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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. Dermal bioaccessibility test 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.

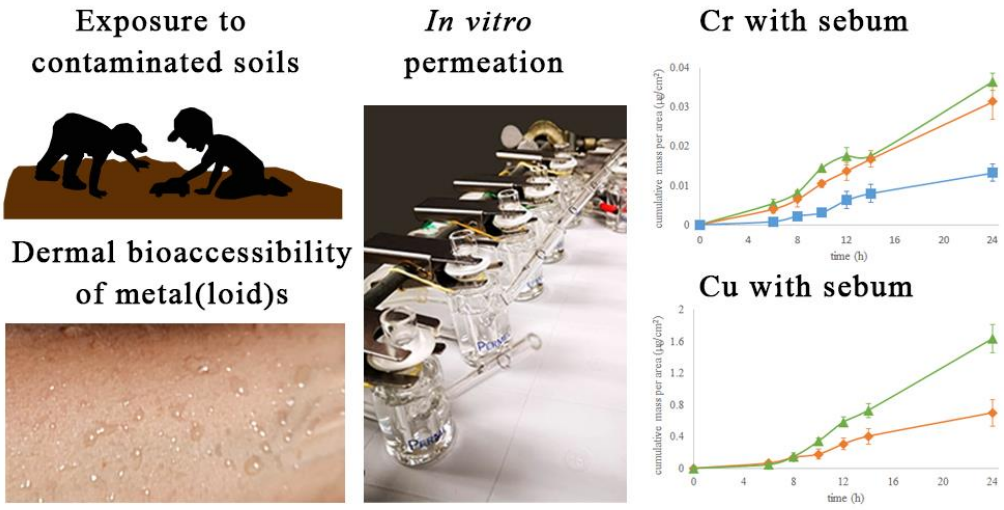
The metal(loid) bioaccessibility percentage was higher for Sweat B (pH 4.7) compared to Sweat A (pH 6.5), attributed to lower pH of sweat B. Among the six elements tested, only chromium and copper permeated the membrane. Permeation coefficient (Kp) was higher for chromium in Sweat A (0.05 to 0.11 cm h^{-1}) than Sweat B (0.0007 to 0.0037 cm h^{-1}) likely due to a higher pH and thus more permeable Cr species. 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.

Key words: contaminated soils, dermal permeation, Franz diffusion cell, in vitro dermal bioaccessibility, synthetic sebum, Strat-M membrane**,** synthetic sweat.

Synopsis: When metal(loid)s found in polluted soils are extractable in sweat they don't indeed permeate a synthetic skin membrane

Graphical abstract TOC



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 [1-4] and *in vitro* dermal bioavailability. [5-8]

It is generally acknowledged that there are very few quantitative and qualitative data on dermal exposure to toxic chemicals present in geological materials [9]. 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 results difficult. [10] 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. [9-11]

The most common method to evaluate dermal diffusion was introduced by Franz [12] 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. [9]

Franken et al.[10] highlighted the importance of the donor solution composition in the Franz cell methodology. The donor solution should be standardized since 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 [7-8]. Even though the influence of synthetic sweat formulation in 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" [6]. 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.[13] Because of these limitations, some studies used animal skin, such as swine, rat, or guinea pig, as human skin surrogates. [10,14] 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 results difficult. [13,15] Moreover, ethical consideration inherent to the use of biological tissues from 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. [16] The Strat-M (EMD Millipore, MA, USA) synthetic membrane is an ultrafiltration membrane made of polyethersulfone composed by multiple layers, including two layers impregnated with synthetic lipids and a very tight top layer, producing diffusion results similar to natural human skin. [16-17] In diffusion studies with human cadaver skin, animal skin, and Strat-M. membrane, Joshi et al.[17] 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 to human skin than the untreated membrane and animal skin. They also exposed the difficulties of correlating diffusion data between animal skin and human skin [17]. In most cases, the correlation between the treated synthetic membrane Strat-M and human skin was better. Moreover, Strat-M membrane showed a high lot-to-lot reproducibility and 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.[5,18] This concentration can be used in conjunction with the permeation data to calculate bioavailability. [5]

To estimate the dermal absorption of contaminants from aqueous media, the USEPA [19] proposes the water approach methodology. It assumes contact with contaminated water and aims to calculate the dermally absorbed dose (DAD) 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. [7,19] Nevertheless, published K_p values involve a high level of uncertainty (since they don't take speciation into account except for chromium) and they are available for metal(loid)s soluble in water but not soluble in sweat. [19] Improvement in K_p determination can reduce uncertainty in the calculation of dermal exposure therefore 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.

