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 Cu^{2+}. 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 Kp for Cu was higher (0.024 to 0.52 cm/h). As, Ni, Pb, and Zn did not permeate the synthetic skin.

Keywords: soil pollution, sweat, chemical equilibrium modelling, Visual MINTEQ, Franz diffusion cell, Strat-M® membrane.

Environmental implication

The manuscript is original as it explains, for the first time in the context of contaminated soils, how speciation of the bioaccessible fraction of metal(loid)s in synthetic sweat affects permeation through a synthetic skin membrane. This manuscript is environmentally relevant and is suitable for publishing in Journal of Hazardous Materials as its subject falls into the journal's subject areas of Environmental Engineering (*in vitro* bioaccessibility, dermal permeation) and Environmental Chemistry (metal(loid)s speciation).

Highlights:

- In both synthetic sweats, As was mainly found as As(V).
- In sweat A, Cr(III) and Cr(VI) were equally present but in sweat B, Cr(III) was prevalent.
- Synthetic skin coating with sebum seems to increase Cr permeation.
- K_p ranged between 0.004 and 0.13 cm/h for Cr and 0.024 and 0.52 cm/h for Cu.
- As, Ni, Pb, and Zn did not permeate the synthetic skin membrane.

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(loids) 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.

2. Materials & Methods

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 ($d < 425$ µm), and five field-collected soil samples ($d < 420$ µm) (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 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 MΩ.cm). Field-collected soil samples were air-dried, gently disaggregated using a mortar and dry sieved to 420 µm using a sieve shaker (Retsch AS-200). Only the fine fraction of soils (< 420 µm) 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 HNO_3 (70% w/w), HF (50% w/w), and $HClO_4$ (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 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 NH_4OAc 1N described in Chapman (1965).

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 S1 of the supplementary material.

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^{o}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^{o}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).

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).

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, Na_2HPO_4 1.44 g/L, and KH_2PO_4 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/cm^2) and plotted against time. The flux (µg/cm^2 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.

3. Results and Discussion

3.1 Soil characterization

Soil characterization and metal(loid) concentrations are shown in Table 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 mg/kg ± 6.8 %) (more than 30 times the C criterion of 50 mg/kg) and S7 (311 mg/kg ± 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 ± 10% of the certified values.

Table 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/100g) of Soils. Precision is Expressed as Mean ± Relative Standard Deviation %.

Parameter	S2	S3	S6	S7*	S8*	SQC001*
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.

3.2 Bioaccessibility of metal(loid)s

While bioaccessible concentration in solution reveals the amount of metal solubilized in synthetic sweat, bioaccessible percentages (Table S2) 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 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 2. Bioaccessible Concentration (mg/L) of As, Cr, Cu, Ni, Pb, and Zn in Soils Using Synthetic Sweat Formulations A and B.

	Soil	As	Cr	Cu	Ni	Pb	Zn
Sweat A	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

3.4 Chemical equilibrium modelling

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).

