Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Medyczne Centrum Innowacji Wrocław Sp. z o.o.

Sadri Rayad

„Zawartość metali toksycznych w zatrzymanych zębach trzecich trzonowych u mieszkańców Legnicko-Głogowskiego Okręgu Miedziowego."

„Toxic metal concentrations in retained third molars in the inhabitants of the Legnica-Głogów Copper Area."

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

Promotor:
Prof. dr hab. Marzena Dominiak
Katedra i Zakład Chirurgii Stomatologicznej UMW

Drugi Promotor:
Dr hab. Maciej Dobrzyński, prof. uczelni
Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej UMW

Wrocław 2024

Chciałbym złożyć podziękowania dla Obu Promotorów:
Prof. dr hab. Marzeny Dominiak
oraz Dr. hab. Macieja Dobrzyńskiego, prof. uczelni.

Bez Waszego wsparcia oraz wielu nieocenionych rad nie byłoby możliwości zrealizowania tego celu.

Dziękuję również za inspiracje powstałe podczas wielu niekończących się rozmów dotyczących zarówno działalności naukowej jak i rozwoju osobistego.

Chciałbym podziękować również wszystkim moim przyjaciołom z Uniwersytetu Medycznego we Wrocławiu, a w szczególności Amadeuszowi Kuźniarskiemu,
Jego wkład we wszystkie moje wyzwania zarówno naukowe jak i życiowe jest nieoceniony. Nasza wspólna droga jest przygodą o wielu płaszczyznach, a przyjaźń każdego dnia mnie zaskakuje.

Prof. dr. hab. Markowi Ziętkowi za to, że od pierwszych dni gdy nasze drogi się przecięły prowadził mnie jak
Prawdziwy Mistrz
i nie dotyczy to tylko sfery naukowej ale również życia zawodowego i prywatnego.

Chciałbym również przy tej okazji szczególnie podziękować Prof. dr. hab. Tomaszowi Konopce. Twoje rady i wielogodzinne rozmowy pozostaną w mojej pamięci na zawsze. Nie zapomnę również, że zasiałeś jako pierwszy myśli o rozwoju naukowym.

Pracę dedykuję moim Rodzicom, bez których mój świat oraz myśli nie miały by formy, która pozwala mi się realizować każdego dnia.

Spis treści

Podstawę niniejszej rozprawy doktorskiej stanowi spójny tematycznie zbiór 4 artykułów opublikowanych w czasopismach naukowych o łącznej punktacji IF=15,00 ; MNSiW=330

1. *Studies on the content of toxic metals in teeth – a narrative review of literature – Dent. Med. Probl. 2024, https://doi.org/10.17219/dmp/193406*
2. *An In-Vitro Evaluation of Toxic Metals Concentration in the Third Molars from Residents of the Legnica-Głogów Copper Area and Risk Factors Determining the Accumulation of Those Metals: A Pilot Study - Appl. Sci. 2023, 13, 2904. https://doi.org/10.3390/app13052904*
3. *Mercury Content in Impacted Wisdom Teeth from Patients of the Legnica–Głogów Copper Area—An In Vitro Pilot Study - J. Xenobiot. 2023, 13, 463–478. https://doi.org/10.3390/jox13030029*
4. *Comparative Analysis of Heavy Metal Content in Impacted Third Molars from Industrial and Non-Industrial Areas and Its Effect on the Isolation, Culture, and Proliferation of Dental Stem Cells (DSCs) - J. Clin. Med. 2024, 13, 5465. https://doi.org/10.3390/jcm13185465*

WYKAZ UŻYWANYCH SKRÓTÓW

L-G - Legnicko–Głogowski Okręg Miedziowy

Pb - Ołów

Cd - Kadm

Cr - Chrom

Ni - Nikiel

Fe - Żelazo

Mn - Mangan

Cu - Miedź

Hg - Rtęć

DSCs - Dental Stem Cells

BMSC - Bone Marrow Stem Cells

BMP - Bone Morphogenetic Protein

AAS - Atomic Absorption Spectrometry Absorpcyjna spektrometria atomowa

STRESZCZENIE

WSTĘP

Pierwsze trzy publikacje dotyczyły możliwości wykorzystania trzecich zębów trzonowych jako wskaźników narażenia na metale ciężkie w określonym środowisku bytowania. Biomonitoring, jako część medynycy środowiskowej, to działania mające na celu ocenę stanu środowiska przy użyciu wskaźników zwanych biomonitorami. Należą do nich m.in. zęby, ślina, włosy, paznokcie czy mocz. Zęby stanowią trwały zapis stylu życia danej osoby w danym środowisku, odzwierciedlając wpływ zanieczyszczeń. W procesie mineralizacji zębów kationy metali ciężkich wbudowują się w hydroksyapatyty tkanek zmineralizowanych zęba. Legnicko-Głogowski Okręg Miedziowy, ze względu na przemysłowy charakter, jest obszarem o zwiększonym ryzyku narażenia na metale ciężkie. Źródłem metali mogą być: zanieczyszczona gleba, powietrze, skażona żywność czy woda pitna. Metale ciężkie mogą powodować różne choroby, w tym układu stomatognatycznego. Badanie ocenia akumulację metali: Pb, Cd, Cr, Ni, Fe, Mn, Cu, Zn i Hg w trzecich zębach trzonowych mieszkańców tego regionu. Zbadano też związek między zawartością metali a danymi demograficznymi, dietą oraz suplementacją witamin. Czwarta publikacja stawia pytanie, czy zawartość metali ciężkich w trzecich zębach trzonowych wpływa na izolację, hodowlę i wzrost komórek macierzystych z miazgi zębowej (DSCs), co może być ważnym ryzykiem przy ich przyszłym zastosowaniu w medycynie regeneracyjnej.