2. Materials & Methods

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. The certified material SQC001 (lot number LRAC0025, produced by Sigma-Aldrich in accordance with ISO 17034 and ISO/IEC 17025 procedures (d < 425 µm)), and two field-collected soil samples (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 10 cm depth. 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^{-1}) HNO_3 and rinsed with deionized water (18.2 MΩ.cm) prior to use. [2-3] 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). Samples were then stored at 4°C.

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. [20] Digestates were transferred to 100-ml volumetric flasks and made up to volume with deionized water. Solutions were filtered (0.45µm) with glass microfiber filters (Whatman) and stored in polypropylene centrifuge tubes with HDPE screw caps. Cr, Cu, Ni, Pb, and Zn concentrations were measured via atomic absorption spectroscopy (AAS) (Perkin- Elmer A200). Detection limits (DLs, determined based on signal-to-noise approach (ratio of 3:1)) in mg kg^{-1} were 0.3, 0.3, 0.2, 1, and 0.1, respectively. Arsenic content was determined via ICP-OES (Varian Vista), with a detection limit (DL) of 0.004 mg kg^{-1}. Soil pH was measured in duplicates in solid-to-liquid ratio 1:2 with deionized water (pH meter: Eutech pH 200 series, probe: Accumet Ag/AgCl) according to method ASTM D4972-13. [21] Total organic carbon content with a detection limit (DL) of 0.1% w/w 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. [22] Cation exchange capacity (CEC) was determined using the sodium acetate method with NaOAc 1N and NH_4OAc 1N.[23]

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 1). The SSFL formulations are further described elsewhere [8] 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 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 [24]

[b] According to standard NIHS 96-10 [25]

[c] According to Wertz [26]

2.3 *In vitro* dermal bioaccessibility test

Tests were started by adding 20 ml of synthetic sweat to 2 g of soil sample (d < 425 µm) 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 36ºC, corresponding to the median skin temperature for humans [5], for 2 hours. The tubes were then centrifuged (Heraeus Megafuge 8, Thermo Fisher) at 10,000 x g for 10 minutes, and the supernatant collected with 60 ml Luer-Lok syringes and filtered with a 0.45 µm PVDF filter fitted to the syringe. The filtered supernatant was transferred into 50-ml Polypropylene centrifuge tubes with HDPE caps and stored at 4ºC until analysis. Samples were analyzed via ICP-AES (Vista, Varian Inc.) to determine As, Cr, Cu, Ni, Pb, and Zn concentrations (with detection limits 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:

$$\%_{bio} = \frac{C_{bio}}{C_{total}} * 100 \quad (1)$$

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. [8]

2.4 Permeation test

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. The temperature of the receptor was maintained at 37 ^{o}C by circulating water from a water bath (Model 2849, Thermo Fisher Scientific) to simulate temperature below the skin.[10, 27] The receptor compartment was filled with Phosphate-Buffered Saline (PBS) solution (Fisher Scientific) at a pH of 7.4 [13] 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, to represent blood salt concentration and blood pH. [10] 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 for human skin. Each membrane was mounted on the Franz diffusion cell with the shiny side in contact with the donor compartment. [13] 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 ml of 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^{o}C until analyzed via ICP-AES (Vista, Varian Inc.) to determine 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 HNO_3, HCl, and HF for 45 minutes 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.

3. Results and Discussion

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 2). 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. [2] Total metal(loid)s concentrations in soil samples S7 and S8 are also shown in Table 2. Values in bold are exceeding Quebec's regulatory limit for industrial land-use (C criterion).[28] 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 %). 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 [2] 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 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 ± 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

3.2 Dermal bioaccessibility of metal(loid)s

Table 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. [8], 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.

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 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. [29]

Table 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	S7	1.4	0.15	0.5	< 0.4	< 0.2	0.05
(pH=6.5)	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	S7	2.8	2.0	7.2	0.5	< 0.2	9.0
(pH=4.7)	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

(b) Bioaccessible concentration (mg l^{-1})

Donor solution	Soil	As	Cr	Cu	Ni	Pb	Zn
Sweat A	S7	0.45	0.05	0.38	< 0.01	< 0.01	0.01
(pH=6.5)	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	S7	0.86	0.76	5.93	0.01	< 0.01	2.34
(pH=4.7)	S8	1.36	0.59	3.13	0.02	< 0.01	0.66
	SQC001	0.88	3.12	5.07	5.02	9.20	46.6

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 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. [7], and Marin Villegas et al. [8]

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 (µg l^{-1}) was converted to the total metal(loid) amount that permeated (µg.cm $^{-2}$) and then plotted against time (Figure 1 and Figure 2). Flux (µg cm^{-2} h^{-1}) was calculated as the slope from the steady-state region of graphs shown on Figures 1 and 2 and 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 (µg cm^{-2} h^{-1}) by the concentration in the donor solution. [30]

Overall, mass balance recovery calculated from donor solutions, synthetic membranes, and receptor solutions yielded satisfactory percentages. 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.

