommended.
- Visual Minteq lacks data on reaction kinetics, which might be crucial for chromium speciation, as some Cr(VI) compounds have a short life and can permeate membranes faster than Cr(III). Additionally, the model does not consider dissolved organic carbon and

proteins, which act as ligands for metal ions. To validate the findings, analytical speciation considering dissolved organic carbon should be performed.

8.2.2 Other recommendations

- Because the soil approach used to calculate the DAD might under predict the risk for specific exposure scenarios, further studies incorporating the water approach using different SSFLs and more soil samples are advised.
- Research on the capacity of Strat-M to characterize deposit of metal(loid)s in the skin is recommended since the accumulation of metal(loid)s in the skin layers, as well as reservoir effects, may contribute to the absorbed dose or produce adverse topical effects.
- Only the Strat-M was used to model skin permeation. However, there are other commercially available skin surrogates. The testing and comparison of other synthetic membranes with Strat-M and human skin in permeation tests is recommended.
- There is uncertainty associated with risk assessment, especially regarding individual human characteristics. For this reason, the use of Monte Carlo simulations and the inclusion of variables as probabilistic distributions rather than discrete values is recommended so that risk is calculated more accurately.

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**APPENDIX A – IN VITRO EVALUATION OF DERMAL
BIOACCESSIBILITY OF METALS USING TWO SYNTHETIC SEBUM
AND THREE SYNTHETIC SWEAT FORMULATIONS**



DÉPARTEMENT DES GENIES CIVIL,
GÉOLOGIQUE ET DES MINES

EXPERIMENTAL

PROTOCOL

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Title: In vitro evaluation of dermal bioaccessibility of metals using two synthetic sebum and three synthetic sweat formulations

Objective

The present protocol aims to explain the procedure for the extraction of potentially toxic elements in contaminated soil samples into synthetic sebum and sweat as an attempt to simulate a more realistic scenario of their bioavailability through the dermal pathway.

Materials

Reagents

General use and others:

- Deionized Water
- 1000 mg/L reference standard solutions for selected metals PerkinElmer (Cr, Ni, Pb, Zn) for spiked samples

Synthetic sebum and sweat chemicals:

- Squalene 98% (w/w), Alfa Aesar CAS 111-02-4
- Jojoba oil, Sigma CAS 61789-91-1
- Triolein 75% (Assay percent range), Alfa Aesar CAS 122-32-7
- Oleic acid 90% (Assay percent range), Alfa Aesar CAS 112-80-1
- Vitamin E 97% (Assay percent range), Alfa Aesar CAS 7695-91-2
- Lanolin, Alfa Aesar CAS 8006-54-0
- Tristearin, Technical grade MP Chemicals CAS 555-43-1
- Palmitic acid 99%, Sigma CAS 57-10-3
- Cholesterol 95% (w/w), Alfa Aesar CAS 57-88-5
- Sodium Chloride Certified ACS, Fisher Scientific CAS 7647-14-5
- Lactic acid 85% (w/w), Fisher Chemical CAS 50-21-5, 97-73-4
- Acetic acid, Fisher Chemicals CAS 64-19-7
- Urea, MP Biomedicals CAS 57-13-6
- Threonine $\geq 98\%$ (HPLC), Sigma CAS 72-19-5
- Methionine 98.5% (w/w), Fisher Chemicals CAS 63-68-3
- Serine 98% (Assay percent range), Fisher Chemicals CAS 1655-64-7
- Alanine 99% (Assay percent range), Acros Organics CAS 338-69-2

- Glycine 98.5% (dry basis), Fisher Chemicals CAS 56-40-6
- Ammonium Chloride (Crystalline/Certified ACS), Fisher Chemicals CAS 12125-02-9

Acid and basic solutions

- Sodium hydroxide, Pellets/Certified ACS, Fisher Chemicals CAS 1310-73-2, 497-19-8
- Nitric acid HNO_3 $\rho = 1.40\text{g/mL}$ 65% (w/w), Fisher Chemicals CAS 7697-37-2

Certified reference soils

- SQC 001, Sigma
- BGS 102, British Geological Survey

Apparatus and materials

General use:

- Analytical balance, 3 digits (x1)
- Magnetic stirrer (1 per SSFL formulation)
- Teflon beakers for acid digestion 100 mL (x1 per soil sample)
- Hotplate (x2)
- Automatic pipette (capacity: 1-10 mL) and disposable sampling tips, Eppendorf brand (x1)

Synthetic sebum and sweat preparation:

- 100 mL glass beaker (x4)
- 100 mL volumetric glass flask (x2)
- 250 mL glass beaker (x3)
- volumetric glass flask [volume synthetic sweat to be prepared] (x3)
- dropper (3)
- Magnetic Stirrer (x6)
- PP Centrifuge tube with cap (50 mL), Fisher Chemicals (x5)
- pH meter, accurate to ± 0.05 pH units (x1)
- Water bath and thermometer (x1)

Bioaccessibility test:

- Incubator adjustable to $36\text{ }^{\circ}\text{C} \pm 0.5\text{ Celsius}$ (x1)
- Centrifuge and corresponding rotor (Highconic) (x1)
- PP Centrifuge tube with cap (50 mL), Fisher Chemicals (x2 per sample)
- Orbital shaker, having variable supports to receive test containers (x1)
- 60 mL (x1 per sweat sample) and 10 mL syringe (x1 per sebum sample), Fisher Chemicals
- $0.45\text{ }\mu\text{m}$ Syringe PVDF filter (x1 per sample), Fisher Chemicals

Preparation of basic solutions**Preparation of 1M NaOH solution:**

Weigh $4\text{ g} \pm 0.01\text{ g}$ of sodium hydroxide into a 100 mL beaker and add 50 mL of deionized water. Stir using a magnetic stirrer and cool to room temperature. Transfer the solution to a 100 mL volumetric flask and complete to volume with deionized water.

Preparation of 0.1M NaOH solution

Weigh $0.4\text{ g} \pm 0.01\text{ g}$ of sodium hydroxide and transfer into a 100 mL beaker and add 50 mL of deionized water. Stir using a magnetic stirrer and cool to room temperature. Transfer the solution to a 100 mL volumetric flask and complete to volume with deionized water.

Theoretical background

The present procedure combines the dermal bioaccessibility approach described in USEPA, 2004 with metal bioaccessibility test performed in synthetic sweat and sebum available in scientific literature.

Determining the amount of soil adhered per cm^2 of skin to establish soil: liquid ratio

To calculate a recommended soil: solution ratio from soil loading based on soil particle size is necessary to know the granulometry of the sample. A schematic of the procedure is shown in figure 1 and an example of calculations is shown in Annex 2. Once granulometry is known, each particle size is multiplied by their respective adherence factor (AF) value, and finally, all AF multiplied by the respective mass is added to obtain an average AF. Theoretical adherence factors were obtained from table 1 in annex 1.

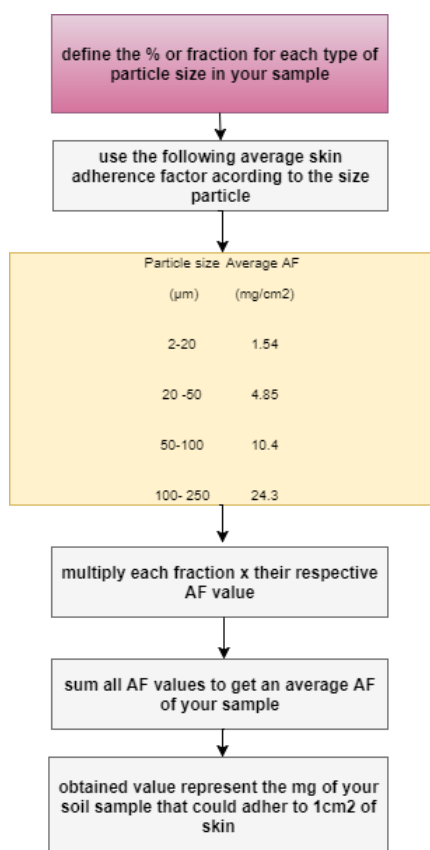


Figure 1. Soil loading calculation per cm² of skin

Determining sweat volume to be used in bioaccessibility test

The volume of sweat is determined assuming that there is a layer of sweat with a thickness of 0.1 cm per 1 cm² of skin, resulting in a volume of 0.1 cm³, or 0.1 mL. To accurately compare sebum and sweat bioaccessibility, the sweat volume and sebum volume is considered the same.