CEL

1. Analiza aktualnych badań i identyfikacja głównych źródeł narażenia na metale ciężkie oraz mechanizmów, za pomocą których metale te odkładają się w tkankach zęba.
2. Ocena akumulacji metali ciężkich: Pb, Cd, Cr, Ni, Fe, Mn, Cu, Zn i Hg w trzecich zębach trzonowych mieszkańców Legnicko-Głogowskiego Okręgu Miedziowego oraz ich korelacja z danymi demograficznymi, klinicznymi, dietą oraz suplementacją witaminową.
3. Ocena wpływu metali ciężkich na proliferację komórek macierzystych pozyskiwanych z miazgi zębowej.

MATERIAŁ I METODY

Ze względu na charakter prac materiały i metody podzielono na 3 części:

1. Analiza aktualnych badań obejmujących stomatologiczny aspekt metali toksycznych: Kompleksowe wyszukiwanie literatury przeprowadzono z wykorzystaniem wybranych baz danych. Wyszukiwanie ograniczono do literatury z lat 1978-2023 i włączono 83 badania.

2. Ocena akumulacji toksycznych metali w zębach:

Badanie przeprowadzono w grupie 72 pacjentów, u których wykonano ekstrakcję zębów trzecich trzonowych. Oprócz usuniętych zębów, od pacjentów pobrano krew oraz uzyskano odpowiedzi w autorskim kwestionariuszu. Pacjenci zostali podzieleni na dwie podgrupy - grupę badawczą pacjentów będących mieszkańcami Legnicko-Głogowskiego Okręgu Miedziowego oraz grupę kontrolną, będących mieszkańcami miasta Wrocławia. Materiał zębowy oceniano pod kątem zawartości Mn, Cr, Ni, Cu, Fe, Cd, Pb, Zn i Hg. Do analizy wielopierwiastkowej wykorzystano metodę atomowej spektrometrii absorpcyjnej.

3. Ocena wpływu metali ciężkich na proliferację DSCs:

Materiał zębowy pobrano od 28 pacjentów - 10 z obszarów przemysłowych L-G i 18 z obszarów nieprzemysłowych (Wrocław). Miazgę zębową ekstrahowano w sterylnych warunkach, a następnie wyizolowane DSCs, poddano hodowli i ocenie.

WYNIKI

Zidentyfikowano istotne czynniki ryzyka, które mogą przyczyniać się do akumulacji toksycznych metali w zębach. Zawartość Fe i Pb w trzecich zębach trzonowych była wyższa wśród mieszkańców Legnicko-Głogowskiego Okręgu Miedziowego. Wykazano istotną korelację między poziomem Cr, Cu i Zn a wiekiem badanych. Stężenie Hg wzrastało wraz z wiekiem i czasem zamieszkania na terenie okręgu miedziowego. Żywotność komórek DSCs pobrana od dawców z L-G była niższa, ze statystycznie istotną różnicą w średnim czasie podwojenia i uzyskanej liczbie komórek.

WNIOSKI

1. Akumulacja toksycznych metali w organizmie człowieka jest istotnym zagadnieniem, na ich zawartość w ludzkich zębach mają wpływ różne czynniki, w tym środowiskowe.

2. Na podstawie uzyskanych wyników można stwierdzić, że zawartość wszystkich badanych metali toksycznych wzrastała wraz z wiekiem pacjentów. Zawartość żelaza i ołowiu była istotnie statystycznie wyższa u pacjentów zamieszkujących L-G niż u mieszkańców Wrocławia.

3. Istnieje znaczący wpływ zanieczyszczenia środowiska na jakość i żywotność komórek macierzystych miazgi zębowej pobranych z zatrzymanych trzecich zębów trzonowych.

ABSTRACT

INTRODUCTION

The first three publications addressed the potential use of third molars as biomarkers of heavy metal exposure in a particular habitat. Biomonitoring is a method of environmental medicine that uses biological indicators to assess environmental conditions. Such biomonitors include teeth, saliva, hair, nails and urine. Teeth provide a permanent, objective record of an individual's lifestyle and the impact of pollution in their specific environment. During the process of tooth mineralisation, heavy metal cations are incorporated into the hydroxyapatites of the tooth mineralised tissues. The Legnica-Głogów Copper Area, due to industrial character, is an area with an elevated risk of exposure to heavy metals. The sources of metals can be classified as follows: soil, air, food and drinking water, all of which may be contaminated. Heavy metals have been linked to a range of health issues, including those affecting the stomatognathic system. The objective of the study is to assess the accumulation of metals. The study examined the accumulation of Pb, Cd, Cr, Ni, Fe, Mn, Cu, Zn and Hg in third molars of residents of the above region. Furthermore, an investigation was conducted into the correlation between metal content and various demographic factors, dietary habits, and the intake of vitamins and supplements. The fourth publication addresses the question of whether heavy metal content in third molars affects the isolation, culture and growth of dental stem cells (DSCs), which may represent a significant risk for their future use in regenerative medicine.

OBJECTIVE

1. Analyse current research and identify the main sources of exposure to heavy metals and the mechanisms by which these metals are deposited in dental tissues; secondly, to evaluate the current understanding of the impact of these metals on dental health.
2. The objective is to assess the accumulation of heavy metals. The objective is to analyse the levels of Pb, Cd, Cr, Ni, Fe, Mn, Cu, Zn and Hg in third molars of residents of the Legnica-Głogów Copper Area and to correlate these levels with demographic and clinical data, diet and vitamin supplementation.
3. To assess the detrimental impact of heavy metals on the proliferation of stem cells extracted from the dental pulp.

MATERIALS AND METHODS

The work was split into three parts:

1. An analysis of current research covering the dental aspect of toxic metals.

A comprehensive literature search was conducted using a selection of databases. The search was limited to literature published between 1978 and 2023, and 83 studies were included.