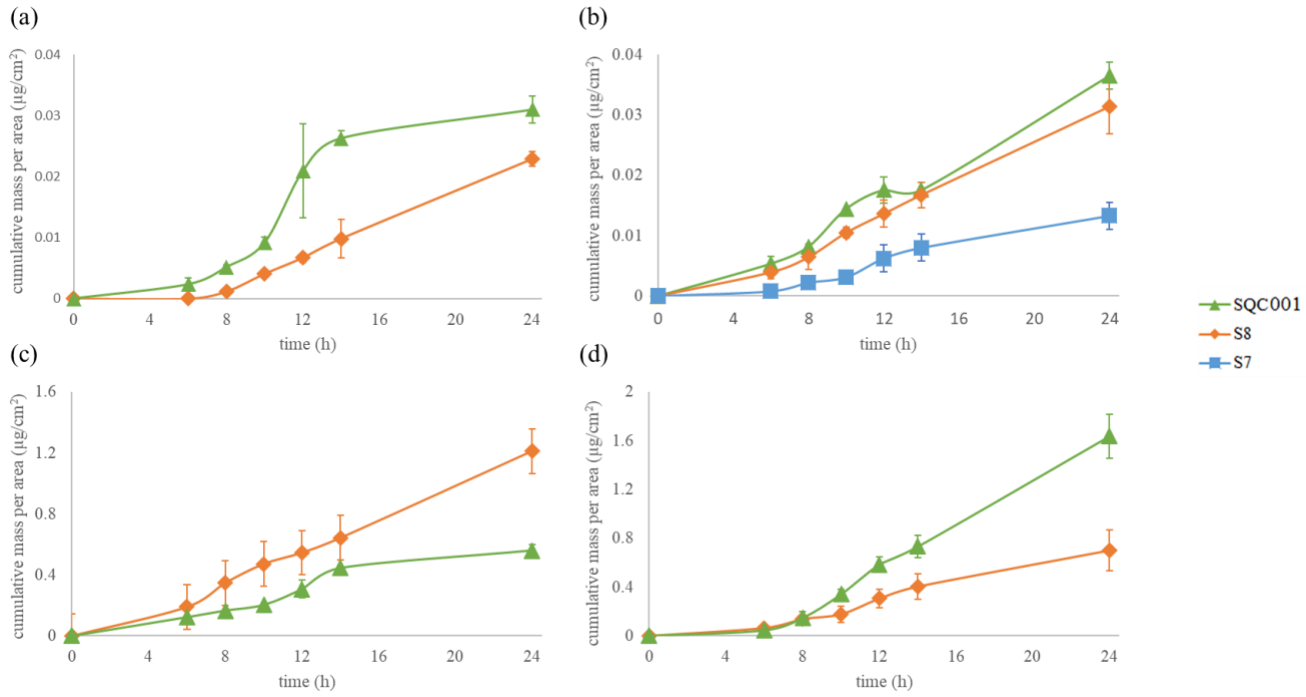


Figure 1

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).

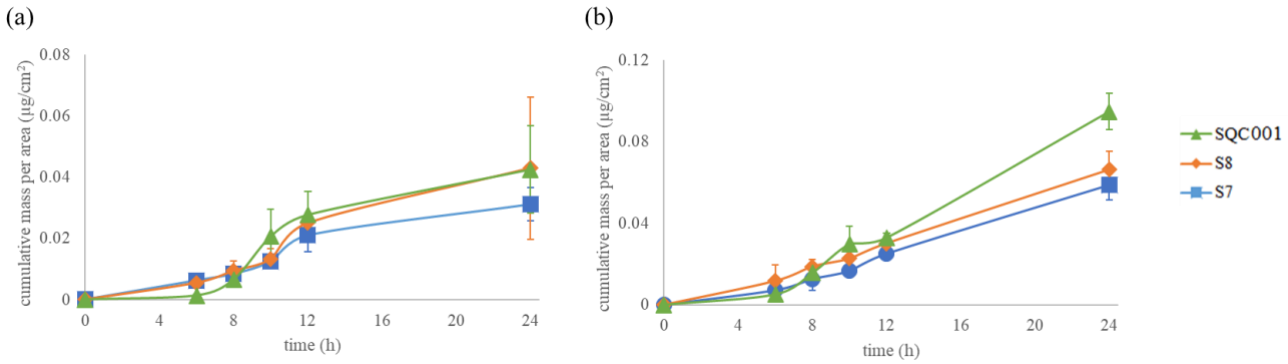


Figure 2

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.