Determining soil to sweat ratio

Soil to sweat ratio is obtained taking in account the soil loading calculation in mg/cm² following the steps from Figure 1 and then divided it into the volume of sweat in 1 cm² of skin, which is 0.1 mL. As an example, AF for sample 1 is 14 mg/cm² according to table 3 from Annex 2; this value is divided into 0.1 mL to obtain soil/sweat, the result is 139.9 mg of soil /mL sweat or 0.14g soil/mL. As proportion sweat/soil varied between 7 and 4 for the samples studied (0.1 mL sweat/0.014 g soil = 7) and (0.1 mL sweat /0.026 soil = ~4) a fixed sweat/soil ratio = 10 has been chosen for all

samples to have a conservative scenario. For different soils samples than the used by Leal et al., (2018) is recommended to repeat the sweat to soil ratio estimation.

Experimental set-up

Material preparation and cleaning

- **General precautions:** As glassware cleaning process is performed using HNO_3 (10% v/v), is essential to consider that this reagent is corrosive thus can cause eye, skin and digestive tract irritation. Therefore, it is mandatory to use all personal protection elements (laboratory glasses, nitrile gloves and coat with long sleeves), as well as use the fume hood to perform the cleaning procedure.
- **Cleaning lab ware procedure:** Clean all glass lab-ware and containers first using hot tap water and phosphate-free laboratory detergent. Rinse with deionized water three times. Soak all glass and plastic lab-ware and containers into 10% (v/v) HNO_3 solution for at least 8 h. Ideally, let all items rest overnight on the acid bath. After soaking, rinse items three times with distilled water, then three times with deionized water. For items not suitable to stay in the acid bath (i.e., stainless-steel items), or when it is not possible to leave items overnight in acid solution, thoroughly wash the items three times with 10% (v/v) HNO_3 solution. After that, rinse three times with distilled water, then three times with deionized water.
- **Calibration of micro-pipettes:** This test is sensitive to volume inaccuracy. To perform micro-pipette calibration in the laboratory:
 - a. Fill a 200 mL beaker with deionized water. Using a thermometer measure the temperature of water for two minutes. Record the temperature.
 - b. Place a weigh boat on the analytical balance and tare it. For calibration, test both the smallest volume and the largest volume the pipette can dispense.
 - c. Aspire a volume of deionized water and place it in the weigh boat. Keep the tip in the pipette for the next measures. Record the weight. Repeat this process 10 times per weight. Repeat at different volumes.
 - d. The formula for calculating the volume dispensed by the pipette is $V = w * Z$ where w is the weight of the water, Z is the conversion factor based on the density of the water (ANNEX 1), and V is the calculated volume of how much water was dispensed.

- e. Calculate the Z variable by looking up the density of water using the temperature recorded at the beginning of the experiment. For example: If the temperature of the water was 23°C, then use a Z value of 1.0035 µg/mg (ANNEX 1).
- f. To average all the values together, add them all up and divide by 10. If did more or fewer trials, add up each trial and divide by the total number of trials.
- g. To calculate the pipette accuracy, use the equation $A = 100 \times V_{\text{avg}}/V_0$, where A is the accuracy of the pipette, V_{avg} is the average calculated volume and V_0 is the value set the pipette to dispense. Accuracy should be between 99-101%.
 - If the pipette is properly calibrated, the calculated value should be very close to the actual value set on the pipette.
 - For example: $A = 100 \times V_{\text{avg}}/V_0 = 100 \times 10.019/10 = 100 \times 1.0019 = 100.19\%$ In this case, the pipette is properly calibrated.
- h. In case the pipette does not pass the calibration test. Stop using it immediately and contact the laboratory responsible so the pipette can be calibrated.

Soil Preparation

- For bioaccessibility essays, the certified reference materials (SQC 001, and BGS 102) require no previous treatment.
- Other soil samples must be air dried and sieved to fractions of less than 425 µm using a vibrator shaker.
- Tailings samples are not sieved, and granulometry curve should be used to determine the adherence factor.

Synthetic sebum preparation

Synthetic sebum preparation according to Wertz (2009)

Synthetic sebum contains:

Table 1. Sebum according to Wertz (2008) content

CHEMICAL	Wt %
Squalene	12.4
Jojoba oil	25

Triolein	44.7
Oleic acid	17
Vitamin e	1

Preparation:

- a. Set a hot bath at 60 °C
- b. Weigh in a 100 mL glass beaker:

Table 2. Preparation quantities Sebum according to Wertz (2008)

	for 25 g of solution	for 50 g of solution	for 100 g of solution
Squalene	3.1 g	6.2 g	12.4 g
Jojoba oil	6.25 g	12.5 g	25 g
Triolein	11.175 g	22.35 g	44.7 g
Oleic acid	4.25 g	8.5 g	17 g
Vitamin e	0.25 g	0.5 g	1 g

- c. Put the mix of chemicals in the hot bath until the consistency is liquid
- d. Remove the glass beaker from the hot bath and stir the mixture using a magnetic mixer until it is homogenous
- e. Transfer the sebum to a 50 mL centrifuge tube and close it
- f. Keep in incubator at 36 °C temperature until use

NOTE: Synthetic sebum should be prepared the same day of the experiment

Synthetic sebum preparation according to Stefaniak (2009) modified

Synthetic sebum contains:

Table 3. Sebum according to Stefaniak (2009) content

CHEMICAL	Wt (%)
Squalene	10.3
Lanolin	24.3
Tristearin	21.4
Triolein	10.7
Palmitic acid	13.8
Oleic acid	13.8
Cholesterol	3.9

- a. Set a hot bath at 60 °C
- b. Weigh in a 100 mL glass beaker:

Table 4. Preparation quantities synthetic Sebum according to Stefaniak (2009)

	for 25 g of solution	for 50 g of solution	for 100 g of solution
Squalene	2.64 g	5.27 g	10.54 g
Lanolin	6.14 g	12.27 g	24.54 g
Tristearin	5.41 g	10.82 g	21.65 g
Triolein	2.74 g	5.47 g	10.95 g
Palmitic acid	3.51 g	7.02 g	14.05 g
Oleic acid	3.51 g	7.02 g	14.05 g
Cholesterol	1.04 g	2.07 g	4.15 g

- c. Put the mix of chemicals in the hot bath until the consistency is liquid
- d. Remove the glass beaker from the hot bath and stir the mixture using a magnetic mixer until it is homogenous
- e. Transfer the sebum to a 50 mL centrifuge tube and close it
- f. Keep in incubator at 36 °C temperature until use

NOTE: Synthetic sebum should be prepared the same day of the experiment.

Synthetic sweat preparation

Synthetic Sweat preparation according to Van Lierde

- a. pour 150 mL of deionized water into a 250-mL glass beaker.
- b. Weigh and add:

Table 5. Preparation quantities synthetic sweat according Van Lierde (2005)

	For 1 L solution	For 500 mL solution	For 250 mL solution
Sodium Chloride (NaCl)	5 ± 0.05 g	2,5 ± 0.025 g	1.25 ± 0.01 g
Lactic acid	1.0 ± 0.01 g	0.5 ± 0.005 g	0.25 ± 0.003 g
Urea	1.0 ± 0.01 g	0.5 ± 0.005 g	0.25 ± 0.003 g
threonine	1.0 ± 0.01 g	0.5 ± 0.005 g	0.25 ± 0.003 g
methionine	1.0 ± 0.01 g	0.5 ± 0.005 g	0.25 ± 0.003 g
serine	1.0 ± 0.01 g	0.5 ± 0.005 g	0.25 ± 0.003 g
Alanine	1.0 ± 0.01 g	0.5 ± 0.005 g	0.25 ± 0.003 g
Glycine	1.0 ± 0.01 g	0.5 ± 0.005 g	0.25 ± 0.003 g

- c. Stir using a magnetic stirrer until the reagents are dissolved
- d. Calibrate the pH meter according to manufacturer's instructions (manual in the lab, drawer close to pH meter)
- e. Immerse the pH electrode into the solution and measure the pH
- f. Maintaining the stirring, adjust the pH to 5.5 ± 0.05 adding drop by drop NaOH 1M until reaching pH 5, subsequently adding NaOH 0,1M
- g. Transfer the solution to a 1000-, 500- or 250-mL volumetric flask according to volume chosen to be prepared
- h. Rinse at least 3 times the beaker mentioned in stage (a) with deionized water and pour it into the volumetric flask that contains the solution
- i. Complete the volume with deionized water
- j. Before using the solution, measure pH to ensure is within the adequate range