2. Evaluation of the accumulation of toxic metals in the dentition.

The study was conducted on a cohort of 72 patients who underwent the extraction of retained third molars. Blood samples were taken from patients and they answered an author-designed questionnaire. The subjects were divided into two distinct subgroups: the study group, comprising patients who resided in the Legnica-Głogów Copper Area, and a control group comprising patients who resided in the city of Wrocław. An assessment of the dental material was conducted for the following elements: manganese (Mn), chromium (Cr), nickel (Ni), copper (Cu), iron (Fe), cadmium (Cd), lead (Pb), zinc (Zn) and mercury (Hg). Heavy metal concentrations in dental tissues were measured using atomic absorption/emission spectrometry.

3. Evaluation of the impact of heavy metals on DSCs proliferation.

Dental specimens were obtained from 28 patients, comprising 10 individuals residing in industrial areas of L-G and 18 individuals residing in non-industrial areas (Wrocław). The dental pulp was extracted in a sterile environment and the isolated DSCs were then cultivated and evaluated.

RESULTS

Significant risk factors that may contribute to the accumulation of heavy metals in teeth were identified. Fe and Pb content in third molars was higher among residents of the Legnica-Głogów Copper Area. A significant correlation was found between Cr, Cu and Zn levels and the age of the subjects. Hg concentration increased with age and time of residence in the Copper Area. Exposure to industrial pollutants negatively affects the viability and proliferation of DSCs.

CONCLUSIONS

1. The accumulation of heavy metals in the human body is an important issue, and their content in human teeth is influenced by both environmental and non-environmental factors.
2. Based on the results obtained, it can be concluded that the content of all toxic metals studied increased with the age of the patients. The content of Fe and Pb in the third molars was higher among residents of the Legnica-Głogów Copper Area.
3 There is a significant effect of environmental pollution on the quality and viability of dental pulp stem cells taken from third molars.

1. WSTĘP

Pierwsze 3 publikacje dotyczyły możliwości wykorzystania zębów trzecich trzonowych jako biowskaźników narażenia na metale ciężkie na terenach zanieczyszczonych.
Biomonitoring, jako część stomatologii środowiskowej, jest definiowany jako zestaw działań mających na celu ocenę stanu środowiska przy użyciu biomonitorów. Badanie zawartości metali toksycznych stanowi podstawę do kształtowania polityki środowiskowej, która wpływa na zdrowie populacji ludzkiej zamieszkującej określony obszar. Biomonitorami narażenia na toksyczne metale mogą być: zęby, ślina, włosy, paznokcie czy mocz [1,2].
Ze względu na łatwość pozyskania materiału biologicznego w postaci zębów, dostępnych jest wiele badań oceniających zawartość metali toksycznych w ich strukturze [3-6]. Zęby są trwałym zapisem przebiegu życia danej osoby w określonym środowisku, odzwierciedlającym wpływ zanieczyszczeń środowiskowych [7,8]. W procesie mineralizacji zębów kationy metali ciężkich są wbudowywane w strukturę hydroksyapatytów. Dostają się one do tkanek zęba za pośrednictwem krwiobiegu.
Środowisko, w którym żyją i pracują ludzie, charakteryzuje się obecnością metali ciężkich. Kategoria ta obejmuje różne pierwiastki, takie jak cynk (Zn), miedź (Cu) i żelazo (Fe), które odgrywają kluczową rolę jako mikroelementy w organizmie człowieka. Obejmuje również metale toksyczne, takie jak kadm (Cd), rtęć (Hg) i ołów (Pb). W związku z tym istotne jest systemowe monitorowanie poziomów metali w środowisku, ponieważ mogą one mieć szkodliwy wpływ na zdrowie [8-10].
Ekstrakcja zębów do celów biomonitoringu jest etycznie niedopuszczalna. Jednak stosunkowo wysoka częstotliwość zabiegów ekstrakcji zębów z powodów ortodontycznych lub chirurgicznych stwarza możliwości ich wykorzystania do tego celu [11]. W związku z powyższym podjęto decyzje o realizacji badań w powyższy sposób. Badany Legnicko-Głogowski Okręg Miedziowy to obszar miejsko-przemysłowy i zagłębie rud miedzi w północnej części województwa dolnośląskiego (Polska), o powierzchni około 2200 km^2. Jest jednym z największych na świecie. Jego rozwój datuje się od 1960 roku, kiedy to po raz pierwszy odkryto tam głębokie złoża miedzi. Obecnie sektor wydobywczy rud miedzi tworzą trzy kopalnie: "Lubin", "Rudna" i "Polkowice-Sieroszowice", wspierane przez "Zakład Wzbogacania Rud" i "Zakład Hydrotechniczny". Z kolei sektor przetwórczy obejmuje trzy huty "Legnica", "Głogów" i "Cedynia". Odpady z rudy miedzi składowane są w ogromnym obiekcie inżynieryjnym "Żelazny Most" o powierzchni około 1400 ha [12].
Eksploatacja złóż wiąże się z emisją do środowiska związków siarki, azotu i węgla, a także pyłów i odpadów zawierających metale ciężkie [13]. Legnicko-Głogowski Okręg Miedziowy w Polsce jest obszarem, w którym ryzyko zanieczyszczenia metalami ciężkimi jest zwiększone ze względu na profil i strukturę przemysłową. Istnieje wiele źródeł toksycznych metali: zanieczyszczona gleba i atmosfera, skażona żywność lub woda pitna. Metale ciężkie mogą powodować różne choroby, w tym układu stomatognatycznego [14,15] oraz wpływać na procesy leczenia. Badanie to jest pierwszym, które ocenia akumulację ciężkich, w tym toksycznych metali: Pb, Cd, Cr, Ni, Fe, Mn, Cu, Zn i Hg w zatrzymanych trzecich zębach trzonowych mieszkańców Legnicko-Głogowskiego Okręgu Miedziowego. W pracy zbadano związek pomiędzy metalami ciężkimi a danymi demograficznymi, klinicznymi, dietą oraz suplementacją witaminową.