3.4.1 Arsenic

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

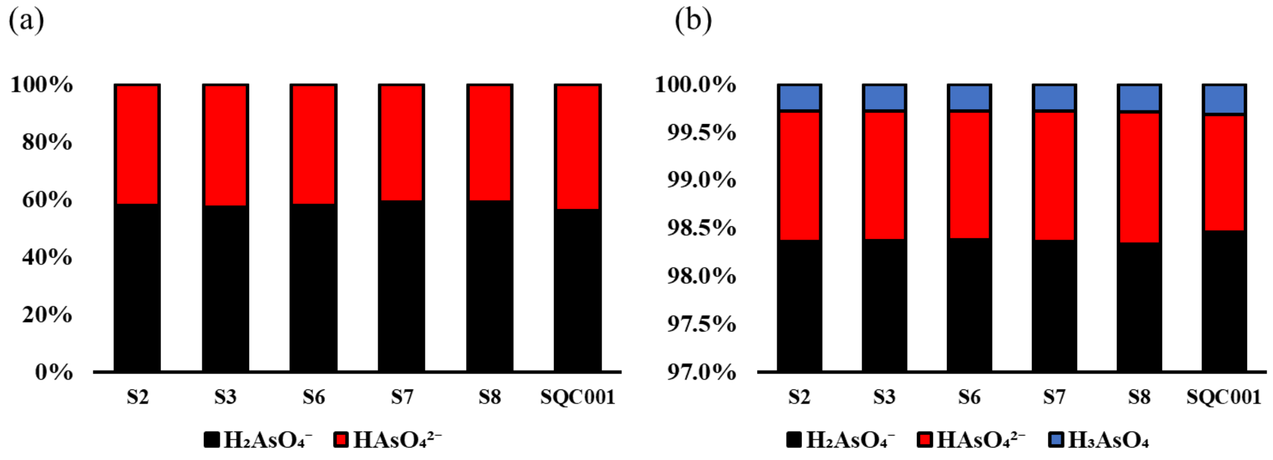

Figure 1. Speciation of As (V) (%): (a) in Sweat A, (b) in Sweat B

3.4.2 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 2.97E-7 ± 2.21E-7 mol/L whereas Cr (VI) mean concentration was 3.97E-7 ± 2.69E-7 mol/L. Cr(III) species were found in similar proportions in bioaccessible extracts from all study soils following extraction with sweat A (Figure 2a) with 38.2% ± 1.6 % of $Cr(OH)_3$ (aq), 36.4% ± 1% of $Cr(OH)_2^+$ and 25% ± 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 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% ± 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 1.79E-5 ± 2.17E-6 mol/L compared with 1.98E-11 ± 7.51E-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 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 2 and Table S2). 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 (3.97E-7 ± 2.69E-7 mol/L) than sweat B (1.98E-11 ± 7.51E-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).

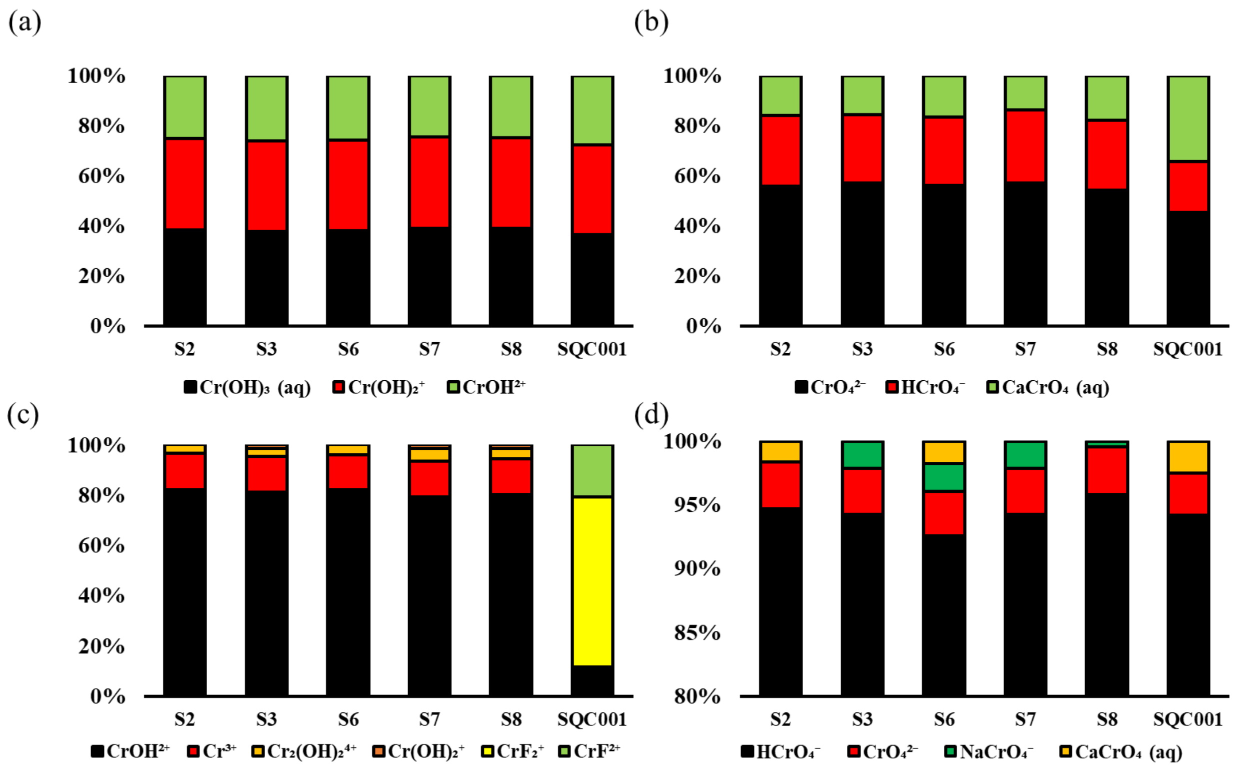

Figure 2. Chromium Speciation (%): (a) Cr(III) in Sweat A, (b) Cr(VI) in Sweat A, (c) Cr(III) in Sweat B, and (d) Cr(VI) in Sweat B.