NOTE: Synthetic sweat should be prepared the same day of the experiment

Synthetic sweat preparation according to norm NF EN 1811 +A1 (2015)

Synthetic sweat contains:

- * 0.5% (m/m) sodium chloride
- * 0.1% (m/m) lactic acid
- * 0.1% (m/m) urea
- * 1M and 0,1 M NaOH solution

Preparation:

- a. pour 150mL of freshly deionized water into a 250-mL glass beaker
- b. Weigh and add:

Table 6. Preparation quantities synthetic sweat according to NF EN 1811 +A1 (2015)

	For 1 L solution	For 500 mL solution	For 250 mL solution
Sodium Chloride	5 ± 0.05 g	$2,5 \pm 0.025$ g	1.25 ± 0.01 g
Urea	1.0 ± 0.01 g	0.5 ± 0.005 g	0.25 ± 0.003 g
Lactic acid	1.0 ± 0.01 g	0.5 ± 0.005 g	0.25 ± 0.003 g

- c. Stir using a magnetic stirrer until the reagents are dissolved
- d. Calibrate the pH meter according to the manufacturer's instructions
- e. Immerse the pH electrode into the solution and measure the pH
- f. Maintaining the stirring, adjust the pH solution to $6,5 \pm 0.05$ start adding drop by drop NaOH 1M until reaching pH 5.5, and subsequently adding NaOH 0,1M
- g. Transfer the solution to a 1000-, 500- or 250-mL volumetric flask according to the weight chosen
- h. Rinse at least 3 times the beaker mentioned in stage (a) with deionized water and pour it into a volumetric flask that contains the solution

- i. Complete to volume with deionized water (deionized water to make up the volume should be at 36 °C). Before using the solution, measure the pH again to ensure that it is in the range of 6.5 ± 0.05 .

Note: Synthetic sweat shall be prepared on the same day of the experiment

Figure 2. shows the main steps to prepare synthetic sweat.

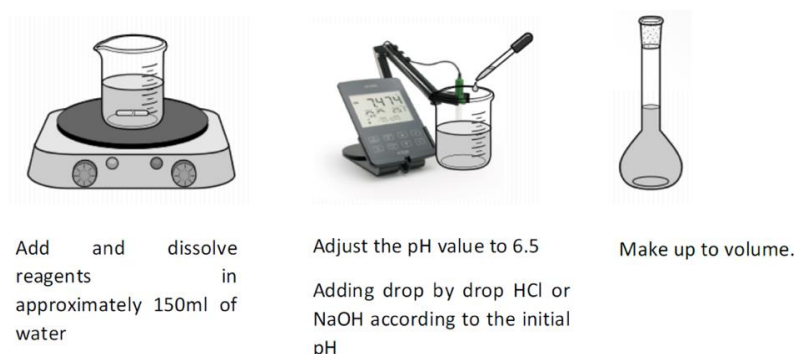


Figure 2. Synthetic sweat preparation EN 1811

Synthetic sweat preparation according to Norm NIHS 96-10

- a. Pour 150 mL of fresh deionized water into a 250 mL glass beaker.
- b. Weigh and add:

Table 7. Preparation quantities synthetic sweat according to Norm NIHS 96-10

	For 1 L solution	For 500 mL solution	For 250 mL solution
Sodium Chloride	20 ± 0.20 g	10 ± 0.10 g	5.0 ± 0.05 g
Urea	5.0 ± 0.05 g	2.5 ± 0.025 g	1.25 ± 0.013 g
Ammonium Chloride	17.5 ± 0.175 g	8.75 ± 0.088 g	4.375 ± 0.044 g
Lactic Acid	15 ± 0.15 g	7.5 ± 0.075 g	3.75 ± 0.038 g
Acetic Acid	2.5 ± 0.025 g	1.25 ± 0.013 g	0.625 ± 0.006 g

- c. Stir using a magnetic stirrer until the reagents are dissolved
- d. Calibrate the pH meter according to the manufacturer's instructions
- e. Immerse the pH electrode into the solution and measure the pH
- f. Maintaining the stirring, adjust the pH solution to 4.7 ± 0.05 start adding drop by drop NaOH 1M until reaching pH 3.5, and subsequently adding NaOH 0,1M

- g. Transfer the solution to a 1000, 500, or 250-mL volumetric flask according to the weight chosen.
- h. Rinse at least 3 times the beaker mentioned in stage (a) with deionized water and pour it into the volumetric flask that contains the solution.
- i. Complete volume with deionized water (deionized water to make up the volume should be at 36 °C). Before using the solution, measure the pH again to ensure that it is in the range of 4.7 ± 0.05 .

Note: synthetic sweat shall be prepared on the same day of the experiment.

Bioaccessibility test

Set the incubator at 36 °C at least 4 hours before proceeding with the experiment, or the day before the assays, in addition to this, place at least 1 liter of deionized water inside the incubator to have it at 36 °C.

The bioaccessibility test should be performed in duplicates. Considering a sweat/soil sample and sebum/soil sample ratio of 10:1.

Bioaccessibility test in synthetic sebum

Step 1: Weigh 0.5 g of each soil sample into a 15 mL centrifuge tubes.

Step 2: Transfer 5 mL of sebum already at 36 °C with a pipette (10 mL) into each tube.

Step 3: Tightly screw the caps in advance to prevent any evaporation or leaking and run the test by setting the speed at 100 RPM in the orbital shaker and the temperature at 36 °C in the incubator (the orbital shaker is inside the incubator). Record the exact time the experiment started and sampling schedule (dates and times) also on a sticky note which is to be placed at the door of the incubator. It is also highly suggested to use a timer as soon as the experiment starts.

Step 4: Let the samples in agitation at 36 °C during the desired time (2h or 8h).

Step 5: Remove the tubes from the orbital shaker inside the incubator, then centrifuge the tubes as follows: carefully place the tubes in the rotor of the centrifuge. Duplicates one in the face of each other to counterbalance the weight. The weight difference between tubes facing each other **must be smaller than 0.1 g** (to be checked with analytical balance **each time**).

Step 6: Fasten the rotor with its screw. Check if the rotor is locked before centrifuging by attempting to lift the rotor (must not move upwards). Set the centrifuge speed to 8,600 RPM (10,000 x g, **never** exceed this value with High comic rotor), time to 10 min 40 sec, select smooth acceleration and deceleration (These settings are already saved under the program #1).

Step 7: Lock the lid of the centrifuge and start the centrifuging using the START button. After the operation is finished,

Step 8: Collect 5 mL of supernatant with an automatic pipette (10 mL) into a 10 mL syringe with a 0.45 μm PVDF filter fitted, use micropipettes to collect remaining small volumes if necessary. Filter the sebum to a centrifuge tube (15 mL) with PP cap. Add 0.25 mL of HNO_3 to the sample using an automatic pipette (10mL).

Keep samples at 4 °C for a maximum of four weeks until the analyses.

Bioaccessibility test in synthetic sweat

Step 1: Weigh 2 g of each soil sample into a 50 mL centrifuge tubes.

Step 2: Transfer 20 mL of sebum already at 36 °C with a pipette (10 mL) into each tube.

Step 3: Tightly screw the caps in advance to prevent any evaporation or leaking and run the test by setting the speed at 100 RPM in the orbital shaker and the temperature at 36 °C in the incubator (the orbital shaker is inside the incubator). Record the exact time the experiment started and sampling schedule (dates and times) also on a sticky note which is to be placed at the door of the incubator. It is also highly suggested to use a timer as soon as the experiment starts

Step 4: Let the samples in agitation at 36 °C during the desired time (2h or 8h).

Step 5: Remove the tubes from the orbital shaker inside the incubator, then centrifuge the tubes as follows: carefully place the tubes in the rotor of th centrifuge. Duplicates one in the face of each other to counterbalance the weight. The weight difference between tubes facing each other **must be smaller than 0.1 g** (to be checked with analytical balance **each time**).