W publikacji czwartej postawiono pytanie, czy zawartość metali ciężkich w zębach może mieć wpływ na izolację, hodowlę i proliferację komórek macierzystych pozyskiwanych z miazgi zębowej (DSCs). Potencjał powyższych komórek w regeneracji tkanek stanowi jedną z dróg nowoczesnej medycyny regeneracyjnej. Badanie czynników ograniczających ich proliferację może być kluczowe dla szukania rozwiązań stanowiących alternatywę lub wsparcie współczesnej transplantacji tkanek [16-18]. Obecnie jednym z powszechnych podejść jest przeszczep kości z wykorzystaniem materiału pochodzącego od pacjenta (autogennego), innego dawcy (allogennego) lub regeneracja kości przy użyciu produktu syntetycznego (ksenogennego). Podczas gdy autologiczny przeszczep kości pozostaje "złotym standardem", pobieranie z miejsc takich jak biodro lub żebro jest inwazyjne, bolesne i obarczone powikłaniami. Współczesna medycyna szuka nowych rozwiązań, kierując się w stronę metod allogenicznych lub ksenogenicznych [16,17].
Materiały allogeniczne przed zabiegiem są oczyszczane przy użyciu różnych protokołów, takich jak obróbka cieplna lub promieniowanie gamma, usuwając ponad 95% leukocytów i składników osocza. Chociaż tradycyjnie uważa się, że ryzyko alloimmunizacji jest niskie, przeszczepy kości zawierające resztki organiczne mogą wywoływać reakcje immunologiczne [19-22].
Przeszczepy kości są niezbędne we współczesnej chirurgii stomatologicznej. Celem stosowania komórek macierzystych jest poprawa regeneracji kości. Przywrócenie jej kształtu, funkcji i ukrwienia. Jest to doskonała opcja w przypadku zaopatrywania ubytku kości spowodowanego urazem lub chorobą [23-15]. Jako pierwsze wykorzystane w medycynie zostały Komórki macierzyste ze szpiku kostnego (BMSC), ale ich pozyskiwanie jest inwazyjne i obarczone ryzykiem [26-28]. W przeciwieństwie do tego, komórki z miazgi zębowej (DSCs) oferują bezpieczniejszą i bardziej wydajną opcję pozyskiwania komórek macierzystych [29]. Komórki te wykazują właściwości multipotencjalne, co czyni je cennymi w leczeniu regeneracyjnym. Ekstrahowane zęby mogą być bogatym źródłem DSCs [30]. Można je wykorzystać do regeneracji ubytków kostnych wyrostka zębodołowego po przeprowadzeniu ich procesowania. Nie bez znaczenia pozostaje fakt, że BMP (Bone Morphogenetic Protein) zidentyfikowano również w macierzy zębiny ludzkiej po demineralizacji [31]. BMP mają zdolność osteoindukcyjną, promując różnicowanie komórek macierzystych mezenchymalnych w osteoblasty [32]. Chociaż BMP pochodzące z kości i BMP pochodzące z zębiny mają różne struktury biochemiczne, ich podobne funkcje w regulacji rozwoju i naprawy tkanki kostnej umożliwiają użycie w zabiegach regeneracyjnych. Wykorzystanie zębów procesowanych, ze względu na swoją biologię, niesie ze sobą wysokie zdolności regeneracyjne i mogłoby być szeroko wykorzystywane w codziennej praktyce stomatologicznej. Jednak zawartość metali ciężkich, może znacząco wpływać na jakość DSCs. Wiedza o potencjalnym narażeniu środowiskowym i jego wpływie na skład struktury zębów może mieć duże znaczenie kliniczne. Badania pokazują, że metale takie jak kadm, ołów i rtęć gromadzą się w tkankach zęba, stanowiąc zagrożenie dla zdrowia. Zęby mogą służyć jako biomarkery narażenia środowiskowego i zawodowego na te metale [33]. Badanie miało na celu przeprowadzenie szczegółowej oceny jakości i ilości komórek uzyskanych od pacjentów, którzy wymagali ekstrakcji trzecich zębów trzonowych.

2. CEL PRACY

1. Analiza aktualnych badań i identyfikacja głównych źródeł narażenia na metale ciężkie oraz mechanizmów, za pomocą których metale te odkładają się w tkankach zęba.
2. Ocena akumulacji metali ciężkich: Pb, Cd, Cr, Ni, Fe, Mn, Cu, Zn i Hg w trzecich zębach trzonowych mieszkańców Legnicko-Głogowskiego Okręgu Miedziowego oraz ich korelacja z danymi demograficznymi, klinicznymi, dietą oraz suplementacją witaminową.
3. Ocena wpływu metali ciężkich na proliferację komórek macierzystych pozyskiwanych z miazgi zębowej.

3. MATERIAŁY I METODY

Ze względu na charakter publikacji materiały i metody podzielono na trzy części.

3.1 Analiza aktualnych badań obejmujący stomatologiczny aspekt metali toksycznych

Kompleksowe wyszukiwanie literatury przeprowadzono z wykorzystaniem następujących baz danych: PubMed; Google Scholar; Polska Bibliografia Lekarska oraz Web of Science. Wyszukiwanie ograniczono do artykułów opublikowanych w latach 1978-2023. Słowa kluczowe i frazy użyte w wyszukiwaniu obejmowały "metale ciężkie w zębach", "metale toksyczne i zdrowie zębów". Do zawężenia wyników wyszukiwania zastosowano operatory logiczne AND i OR. Wyszukiwanie ograniczono do artykułów opublikowanych w języku angielskim i polskim. Artykuły nierecenzowane, artykuły niedostępne w pełnym tekście oraz badania niezwiązane z tematem zostały wykluczone z analizy. Artykuły pełnotekstowe zostały ponownie przejrzane pod kątem trafności, w wyniku czego włączono 83 publikacje. Ekstrakcja danych obejmowała przegląd abstraktów i pełnotekstowych artykułów w celu zidentyfikowania odpowiednich badań. Wyodrębnione informacje obejmowały projekt badania, wielkość próby, badane rodzaje metali i kluczowe ustalenia związane z wpływem metali ciężkich na organizm. Zastosowano podejście syntezy narracyjnej, aby zintegrować i podsumować wyniki badań. Jakość włączonych badań została oceniona na podstawie projektu badania, wielkości próby i rygoru metodologicznego.