3.3.1 Arsenic

Arsenic did not permeate the synthetic skin membrane for all sweat formulation and soil sample 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. [31-32] Experimental data indicate that penetration of metal(loid)s through the skin is significantly dependent on ion mobility and charge. [33]

3.3.2 Chromium

Figure 1 and Figure 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). Results (Table 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 [10, 34 ,35], which explains the higher Flux and K_p of Cr in Sweat A.

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 $Cr(OH)_3(aq)$, whereas in Sweat B due to a more acidic pH, Cr(III) is expected to be the main Cr oxidation state. [36] This hypothesis is backed by the fact that for Sweat A, there is no visible influence of sebum in the permeation parameters. However, other variables such as molecular size, chemical reactivity, and counterions could affect permeation of Cr through the skin membrane [33, 37] 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, absolute absorption can reach a plateau value and then decrease with an increment in concentration. [37] This phenomenon happens 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. [37] As previously reported, [8] 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 bioaccessibility test (Table 3) producing different diffusion profiles (Figure 1 and Figure 2).

Table 4

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

(a)

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

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 1 and Table 4). It should be noted that Cu was also detected in the receptor solution following

355 permeation with sample S7 extracted with Sweat A (0.38 mg l^{-1}) in the presence of sebum at
356 the end of the sampling period (t=12h and t=24h). However, there was not enough data to
357 build the cumulative mass per area versus time curve and calculate the permeation
358 parameters. Copper did not permeate the membrane when extracted with sweat B even
359 though bioaccessible concentrations (donor solution) were much higher (3.13 mg l^{-1} for S8
360 and 5.07 mg l^{-1} for SQC001). This suggests that Cu speciation and complexation, which
361 depends on pH, and sweat formulation among other parameters, might influence the
362 permeation of Cu.

363 The K_p for Cu was similar for soils S8 and SQC001 in the absence of sebum. However, in
364 the presence of sebum, the K_p was around five times higher for SQC001. Lag times before
365 Cu permeation were longer in the presence of sebum for S8 and SQC001, increasing from
366 0.2 to 4.6 h and from 0.6 to 5.9 h, respectively. This could be caused by the added layer
367 formed by the sebum, causing the Cu flux to take a longer time to reach equilibrium.

368 **3.3.4 Nickel**

369 High bioaccessible Ni concentrations (2.29-5.02 mg l^{-1}) were obtained when extracting
370 SQC001 with both sweat formulations but low to below detection (< 0.01 mg l^{-1})
371 bioaccessible Ni concentrations were measured when extracting field-collected soil samples
372 S7 and S8 (Table 3). In all cases, Ni did not permeate the synthetic skin membrane.
373 Depending on Ni activity, Ni in solutions with a pH < 8 is expected to be mostly found as
374 Ni^{2+}. [38] Fullerton et al. [39] reported a strong influence of the vehicle in the permeation of $NiCl_2$
375 and $NiSO_4$ through the skin and lag times of 50 h (our permeation test lasted 24 h). In another
376 study, Larese et al. [30], tested permeation through human abdominal skin of Ni powder in
377 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.

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 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. [10, 40] The Strat-M membrane is designed to specifically simulate the diffusion pathway.

3.3.6 Zinc

Zinc did not permeate the synthetic skin membrane even if very high bioaccessible concentrations (Table 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. [41] 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. [10,42,43]

3.3.7 Comparison of K_p values with published values

The USEPA [19] recommends K_p values (cm hr^{-1}) for some metals (Cr(III): 0.001, Cr(VI): 0.002, Ni:0.0002, Pb:0.0001, Zn: 0.0006, and other non-specified inorganics: 0.001). These values have been adapted from Hostýnek et al. [44] Because of its conservative approach,

USEPA [19] listed the highest reported permeability coefficient. Nonetheless, other studies reported higher permeability coefficients than the ones recommended by USEPA. [19] Examples of these are Filon et al.[45], who reported a K_p for Cr of 0.0124, and Fullerton et al.[39], who reported K_p for Ni of 0.0015 for epidermis and 0.23 for the dermis.

The K_p 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. [19] 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 Kp 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. 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 bioaccessibility test in synthetic sweat, and Cr and Cu seem to have a 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, 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 with dermal exposure.

The results obtained in this study showed that membrane Strat-M 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 to simulate reservoirs of metal(loids) in the stratum corneum and other layers of the skin. Further research to compare 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.

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5. References

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