Step 6: Fasten the rotor with its screw. Check if the rotor is locked before centrifuging by attempting to lift the rotor (must not move upwards). Set the centrifuge speed to 8,600 RPM (10,000 x g, **never** exceed this value with High comic rotor), time to 10 min 40 sec, select smooth acceleration and deceleration (These settings are already saved under the program #1).

Step 7: Lock the lid of the centrifuge and start the centrifuging using the START button. After the operation is finished,

Step 8: Collect 20 mL of supernatant with an automatic pipette (10 mL) into a 10 mL syringe with a 0.45 μm PVDF filter fitted, use micropipettes to collect remaining small volumes if necessary. Filter the sebum to a centrifuge tube (50 mL) with PP cap. Add 1 mL of HNO_3 to the sample using an automatic pipette (10mL).

Keep samples in 4 °C for a maximum of four weeks until the analyses.

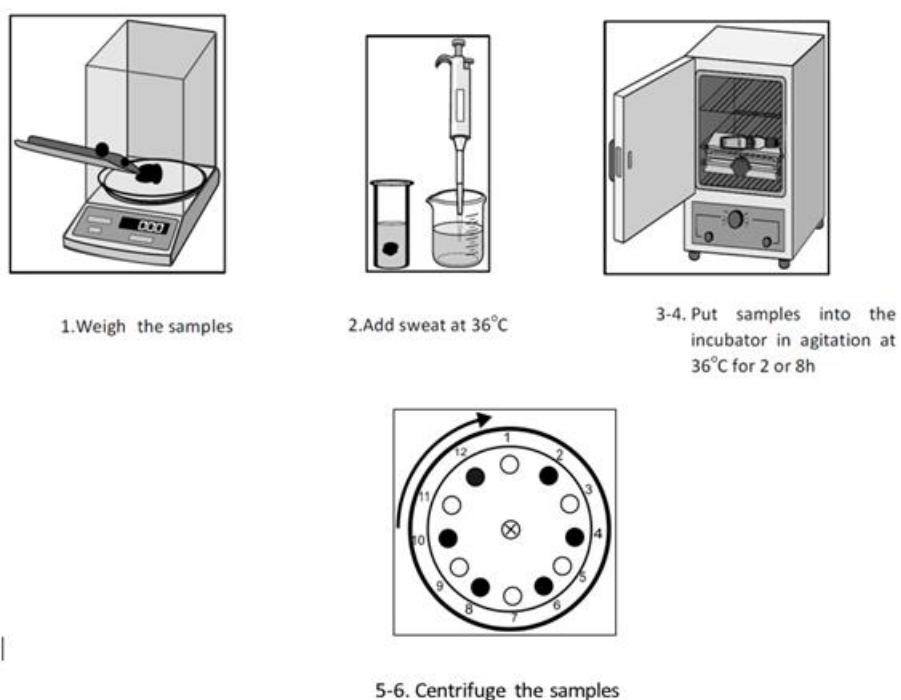


Figure 3. Bioaccessibility test main steps.

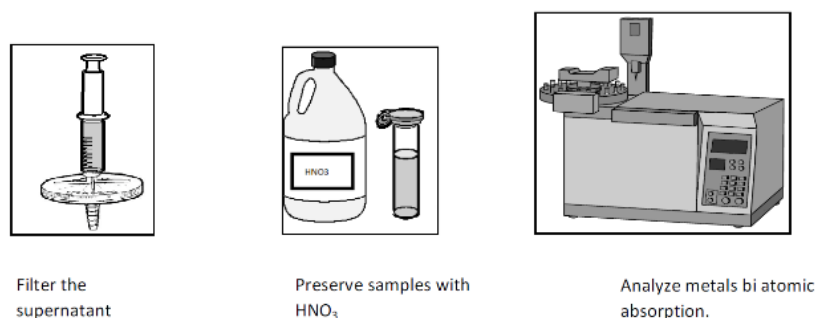


Figure 4. Sample collection analysis

Digestion of sebum samples

After the bioaccessibility test, the sebum samples should be digested with HNO_3 and H_2O_2 prior to the ICP analysis. The steps for the digestion are:

- a. Weigh precisely about 0.15 g of homogenized sample in a 150 mL beaker.
- b. Add 5 mL of nitric acid and stir. Let one night at room temperature.
- c. Add 1 mL of 30% hydrogen peroxide (v/v).
- d. Place the beaker on a hot plate and heat for 2 h (about 90-95 °C).
- e. Cool, filter and rinse the beaker in a 50 mL volumetric flask.
- f. Complete the volume with deionized water.

Note: the digestion of the sample and ICP analysis is performed by a third party (Institut National de la Recherche Scientifique, Centre Eau Terre Environnement). Person of contact: Lan H. Tran, lan.huong.tran@ete.inrs.ca

Quality control (spiked solutions)

Transfer commercially available standard solutions (present in the laboratory, ask technician) to transfer caps.

Prepare a solution with final concentration of 10 mg/L of each of metals Cr, Ni, Zn, and Pb, by adding 1 mL of each standard solution with concentration 1000 mg/L to a volumetric flask of 100 mL, then add 2 mL of concentrated nitric acid 68% (w/w), and complete volume with deionized water.

For synthetic sebum, weigh 0.5 g of any of the soil samples in a centrifuge tube and add 0.25 mL of spiked solution, then add 5 mL of synthetic sebum. Treat spiked samples according to section 3.5.

For synthetic sweat, weigh 2 g of any of the soil samples in a centrifuge tube and add 1 mL of spiked solution, then add 20 mL of synthetic sweat. Treat spiked samples according to section 3.5.

Besides the samples with spiked soil, a blank should be included for each set of experiment in each synthetic sebum or sweat and, a blank with spiked solution to estimate recovery. Duplicates should also be included.

Sample analysis

ICP-AES is recommended at INRS-ETE. At the time, the cost for analysis per sample is 15 CAD for the first metal and 2 CAD for each additional metal (25 CAD per sample to analyse 6 metals). Contact at INRS is Tran Lang Huong (Lan.Huong.Tran@ete.inrs.ca).

AAS can also be performed (not for arsenic). Consult with Professor Gerald Zagury. Make sure to inquire for updated cost for both ICP and AAS.

Calculations

Calculate DA_{event} according to equation 1.

$$DA_{event} = K_p * C_{sweat} * t \quad \text{equation 1.}$$

where:

DA_{event} = absorbed dose per event ($mg/cm^2 * event$)

K_p = dermal permeability coefficient of compound in water (cm/h)

C_{sweat} = chemical concentration in sweat or sebum mg/cm^3

t = event duration (hr/event)

then calculate dermal absorbed dose according to equation 2.

$$DAD = DA_{event} * EV * ED * EF * SA / BW * AT \quad \text{equation 2.}$$

Where:

DAD = Dermal Absorbed Dose ($mg/kg * day$)

DA_{event} = Absorbed dose per event ($mg/cm^2 event$)

SA = Skin surface area available for contact (cm^2)

EV = Event frequency (events/day)

EF = Exposure frequency (days/year)

ED = Exposure duration (years)

BW = Body weight (kg)

AT = Averaging time (days)

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APPENDIX B –DERMAL BIOACCESSIBILITY OF METALS: IN VITRO PERMEATION OF METALS FROM SOILS THROUGH SYNTHETIC SKIN (STRAT M. MEMBRANE)

 ÉCOLE POLYTECHNIQUE MONTRÉAL	
DÉPARTEMENT DES GENIES CIVIL, GÉOLOGIQUE ET DES MINES	EXPERIMENTAL PROTOCOL
Protocole :	Number of pages: 20
Version : 1/1	Date : 05-01-2021
Author(s): Carlos A. Marin V	
Dermal bioaccessibility of metals: In Vitro permeation of metals from soils through synthetic skin (Strat M. membrane)	
Keywords: EN 1811, NIHS 96-10, Synthetic sweat, Synthetic sebum, bioaccessibility,	

Objective

The present protocol explains the procedure for the determination of the diffusion parameters of the bioaccessible fraction of potentially toxic metal(loid)s in contaminated soil samples using the Static Franz cell methodology and synthetic skin (membrane Strat-M).