3.2 Ocena akumulacji toksycznych metali w zębach

Badanie przeprowadzono w pełnej zgodności z Deklaracją Helsińską, w grupie 72 pacjentów, u których ekstrakcji poddano trzecie zęby trzonowe jako główny materiał badawczy. Oprócz usuniętych zębów, od pacjentów pobrano krew i uzyskano odpowiedzi w autorskim kwestionariuszu. Przed przystąpieniem do badania uzyskano zgodę Komisji Bioetycznej Uniwersytetu Medycznego we Wrocławiu (numer zgody: KB-246/2019). Pacjenci zostali podzieleni na dwie podgrupy - grupę badawczą pacjentów będących mieszkańcami Legnicko-Głogowskiego Okręgu Miedziowego oraz grupę kontrolną będących mieszkańcami miasta Wrocławia. Zebrano trzecie zęby trzonowe od pacjentów z obu grup. W ramach badania pozyskano również dane dotyczące płci, wieku, stałego miejsca zamieszkania oraz stosowania suplementów diety. Najważniejszym kryterium włączenia i wykluczenia było to, że badani pacjenci mieszkali od urodzenia na terenie Legnicko-Głogowskiego Okręgu Miedziowego lub we Wrocławiu. Próbka składała się z całego usuniętego zęba. Aby uniknąć obróbki chemicznej materiału przed testami analitycznymi, materiał był przechowywany w temperaturze -20^{o}C w warunkach sterylnych, a następnie oceniany pod kątem zawartości Mn, Cr, Ni, Cu, Fe, Cd, Pb, Zn i Hg.

Do analizy wielopierwiastkowej materiału zębowego wykorzystano metodę atomowej spektrometrii absorpcyjnej (AAS). Informacje zebrane za pomocą autorskiego kwestionariusza obejmowały: pochodzenie, wiek, miejsce zamieszkania, narażenie zawodowe, nałóg palenia tytoniu, występowanie alergii, chorób ogólnych oraz stosowaną suplementację diety. Zawartość wybranych składników mineralnych i toksycznych pierwiastków śladowych w tkance kostnej oznaczono w certyfikowanym laboratorium AAS Katedry Żywienia Człowieka Uniwersytetu Przyrodniczego we Wrocławiu. Szczegółowy opis procesu mineralizacji i badań na zawartość poszczególnych metali ciężkich oraz metodologii badań statystycznych zawarto w załączonych publikacjach.

3.3 Ocena wpływu metali ciężkich na proliferację DSCs

Trzecie zęby trzonowe pobrano od 28 pacjentów - 10 z obszarów przemysłowych L-G i 18 z obszarów nieprzemysłowych (Wrocław). Pacjentów podzielono na dwie grupy wiekowe: 18-27 lat i 28-38 lat. Miazgę zębową ekstrahowano w sterylnych warunkach, a DSCs izolowano i hodowano. Szczegółowy opis procesu ekstrakcji miazgi, izolacji komórek macierzystych, oceny ich żywotności i indukcji ich różnicowania oraz metodologii badań statystycznych został opisany w załączonej pracy.

4. WYNIKI

4.1 Analiza aktualnych badań

4.1.1 Wiek i płeć mogą mieć wpływ na zawartość metali toksycznych w ludzkich zębach.
4.1.2 Choroby ogólnoustrojowe, zaburzenia neurorozwojowe oraz alergie pokarmowe mogą mieć wpływ na stężenie metali ciężkich w organizmie m.in. ze względu na stosowanie restrykcyjnych diet lub być spowodowane zaburzeniami metabolicznymi.
4.1.3 Zanieczyszczenie środowiska ma kluczowe znaczenie dla procesu akumulacji metali toksycznych w organizmie człowieka.

4.2 Ocena akumulacji toksycznych metali

4.2.1 Największy odsetek grupy badanej stanowiły młode osoby niepalące w przedziale wiekowym 16-26 lat, ogólnie zdrowe, z przewagą kobiet. Nie stwierdzono istotnych różnic w rozkładzie płci, wieku, palenia tytoniu, zawodu, występowania chorób, suplementacji diety pomiędzy grupą badaną i kontrolną ($p > 0{,}05$ dla wszystkich parametrów).
4.2.2 Zawartość żelaza i ołowiu była istotnie wyższa w zębach usuniętych u pacjentów z L-G niż z grupy kontrolnej (odpowiednio $p = 0{,}016$ i $p = 0{,}002$)
4.2.3 Zawartość manganu istotnie zależała od wykonywanego przez pacjentów zawodu ($p = 0{,}043$). W grupie studentów mediana wyniosła 0,14 µg/g, natomiast wśród pracowników umysłowych 0,25 µg/g - oba wyniki były istotne statystycznie w parze podgrup.
4.2.4 W przypadku zawartości chromu i niklu w usuniętych trzecich zębach trzonowych wykonywany przez pacjenta zawód wykazywał tendencję do istotności statystycznej.
4.2.5 Zawartość miedzi w badanych próbkach wzrastała wraz z wiekiem pacjentów – jej wartości wykazywały tendencję do istotności statystycznej.
4.2.6 W grupie pacjentów, którzy mieszkali przez 21-30 lat w L-G, mediana zawartości manganu była najwyższa (0,26 (µg/g)), jednak nie została określona jako istotna statystycznie.