3.4.3 Copper

In sweat A, Cu was mainly present as Cu(II). The concentration of Cu(II) and Cu(I) in mol/L were 4.93E-6 ± 1.18E-6 and 4.93E-10 ± 3.14E-10, respectively. Most of Cu(II), was found as Cu^{2+} (Figure 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 5.28E-5 ± 2.47E-5 and 3.67E-8 ± 1.80 E-8, respectively. Similarly to sweat A, the most abundant compound was Cu^{2+} with a proportion of 72.3 % ± 19.2%. However, $CuCl^+$ at 27% ± 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 3b) and $CuCl^+$ at 8.15%. This much higher proportion of Cu^{2+} ion helps explain the 61.3 % bioaccessible percentage of Cu (Table S2) in the reference material when extracted with the more acidic sweat B compared to a significantly lower average dermal bioaccessibility of 2.6 % ± 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).

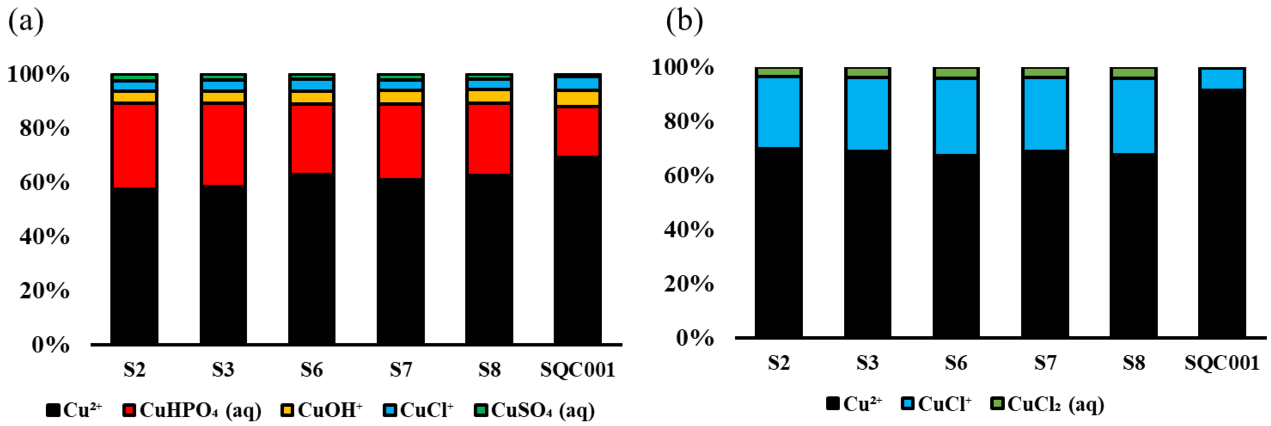

Figure 3. Copper Speciation (%): (a) Cu(II) in Sweat A (b) Cu(II) in Sweat B

3.4.4 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 S1). 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 S1).

3.4.5 Lead

As shown in Table 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 $PbCl_2$(aq), $PbSO_4$(aq), and $PbOH^+$ were also present but in lower proportions (< 10%) (Figure S2).

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. $PbCl_2$ (aq) represented 28.5% ± 0.5% in the bioaccessible extracts of soils S2, S3, and S6 but only 8.5 % in reference soil SQC001 (8.5%) (Figure S2).

3.4.6 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 S2)). Zinc was primarily found as Zn^{2+} (> 80%) in sweat A (Figure S3). In sweat B, the most abundant species was also Zn^{2+} (44% - 48%) but followed by $ZnCl^+$ (25% ± 1%) and $ZnCl_2$ (aq) (11% ± 1%) for all soils except SQC001. In SQC001 extract (sweat B), Zn^{2+} comprised more than 87 % of Zn (Figure S3) explaining the 91 % dermal bioaccessibility (Table S2).

3.3 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 (µg/cm^2) was assessed and plotted against time (Figure 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 µg/cm^2) was found in the membranes following digestion.

3.3.1 Chromium

Bioaccessible Cr extracted from all soil samples permeated through the synthetic skin (Table 3 and Figure 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 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 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 3). The mean flux (1.4E-3–1.9E-3 µg/cm^2 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 3. Flux (µg/cm^2 h), Lag Time (h), and Permeation Coefficient (cm/h) for Cr and Cu (Data Expressed as Mean ± Standard Deviation)

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

* From Marin Villegas & Zagury (2021)

3.3.2 Copper

Along with Cr, Cu was the only other trace element that permeated the membrane (Table 3 and Figures 4c and 4d). Copper extracted from all soil samples (S2, S3, S6, S7, S8, and SQC001) permeated the membrane (Figure 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=12h and t=24h). 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 3a). Moreover, most of Cu(II), was found as Cu^{2+} (Figure 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 2). This suggests that Cu species present in sweat A and absent in sweat B might permeate the membrane. $CuHPO_4$(aq), $CuOH^+$, and $CuSO_4$(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 that 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 µg/cm^2), Cu content in human skin following permeation reported by Franken et al. (2015) was much higher and ranged from 53-290 µg/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.