Materials**Reagents****General use and others**

- Deionized water
- Phosphate buffered saline pH 7.4, Fisher Scientific (Receptor solution)
- Synthetic sweat containing metals after bioaccessibility test (see experimental protocol “In vitro evaluation of dermal bioaccessibility of metals using two synthetic sebum and three synthetic sweat formulations”) 20 mL per sweat composition (Donor solution). Each set of experiment (1 soil, one synthetic sweat) needs 6 mL of donor solution.
- Fresh Synthetic sweat for blank cells, 20 mL per sweat composition (see experimental protocol “In vitro evaluation of dermal bioaccessibility of metals using two synthetic sebum and three synthetic sweat formulations”)
- Sebum for coating membranes (for preparation of sebum, see experimental protocol “In vitro evaluation of dermal bioaccessibility of metals using two synthetic sebum and three synthetic sweat formulations”)

Digestion of membranes

- Nitric acid (70% v/v) 4 mL per sample, Fisher Scientific
- Hydrochloric acid (50% v/v) 10 mL per sample, Fisher Scientific
- Hydrofluoric acid (50% v/v) 10 mL per sample, Fisher Scientific
- Deionized water
- Acetone, Fisher Chemical BPA181

Apparatus and materials

- Analytical balance 3 digits, Cole-Palmer Model TA-64

- Jacketed Franz Cell including receptor $A=0.64\text{ cm}^2$, donor, o-ring, clam and stirrer, Permegear (x6)
- Brand new rubber bands (x6)
- Magnetic stirrer base (Variomag) 15 slots
- Hot bath and thermometer adjustable to $37\text{ }^{\circ}\text{C}$, Precision 280 Series Thermo Electron Corp.
- Silicone tubing 10 cm to connect jackets Receptor compartments (x12)
- Silicone tubing 50 cm to connect the last cell back to the hot bath (x1)
- Silicone tubing 50 cm with adapter to connect pump to first cell (x1)
- Laboratory stand (x2)
- Laboratory clams 90° (x2)
- Aluminium bars (x2)
- Aquarium submersible water pump, VicTsing NP-600
- Automatic pipette (capacity: 10 mL and 1000 μl) and disposable sampling tips, Eppendorf brand
- Sterile syringe 10 ml with 10 cm needle (1 per cell per set)
- Droppers, Fisher Scientific (x6)
- Chronometer, Fisher Scientific
- Centrifuge tube with cap 15 mL, Fisher Scientific (x1 per sample)
- Centrifuge tube with cap 50 mL, Fisher Scientific (x1 per membrane sample)
- Teflon beaker for digestion 100 mL (x1 per membrane to be digested)
- Hot plate (for digestion of membrane)
- Filtering system vacuum (x1 per membrane to be digested)
- Glass microfibre filters 42.5 mm, Whatman (x1 per membrane to be digested)
- 100 mL volumetric flask
- Thermometer, Fisher Scientific
- Glass beaker 200 mL (x1)

Theoretical background

This test is based on the one proposed by Franz (1975) and modified by A Franken et al. (2015); Haq et al. (2018) and Larese et al. (2007). The main objective is to experimentally identify the

main parameters (K_p , J , Lag time) of the diffusion of the bioaccessible fraction of a toxic metal present in synthetic sweat through a membrane that acts as a surrogate to human skin.

The absorption of substances through skin happens by dissolution and molecular diffusion through the skin in a protein-lipid interaction (Beriro et al., 2016; Michaels et al., 1975). There are three dermal absorption pathways: (1) intracellular bulk pathways, (2) Interstitial bulk pathways, and (3) Shunt pathways (Beriro et al., 2016; Price et al., 2008). The most important route among the three pathways of dermal absorption is the interstitial pathway. The lipids facilitate this pathway in the stratum corneum (Beriro et al., 2016; Michaels et al., 1975).

Percutaneous absorption can be defined as the process where a chemical penetrates the skin to reach the systemic blood supply. This definition differs from dermal absorption, which includes the chemical remaining in the skin depot that is not considered bioavailable systematically for the duration of the exposure period. However, the depot may become bioavailable at more extended exposure periods (Moody et al., 2007).

Two factors determine the dermal bioavailability of a contaminant in soils: (1) transfer of the contaminant from the vehicle, and (2) the capacity for penetration of the contaminant through the skin (Pugh et al., 2008). For contaminants to be absorbed into the skin and be available for systemic circulation, both processes need to occur. However, just the presence of the contaminant in the skin can create localized effects (Beriro et al., 2016). Because the sole presence of a contaminant can cause localized effects, bioavailability in dermal exposure should be defined as a substance “absorbed into any part of the epidermis” rather only the fraction available to systemic absorption (Beriro et al., 2016).

Depending on the dosage and the physio-chemical properties of the substance being absorbed by the skin, exposure may be under equilibrium or non-equilibrium conditions (Beriro et al., 2016). There is a maximum concentration of a substance that the skin can absorb, this is relative to time and is referred as penetration rate or flux J [$\text{g cm}^{-2}\text{h}^{-1}$] (Beriro et al., 2016; Pugh et al., 2008). This flux happens in a theoretically unlimited supply of the substance, referred to as the infinite dose. If the amount of substance available limits the exposure scenario, the exposure is instead referred to as the finite dose (Beriro et al., 2016). In order to calculate kinetic parameters such as flux (J), the permeability coefficient (K_p) and diffusion coefficient (D), the infinite dose approach is preferred. For chronic exposure to soil, the finite dose should be used instead (Beriro et al., 2016; Jeffries et

al., 2009). After a lag phase (T_{lag}), steady-state flux is reached (J_{ss}). The lag face is graphically measurable as the intercept in “x” of the steady state region.

The dose across skin or a permeable membrane can be expressed mathematically by Fick’s First Law of diffusion (Equation 1 and Equation 2) where J is the flux, dc the concentration gradient, dx the distance traveled and D the diffusion coefficient. J_{ss} is the steady state flux per unit area, K_p the permeability coefficient in distance and time for a given compound in soil and C_o the concentration of the compound in soil (Beriro et al., 2016).

$$J = -D \frac{dc}{dx} (1)$$

$$J_{ss} = K_p * C_o (2)$$

Properties such as molecular weight, solubility, charge, and hydrogen bonding affect dermal bioavailability of contaminants (Brain et al., 2008).

The Franz diffusion cell method (Franz, 1975) has been used since the mid 70’s to assess skin permeation. This method was initially developed to be used by the pharmaceutical and cosmetics industry to determine penetration rates and absorbed fractions of transdermal medications (A. Franken et al., 2015). The Franz cell has been adapted to be used in other fields such as occupational toxicology, where it helped to quantify the absorption of metals and other toxins through the skin. Generally, the apparatus consists of two compartments, (donor and receptor) with a permeable membrane clamped in between. Areas exposed to the membrane in the Franz cell may vary from 0.55 to 10 cm² (A. Franken et al., 2015). The receptor solution temperature ranged between 32 and 37 °C with 32 °C corresponding to the surface of the skin and 37 °C to body temperature beneath the skin (Franz, 1975). During the experiment, the receptor solution can be tested at different times to measure the content of the studied substance. Once the test reaches completion, the membrane can be digested to measure the content of the substance studied and verify mass balance. The receptor is recommended to be filled with a solution that contains salt concentration similar to what is found in blood and a pH of 7.35 (Larese Filon et al., 2013). For studies related with toxicity and exposure, it is recommended to use synthetic sweat as the donor with pH ranging between 4.5 and 6.5 (Filon et al., 2009; Larese et al., 2007). The diffusion assay using a Franz cell can produce results that can be reported as flux or permeability coefficient; lag time; total cumulative diffused;

or percentage diffused and the total mass retained in membranes or percentage retained (A. Franken et al., 2015).

Experimental set-up

Material preparation and cleaning

- **General precautions:** As glassware cleaning process is performed using HNO_3 (10% v/v), is essential to consider that this reagent is corrosive thus can cause eye, skin and

digestive tract irritation. Therefore, it is mandatory to use all personal protection elements (laboratory glasses, nitrile gloves and coat with long sleeves), as well as use the fume hood to perform the cleaning procedure.

- **Cleaning lab ware procedure:** Clean all glass lab-ware and containers first using hot tap water and phosphate-free laboratory detergent. Rinse with deionized water three times.

Soak all glass and plastic lab-ware and containers into 10% (v/v) HNO_3 solution for at least 8 h. Ideally, let all items rest overnight on the acid bath. After soaking, rinse items three times with distilled water, then three times with deionized water. For items not suitable to stay in the acid bath (i.e., stainless-steel items), or when it is not possible to leave items overnight in acid solution, thoroughly wash the items three times with 10% (v/v) HNO_3 solution. After that, rinse three times with distilled water, then three times with deionized water.