4.2.7 W przypadku zawartości chromu i niklu w usuniętych trzecich zębach trzonowych wykonywany przez badanych zawód wykazywał tendencję do istotności statystycznej.
4.2.8 Zawartość miedzi w badanych próbkach wzrastała wraz z wiekiem pacjentów natomiast najwyższe wartości dla tego pierwiastka odnotowano u pacjentów mieszkających w L-G od ponad 31 lat (0,22µg/g).
4.2.9 Nie zaobserwowano statystycznie istotnego wpływu wszystkich badanych parametrów na zawartość żelaza w usuniętych zębach.
4.2.10 Stwierdzono statystycznie istotny wpływ płci pacjenta na zawartość kadmu ($p = 0,037$).
4.2.11 Zawartość ołowiu w usuniętych zębach istotnie wzrastała wraz z wiekiem pacjentów ($p = 0,032$); w grupie wiekowej 38-45 lat zawartość tego metalu była istotnie wyższa niż w grupie wiekowej 16-26 lat.
4.2.12 Zawartość cynku w badanych zębach wzrastała wraz z okresem zamieszkiwania w L-G.
4.2.13 W grupie wiekowej 38-45 lat zawartość ołowiu w usuniętych zębach była istotnie wyższa niż w pozostałych grupach wiekowych.
4.2.14 Zawartość cynku w badanych próbkach wzrastała wraz z wiekiem pacjentów i okresem zamieszkania w L-G.
4.2.15 Zaobserwowano statystycznie istotną dodatnią korelację między zawartością chromu, miedzi i cynku a wiekiem pacjentów zamieszkujących w L-G ($p < 0,05$).
4.2.19 Pacjenci z L-G mieli nieco wyższą zawartość rtęci w usuniętych zębach w porównaniu z grupą kontrolną (0,389 i 0,341µg/g); nie było to jednak istotne statystycznie.

4.3 Ocena wpływu metali ciężkich na proliferację DSCs

4.3.1 Żywotność komórek była statystycznie istotnie niższa w próbkach miazgi zębowej z obszarów L-G.
4.3.2 Średni czas podwojenia hodowli był statystycznie istotnie krótszy w hodowlach DSCs izolowanych z miazgi z obszarów nieprzemysłowych: 41,2 ± 22,0 h na obszarach przemysłowych L-G w porównaniu do 17,1 ± 4,8 h na obszarach nieprzemysłowych.
4.3.3 Liczba komórek po pierwszym pasażu była statystycznie istotnie wyższa w grupie z obszarów nieprzemysłowych. Średnia liczba komórek po pierwszym pasażu wynosiła 1,479 ± 0,250 mln dla miazgi zębów z obszarów przemysłowych L-G i 1,746 ± 0,150 mln dla materiału z obszarów nieprzemysłowych.

Wszystkie szczegółowe wyniki dla poszczególnych badań zostały zamieszczone w załączonych publikacjach.

5. PODSUMOWANIE I WNIOSKI

1. Akumulacja toksycznych metali w organizmie człowieka jest istotnym zagadnieniem ze względu na możliwość wywoływania różnych niekorzystnych skutków zdrowotnych. Na zawartość metali ciężkich w ludzkich zębach mają wpływ różne czynniki, w tym środowiskowe. Wyniki przeglądu piśmiennictwa jak i badań własnych sugerują, że zęby mogą być cennym narzędziem do monitorowania obecności zanieczyszczeń w środowisku.
2. Interdyscyplinarna współpraca między naukowcami, pracownikami służby zdrowia i ekspertami zdrowia publicznego ma kluczowe znaczenie dla opracowania skutecznych strategii łagodzenia wpływu toksycznych metali na zdrowie ludzi. Integrując wyniki badań można uzyskać bardziej kompleksowe zrozumienie wpływu metali ciężkich na różne aspekty zdrowia.
3. Na podstawie uzyskanych wyników można stwierdzić, że zawartość badanych metali ciężkich wzrastała wraz z wiekiem pacjentów. Zawartość żelaza i ołowiu była istotnie statystycznie wyższa u pacjentów zamieszkujących Legnicko - Głogowskie Zagłębie Miedziowe niż u mieszkańców Wrocławia, co może wskazywać na większe zanieczyszczenie środowiska w L-G. Należy prowadzić dalsze badania dotyczące wpływu środowiska na zawartość metali ciężkich w organizmie człowieka, szczególnie w zakresie tkanek zmineralizowanych.
4. Istnieje znaczący wpływ zanieczyszczenia środowiska na jakość i żywotność komórek macierzystych miazgi zębowej pobranych z trzecich zębów trzonowych. Porównując DSCs od pacjentów z obszarów przemysłowych i nieprzemysłowych, badanie ujawniło niekorzystny wpływ metali ciężkich na proliferację komórek macierzystych.

6. OGRANICZENIE BADANIA

Materiał został zebrany w okresie od czerwca 2020 roku do czerwca 2021 roku (podczas pandemii COVID-19), dlatego liczba pacjentów w grupie kontrolnej, która była dopasowana pod względem wieku i płci do grupy badanej, była niewielka. Przeprowadzono analizy mocy testu dla obu grup i zastosowano testy statystyczne. Na podstawie obliczeń stwierdzono, że liczba osób w grupie badanej była prawidłowa, natomiast liczebność grupy kontrolnej była nieco niższa niż wymagana.