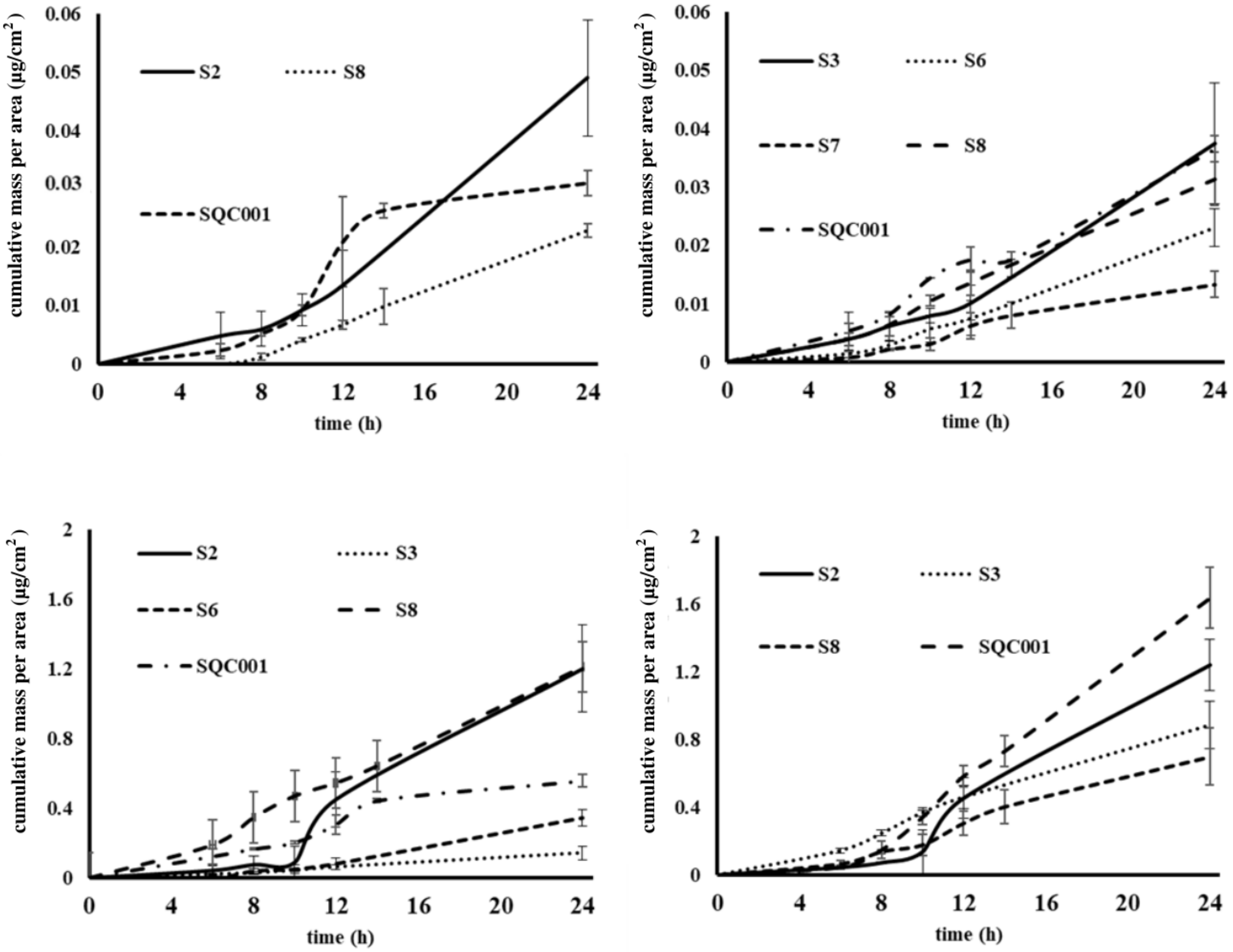

Figure 4. Mean Cumulative Mass per Area and Standard Deviation of Metal in Sweat A (pH = 6.5) That Permeated Through the Strat-M Membrane for (a) Chromium (Not Coated with Sebum), (b) Chromium (Coated with Sebum), (C) Copper (Not Coated with Sebum), and (d) Copper (Coated With Sebum). Results of S7, S8, And SQC001 are Extracted from Marin Villegas & Zagury (2021)

3.4 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.

4. 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(loids) 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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