- **Calibration of micro-pipettes:** This test is sensitive to volume inaccuracy. To perform micro-pipette calibration in the laboratory:
 - i. Fill a 200 mL beaker with deionized water. Using a thermometer measure the temperature of water for two minutes. Record the temperature.
 - j. Place a weigh boat on the analytical balance and tare it. For calibration, test both the smallest volume and the largest volume the pipette can dispense.
 - k. Aspire a volume of deionized water and place it in the weigh boat. Keep the tip in the pipette for the next measures. Record the weight. Repeat this process 10 times per weight. Repeat at different volumes.
 - l. The formula for calculating the volume dispensed by the pipette is $V = w * Z$ where w is the weight of the water, Z is the conversion factor based on the density of the water (ANNEX 1), and V is the calculated volume of how much water was dispensed.

- m. Calculate the Z variable by looking up the density of water using the temperature recorded at the beginning of the experiment. For example: If the temperature of the water was 23°C, then use a Z value of 1.0035 µg/mg (ANNEX 1).
- n. In order to average all the values together, add them all up and divide by 10. If did more or fewer trials, add up each trial and divide by the total number of trials.
- o. To calculate the pipette accuracy, use the equation $A = 100 \times V_{\text{avg}}/V_0$, where A is the accuracy of the pipette, V_{avg} is the average calculated volume and V_0 is the value set the pipette to dispense. Accuracy should be between 99-101%.
 - If the pipette is properly calibrated, the calculated value should be very close to the actual value set on the pipette.
 - For example: $A = 100 \times V_{\text{avg}}/V_0 = 100 \times 10.019/10 = 100 \times 1.0019 = 100.19\%$ In this case, the pipette is properly calibrated.
- p. In case the pipette does not pass the calibration test. Stop using it immediately and contact the laboratory responsible so the pipette can be calibrated.

Set-up

This procedure has been conceived to compare the diffusion parameters of Cr, Ni, Zn, Cu, Pb, and As present in three synthetic sweat formulations (Sweat A, Sweat B, Sweat C) precedent from three geological material after conducting bioaccessibility test (2 h). The geological material first should be subjected to dermal *in vitro* bioaccessibility test to assess the bioaccessible fraction of Cr, Ni, Zn, Cu, Pb and As and have the metals in solution in the synthetic sweat.

The procedure should be done in duplicates and including two blanks. The blanks should be treated the same way as the other samples with the difference that the donor solution is the blank from the bioaccessibility test instead of synthetic sweat containing metals after bioaccessibility test.

In total, six cells should be used each set. For each set one combination of synthetic sweat and soil can be tested. Table 1 shows the sweat formulation and soils used in each set for testing three sweat formulation and three geological materials.

	Set 1	Set 2	Set 3
Sample 1	Sweat A + soil 1	Sweat A + soil 2	Sweat A + soil 3
Sample 2	Sweat A + soil 1 duplicate	Sweat A + soil 2 duplicate	Sweat A + soil 3 duplicate
Sample 3	Sweat A + soil 1 sebum coated membrane	Sweat A + soil 2 sebum coated membrane	Sweat A + soil 3 sebum coated membrane
Sample 4	Sweat A + soil 1 sebum coated membrane duplicate	Sweat A + soil 2 sebum coated membrane duplicate	Sweat A + soil 3 sebum coated membrane duplicate
Sample 5	Sweat A Blank	Sweat A Blank	Sweat A Blank
Sample 6	Sweat A sebum coated blank	Sweat A sebum coated blank	Sweat A sebum coated blank
	Set 4	Set 5	Set 6
Sample 1	Sweat B + soil 1	Sweat B + soil 2	Sweat B + soil 3
Sample 2	Sweat B + soil 1 duplicate	Sweat B + soil 2 duplicate	Sweat B + soil 3 duplicate
Sample 3	Sweat B + soil 1 sebum coated membrane	Sweat B + soil 2 sebum coated membrane	Sweat B + soil 3 sebum coated membrane
Sample 4	Sweat B + soil 1 sebum coated membrane duplicate	Sweat B + soil 2 sebum coated membrane duplicate	Sweat B + soil 3 sebum coated membrane duplicate
Sample 5	Sweat B Blank	Sweat B Blank	Sweat B Blank
Sample 6	Sweat B sebum coated blank	Sweat B sebum coated blank	Sweat B sebum coated blank
	Set 7	Set 8	Set 9
Sample 1	Sweat C + soil 1	Sweat C + soil 2	Sweat C + soil 3
Sample 2	Sweat C + soil 1 duplicate	Sweat C + soil 2 duplicate	Sweat C + soil 3 duplicate
Sample 3	Sweat C + soil 1 sebum coated membrane	Sweat C + soil 2 sebum coated membrane	Sweat C + soil 3 sebum coated membrane
Sample 4	Sweat C + soil 1 sebum coated membrane duplicate	Sweat C + soil 2 sebum coated membrane duplicate	Sweat C + soil 3 sebum coated membrane duplicate
Sample 5	Sweat C Blank	Sweat C Blank	Sweat C Blank
Sample 6	Sweat C sebum coated blank	Sweat C sebum coated blank	Sweat C sebum coated blank

Figure 1. Experimental sets

To start the procedure:

- Fill the **hot bath** and set the temperature at 37 °C. let it stabilize for at least 30 mins.
- Submerge the **water pump** in the hot bath, plug it and turn it on. Make sure water flows through the pump. Unplug the pump until the cells are connected, and the water circuit is closed. Connect the pipe (50 cm) with the adapters to the water pump.
- Connect the **Franz cells receptors compartments** in parallel using the silicone pipes to circulate water (10 cm pipes). The bottom plug should be the water input and the upper plug the outlet to the next cell (See figure 1). The first cell should be connected to the water pump (water pump to cell 1 using the 50 cm pipe with adapters) and the last cell should discharge back to the hot bath (cell 6 to hot bath using the 50 cm pipe).