PIŚMIENNICTWO

1. Tsz-Shan Lum, J.; Chan, Y.-N.; Sze-Yin Leung, K. Current applications and future perspectives on elemental analysis of noninvasive samples for human biomonitoring. Talanta 2021, 234, 122683.
2. Esteban, M.; Castaño, A. Non-invasive matrices in human biomonitoring: A review. Environ. Int. 2009, 35, 438–449.
3. Barton, H.J. Advantages of the use of deciduous teeth, hair, and blood analysis for lead and cadmium bio-monitoring in children. A study of 6-year-old children from Krakow (Poland). Biol. Trace Elem. Res. 2011, 143, 637–658.
4. Alomary, A.; Al-Momani, I.F.; Obeidat, S.M.; Massadeh, A.M. Levels of lead, cadmium, copper, iron, and zinc in deciduous teeth of children living in Irbid, Jordan by ICP-OES: Some factors affecting their concentrations. Environ. Monit. Assess. 2013, 185, 3283–3295.
5. Fischer, A.;Wiechuła, D.; Przybyła-Misztela, C. Changes of concentrations of elements in deciduous teeth with age. Biol. Trace Elem. Res. 2013, 154, 427–432.
6. Tvinnereim, H.M.; Eide, R.; Riise, T. Heavy metals in human primary teeth: Some factors influencing the metal concentration. Sci.Total Environ. 2000, 255, 21–27.
7. Leventouri, T.; Antonakos, A.; Kyriacou, A.; Venturelli, R.; Liarokapis, E.; Perdikatsis, V. Crystal structure studies of human dental apatite as a function of age. Int. J. Biol. Mater. 2009, 2009, 1–17.
8. Bryła, E.; Dobrzyński, M.; Konkol, D.; Kuropka, P.; Styczyńska, M.; Korczyński, M. Toxic Metals Content in Impacted Third Molars and Adjacent Bone Tissue in Different Groups of Patients. Materials 2021, 14, 793.
9. Rayad S, Dobrzyński M, Kuźniarski A, et al. An in-vitro evaluation of toxic metals concentration in the third molars from residents of the Legnica–Głogów Copper Area and risk factors determining the accumulation of those metals: A pilot study. Appl Sci. 2023;13(5):2904.
10. Rayad S, Dobrzyński M, Kuźniarski A, et al. Mercury content in impacted wisdom teeth from patients of the Legnica–Głogów Copper Area – an in vitro pilot study. J Xenobiot. 2023;13(3):463–478
11. Malara, P.; Fischer, A.; Malara, B. Selected toxic and essential heavy metals in impacted teeth and the surrounding mandibular bones of people exposed to heavy metals in the environment. J. Occup. Med. Toxicol. 2016, 11, 56.
12. Kołacz, R.; Dobrzański, Z.; Kupczyński, R.; Cwynar, P.; Opaliński, S.; Pogoda-Sewerniak, K. Impact of the copper industry on the content of selected heavy metals and biochemical indicators in the blood of dairy cows. Med. Weter 2017, 73, 171–175.
13. Strzelec, Ł.; Niedźwiecka, W. Natural environment in the area of copper smelter plants. Trend Chang. Med. Sr. Environ. Med. 2012, 15, 21–31.
14. Tsushima, F.; Sakurai, J.; Shimizu, R.; Kayamori, K.; Harada, H. Oral lichenoid contact lesions related to dental metal allergy may resolve after allergen removal. J. Dent. Sci. 2022, 17, 1300–1306.
15. Lucchese, A.; Dolci, A.; Minervini, G.; Salerno, C.; DIStasio, D.; Minervini, G.; Laino, L.; Silvestre, F.; Serpico, R. Vulvovaginal gingival lichen planus: Report of two cases and review of literature. Oral. Implantol. 2016, 9, 54–60.
16. Ciszyński, M.; Dominiak, S.; Dominiak, M.; Gedrange, T.; Hadzik, J. Allogenic Bone Graft in Dentistry: A Review of Current Trends and Developments. Int. J. Mol. Sci. 2023, 24, 16598.
17. Hadzik, J.; Błaszczyszyn, A.; Gedrange, T.; Dominiak, M. Soft-Tissue Augmentation around Dental Implants with a Connective Tissue Graft (CTG) and Xenogeneic Collagen Matrix (CMX)—5-Year Follow-Up. J. Clin. Med. 2023, 12, 924.

18. Glowacka-Sobotta, A.; Ziental, D.; Czarczynska-Goslinska, B.; Michalak, M.; Wysocki, M.; Güzel, E.; Sobotta, L. Nanotechnology for Dentistry: Prospects and Applications. Nanomaterials 2023, 13, 2130.
19. Ward,W.G.; Gautreaux, M.D.; Lippert, D.C.; Boles, C. HLA Sensitization and Allograft Bone Graft Incorporation. Clin. Orthop. Relat. Res. 2008, 466, 1837–1848.
20. O'Sullivan, E.D.; Battle, R.K.; Zahra, S.; Keating, J.F.; Marson, L.P.; Turner, D.M. Allosensitization Following Bone Graft. Am. J. Transplant. 2017, 17, 2207–2211.
21. Lorenz, J.; Schlee, M.; Al-Maawi, S.; Chia, P.; Sader, R.; Ghanaati, S. Variant Purification of an Allogeneic Bone Block. Acta Stomatol. Croat. 2017, 51, 141–147.
22. Ghanaati, S.; Barbeck, M.; Booms, P.; Lorenz, J.; Kirkpatrick, C.J.; Sader, R.A. Potential Lack of "Standardized" Processing Techniques for Production of Allogeneic and Xenogeneic Bone Blocks for Application in Humans. Acta Biomater. 2014, 10, 3557–3562.
23. Charavet, C.; Lecloux, G.; Vandenberghe, B.; Lambert, F. Buccal Bone Regeneration Combined with Piezocision in Adult Orthodontic Patients: Clinical, 3D Radiographic, and Patient-Reported Outcomes. J. Stomatol. Oral Maxillofac. Surg. 2021, 122, 549–556.
24. Dominiak, M.; Dominiak, S.; Targonska, S.; Gedrange, T. Three-Dimensional Bone Block Planning for Mandibular Sagittal Bone Defect Reconstruction. J. Healthc. Eng. 2020, 2020, 8829288.
25. Dominiak, M.; Hnitecka, S.; Olchowy, C.; Dominiak, S.; Gedrange, T. Possible Treatment of Severe Bone Dehiscences Based on 3D Bone Reconstruction—A Description of Treatment Methodology. Appl. Sci. 2021, 11, 10299.
26. Fraser, J.K.; Schreiber, R.; Strem, B.; Zhu, M.; Alfonso, Z.;Wulur, I.; Hedrick, M.H. Plasticity of Human Adipose Stem Cells toward Endothelial Cells and Cardiomyocytes. Nat. Clin. Pract. Cardiovasc. Med. 2006, 3, S33–S37.
27. Wang, S.; Qu, X.; Zhao, R.C. Clinical Applications of Mesenchymal Stem Cells. J. Hematol. Oncol. 2012, 5, 19.
28. Mizuno, H. Adipose-Derived Stem Cells for Tissue Repair and Regeneration: Ten Years of Research and a Literature Review. J. Nippon. Med. Sch. 2009, 76, 56–66.
29. Shi, S.; Robey, P.G.; Gronthos, S. Comparison of Human Dental Pulp and Bone Marrow Stromal Stem Cells by CDNA Microarray Analysis. Bone 2001, 29, 532–539.
30.Baranova, J.; Büchner, D.; G.tz, W.; Schulze, M.; Tobiasch, E. Tooth Formation: Are the Hardest Tissues of Human Body Hard to Regenerate? Int. J. Mol. Sci. 2020, 21, 4031.
31. Bessho, K.; Tanaka, N.; Matsumoto, J.; Tagawa, T.; Murata, M. Human dentin-matrix-derived bone morphogenetic protein. J. Dent. Res. 1991, 70, 171–175.
32.Rijal, G.; Shin, H.I. Human tooth-derived biomaterial as a graft substitute for hard tissue regeneration. Regen. Med. 2017, 12, 263–273.
33. Malara, P.; Fischer, A.; Malara, B. Selected Toxic and Essential Heavy Metals in Impacted Teeth and the Surrounding Mandibular