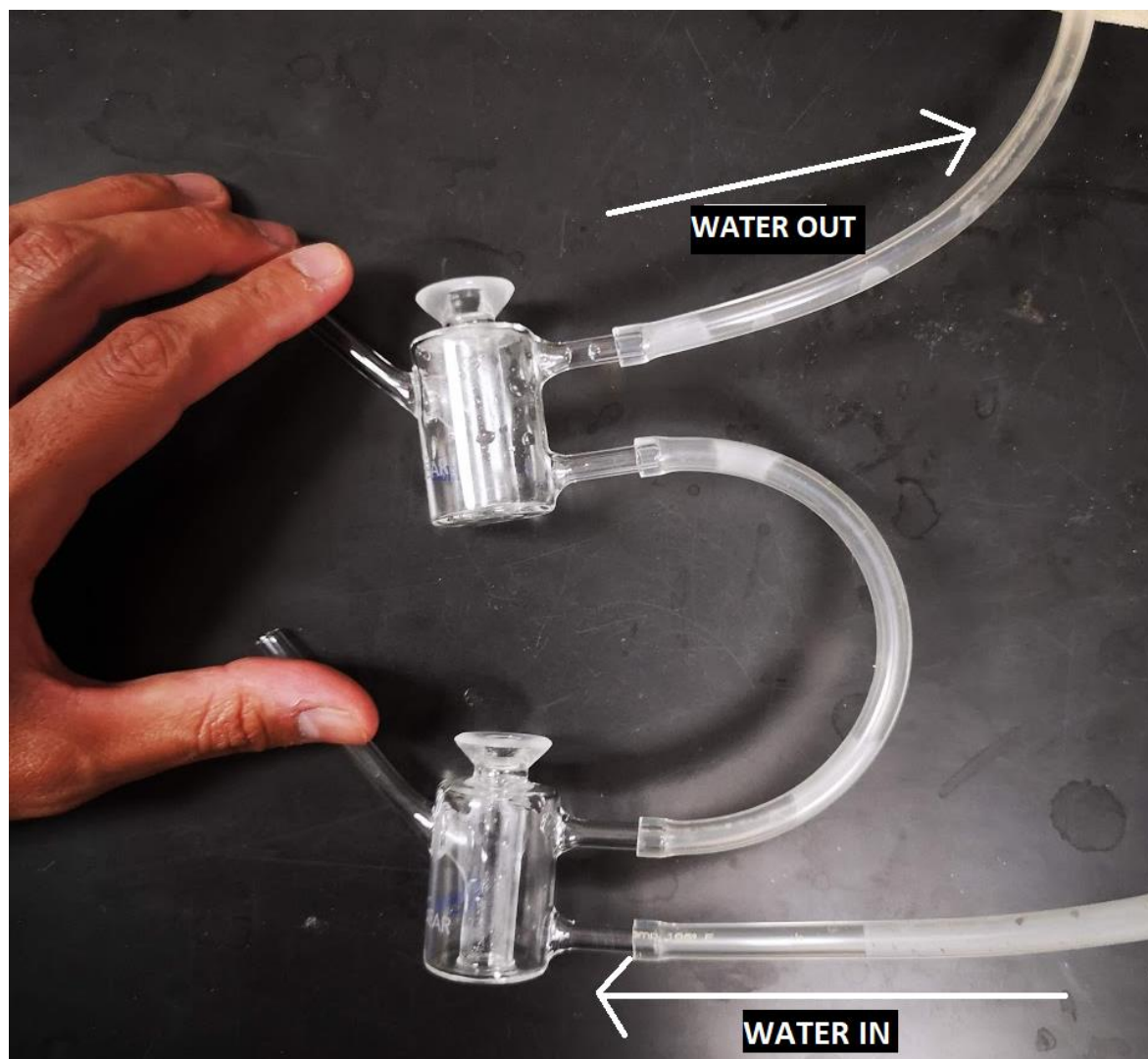


Figure 2. Franz Cells and pipes.

- d. Place the cells over the **magnetic stirrer base**, use the **stands, rubber bands** and **bars** to fix the position of the cells (figure 3) of the cells making sure they are horizontal and steady. Add the magnetic **stirrer** inside the receptor compartment.
- e. Start the **magnetic stirrer base** to make sure all the magnetic stirrers move properly at 300 rpm. If not, adjust the position of the cells.



Figure 3. Franz cells in magnetic stirrer arrangement

- f. Plug and start the **water pump** so water from the hot bath flows and fill the jackets of the cells with water at 37 °C. Make sure there are no leaks, the pipes are well adjusted, and the last pipe (drain) is secured to the **hot bath** as it might move.
- g. Open the clam and remove the **donor compartment**, using **tweezers** place the **membrane** with the shiny side up and then the **O-ring** (on top of the membrane), place the donor compartment on top of the O-ring and secure it with the clam (Figure 3).

NOTE: see section 3.4 for Sebum coated membranes.

- h. Using a micropipette, transfer 5 mL of Phosphate Buffered Saline (PBS) to a **10 mL syringe** with a 10 cm needle. Add the content of the syringe to the receptor compartment. Incline the cell to ensure no air is caught inside the donor compartment.
- i. Let the system equilibrate for 30 minutes in order to bring the receptor solution to 37 °C before adding the donor solution.
- j. Add 1 mL of the **donor solution** with a 1000 μL **micropipette** to the donor compartment. Take 5 mL of the donor solution (synthetic sweat following bioaccessibility test) with a 10 mL micropipette for analysis ($t=0$) in a 15 mL centrifuge tube.
- k. Record the starting time, use a chronometer.

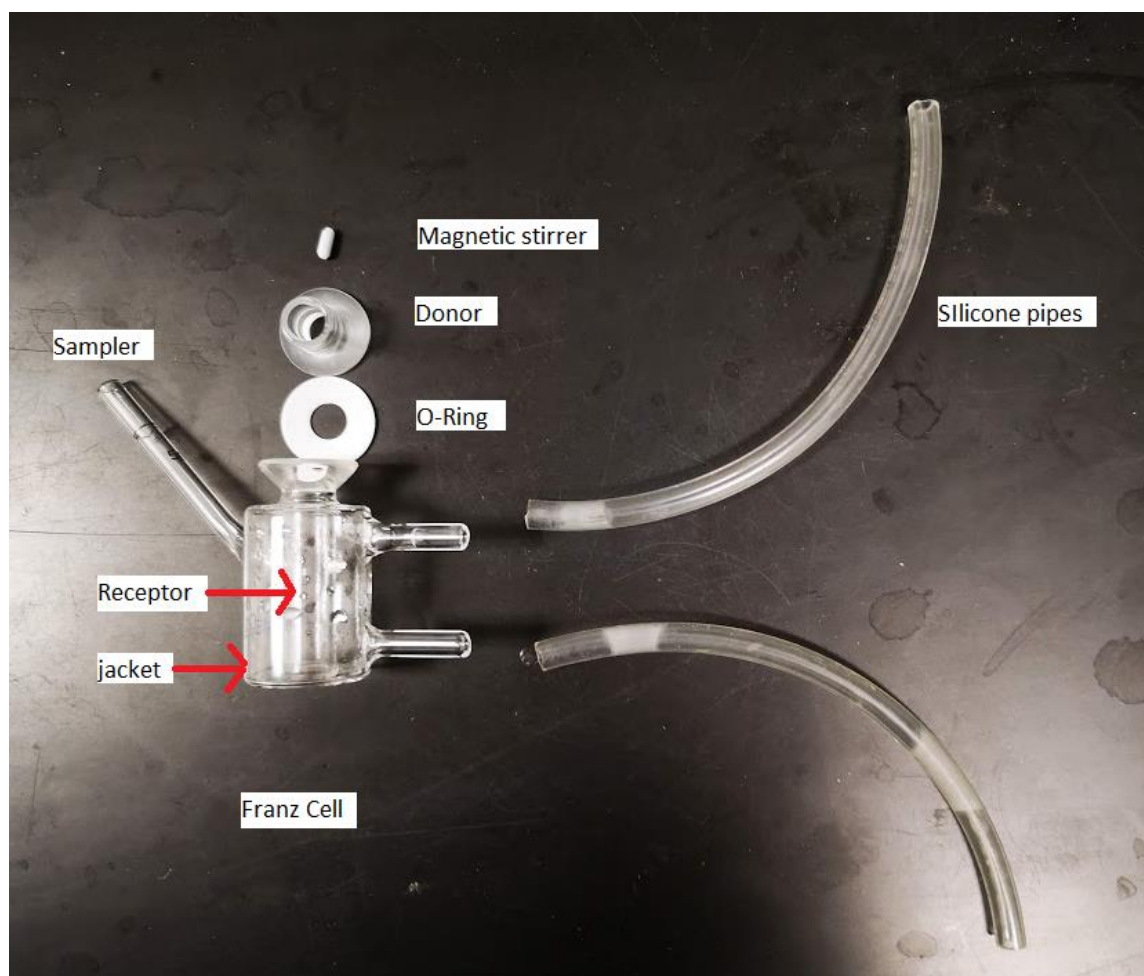


Figure 4. Components of Franz cell

Sampling

- a. The sampling should be performed at 6, 8, 10, 12, 14, and 24 h of the test.
- b. Insert a 10 cm needle fitted to a **10 mL syringe** to the receptor through the sampler.
- c. Remove all receptor solution and store it in a 15 mL **test tube**.
- d. Using the same syringe and needle, add exactly 5 mL (measure with a micropipette, then transfer to the syringe) of fresh donor solution (PBS) to the donor chamber to rinse any residues of metals, remove it with the syringe and place it in the same test tube as step c.
- e. With a new syringe and needle, fill the receptor with exactly 5 mL of fresh PBS receptor solution the same way as **set up step h**. make sure no air is trapped under the membrane and no donor solution is spilled.
- f. Make sure to properly label every test tube with the time, sweat and soil. Many samples will be collected, and good organization is essential. Store the samples at 4 °C.

Sampling 24 h (last sampling event) to perform mass balance analysis

- g. Collect the donor solution normally, no need to refill the receptor
- h. Using a dropper, collect the remain donor solution from the donor compartment. Store it in a 15 mL test tube.
- i. Carefully open the **clam** and remove the donor compartment, rinse (using a micropipette) the donor compartment, ring and membrane 4 times with 1 mL of **deionized water**. Store it in the same 15 mL tube as step h. It is recommended to use a **funnel** to ensure the collection of all the rinsing solution.
- j. Store the membrane in a 15-mL tube.
- k. Store the samples at 4 °C.

Note: This procedure is only necessary for **one sample per set** and is performed for quality purposes to quantify the metal mass that did not permeate. Figure 6 shows a flowchart of the process.

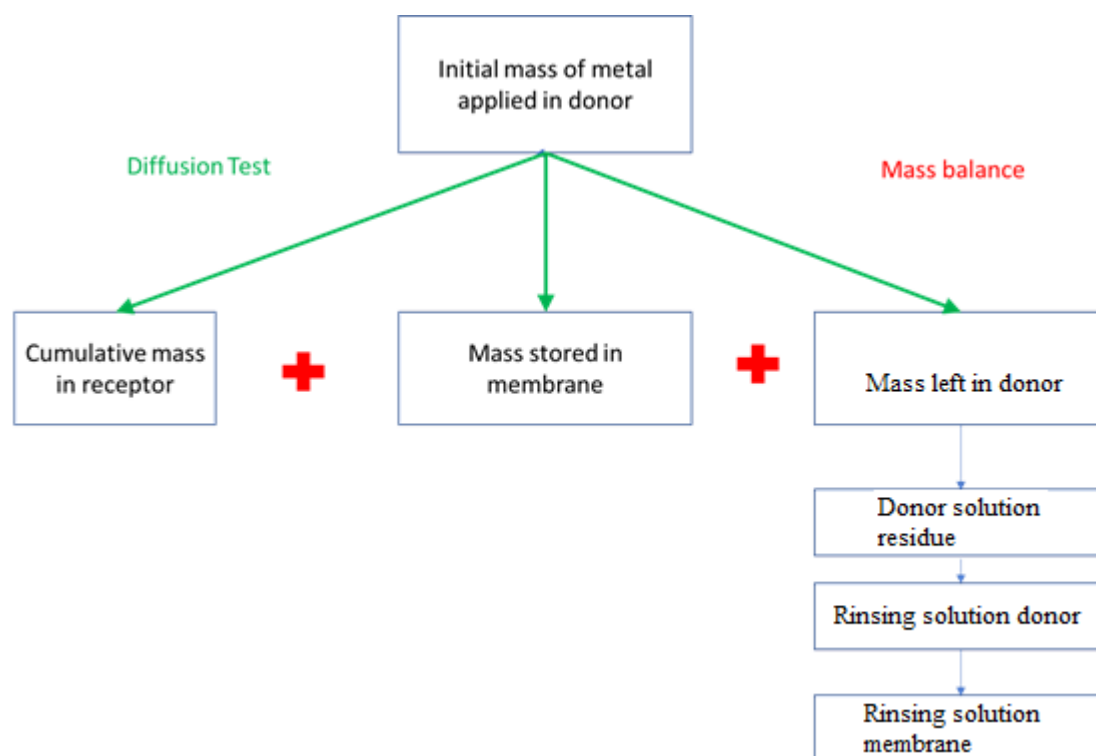


Figure 6. Flowchart of diffusion test and mass balance

Membrane treatment with sebum

- Prepare **Sebum B** as explained in the bioaccessibility protocol.
- After placing the membrane in the Franz cell (set up, step g). Using a micropipette, apply 0.1 mL (100 µl) of sebum on top of the membrane (shiny side).
- Continue the procedure normally.

Digestion of membrane and mass balance analysis

This method has been taken and modified from “Détermination des métaux: méthode par spectrométrie de masse a source au plasma d’argon” (Centre d’expertise en analyse environnementale du Québec, 2014). Include a procedure blank.

- In a Teflon beaker for digestion, add 4 mL of nitric acid (70 % v/v) and 10 mL of hydrochloric acid (50% v/v).
- Remove the membrane to be digested from the test tube with tweezers, rinse the tube with 5 mL of acetone to remove any residue to the vial and add it to the digestion beaker (A Franken et al., 2015).

- c. Cover the beaker with a glass lid and place it on top of a hot plate. Apply heat (250 °C) for 45 mins. If is boiling vigorously, lower the temperature.
- d. Let it cool down at room temperature for 30 mins.
- e. Add 10 mL of HF and apply heat (250 °C) for 30 mins.
- f. Let cool down at room temperature for 30 mins.
- g. Filter using a vacuum filter system (42.5 mm glass microfibre filter)
- h. Add approximately 80 mL of deionized water.
- i. Transfer content to a 100 mL volumetric flask, make up volume with deionized water.

NOTE: the membrane will not totally degrade

Analysis of samples

ICP-AES is recommended at INRS-ETE. At the time, the cost for analysis per sample is 15 CAD for the first metal and 2 CAD for each additional metal (25 CAD per sample to analyse 6 metals). Contact at INRS is Tran Lang Huong (Lan.Huong.Tran@ete.inrs.ca)

Calculations

Metals in donor and receptor solutions

Receptor, donor and membrane digestion solutions should be analysed by ICP-AES (A Franken et al., 2015) to establish the concentration of metals.

- a. Convert concentration of metals in the receptor to total mass that permeated through the exposed membrane area [mass/area].
- b. Plot against time: cumulative mass of metal that permeated per area of skin vs time. Determine the steady state region.
- c. The slope for this plot in the steady state region gives the flux through the skin ($\mu\text{g cm}^{-2}\text{h}^{-1}$) and the intercept of the curve with the x axis gives the lag time (h).
- d. The permeation coefficient (K_p) is calculated from the linear steady state region of the plots by dividing the flux through the skin ($\mu\text{g cm}^{-2}\text{h}^{-1}$) by the concentration used (Larese et al., 2007).
- e. Report flux ($\mu\text{g cm}^{-2} \text{h}^{-1}$) lag time (h) and K_p (cm h^{-1}).

EXAMPLE:

The data for this example has been taken from A. Franken et al. (2015)

From a permeation test, the following concentrations of platinum have been found after ICP analysis of the donor chamber. The initial concentration of platinum in the donor is **0.3 mg/l**:

Time (h)	Cumulative mass (ng/cm ²)
0	0
6	3
8	4
10	6
12	9
14	14
24	37

The first step is to plot the cumulative mass vs time (Figure 6). Localize the steady state section and find the slope for this region.

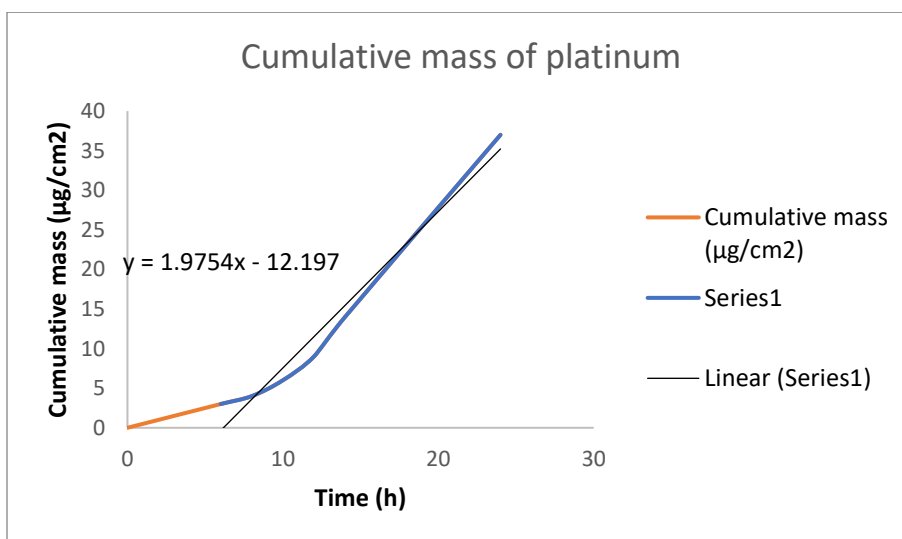


Figure 7. Cumulative mass of platinum

The slope is the flux ($\mu\text{g}/\text{cm}^2/\text{h}$) = 1.9754

Lag time $\rightarrow$ intercept with x axis

$T = 6.17 \text{ h}$

Permeability coefficient K_p (cm/h) = flux/concentration in donor = $1.9754/(0.3 \times 1000 \times 1000) = 6.58 \times 10^{-6} \text{ cm}/\text{h}$

Mass balance analysis

The mass balance analysis should be made with one sample per set for quality purposes.

Mass of metals in receptor (cumulative), mass that depot in the membrane (measured via membrane digestion) and mass that remained in donor should be calculated after test finished. These masses should be expressed as a percentage of the initial mass added in the donor solution at $t=0$. Reported admissible values range from 80% to 120%.

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