II ZAŁĄCZNIKI

5. Prace stanowiące podstawę rozprawy doktorskiej
6. Informacja o indywidualnym wkładzie współautorów
7. Opinia komisji bioetycznej
8. Wykaz publikacji autora

Review

AHEAD OF PRINT

Studies on the content of toxic metals in teeth: A narrative review of literature

Sadri Rayad[1,A–E], Sylwia Klimas[2,B–D], Maciej Janeczek[3,B–D], Agata Małyszek[3,B–D], Marta Bort[4,D,E], Andrzej Małysa[4,D,E], Marzena Dominiak[5,E,F], Maciej Dobrzyński[2,A,E,F]

[1] University Dental Center of Wroclaw, Medical Innovation Centre Ltd., Wroclaw, Poland
[2] Department of Pediatric Dentistry and Preclinical Dentistry, Wroclaw Medical University, Poland
[3] Department of Biostructure and Animal Physiology, Wrocław University of Environmental and Life Sciences, Poland
[4] Department of Experimental Dentistry, Wroclaw Medical University, Poland
[5] Department of Dental Surgery, Wroclaw Medical University, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.*

Address for correspondence
Marta Bort
E-mail: bort.marta@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on August 20, 2024
Reviewed on September 1, 2024
Accepted on September 14, 2024

Published online on September 26, 2024

Abstract

The presence of toxic metals in the human environment can have detrimental effects on people's well-being. This literature review examines the ways in which various environmental and non-environmental factors can contribute to the accumulation of heavy metals in hard dental tissues. It is of the utmost importance to ensure the safety of the environment by restricting the presence of toxic metals originating from both industrial and non-industrial sources. The aim of this study is to analyze current research and identify the primary sources of heavy metal exposure and the mechanisms by which these metals are deposited in dental tissues. Moreover, the objective of this review is to synthesize data from various studies to determine the main environmental and non-environmental sources of toxic metal exposure that contribute to their presence in dental tissues, as well as the biological and chemical processes that are responsible for the deposition of heavy metals in hard dental tissues. Additionally, the review aims to assess the impact of heavy metal accumulation on dental health and its potential systemic effects on overall well-being. The accumulation of heavy metals in the teeth is influenced by a number of factors, such as age, systemic conditions, the nutritional status, and dental caries. The presence of supernumerary teeth results in altered levels of microelements, including an increase in cadmium (Cd) and copper (Cu). Additionally, smoking exacerbates toxic metal accumulation, especially Cd and lead (Pb), and disrupts the balance of essential minerals within the teeth. These findings underscore the impact of environmental pollution on dental health and highlight the potential of teeth as biomarkers of environmental exposure, emphasizing the need for continued research to address the health risks associated with environmental toxins.

Keywords: teeth, biomonitoring, toxic metals, bioelements

Cite as
Rayad S, Klimas S, Janeczek M, et al. Studies on the content of toxic metals in teeth: A narrative review of literature [published online as ahead of print on September 26, 2024]. *Dent Med Probl.* doi:10.17219/dmp/193406

DOI
10.17219/dmp/193406

Introduction

The environment in which humans reside and work is characterized by a notable and pervasive presence of heavy metals. This category encompasses a variety of elements, such as zinc (Zn), copper (Cu) and iron (Fe), that play crucial roles as micronutrients in the human body. It also includes metals that are not essential for life processes, such as cadmium (Cd), mercury (Hg) and lead (Pb). It is vital to carefully manage the levels of these metals as trace elements, since both deficiencies and excesses can have harmful effects on human health.[1–3]

The role of Zn in the body is crucial for the production of insulin, as well as the synthesis of proteins and nucleic acids. Insufficient levels of Zn within the body may contribute to the development of obesity and diabetes. The uneven distribution of Zn may impede the onset of metabolic disorders and diabetes by regulating several biological processes. The influence of Zn on a range of hormones, including testosterone, growth hormone and gonadotropins, is widely recognized. Zinc also takes part in the synthesis, storage and secretion of insulin. Additionally, it is a crucial component of thymulin, which is responsible for T cell maturation and differentiation. Recent studies have indicated that Zn is crucial in the development and maintenance of bone tissue homeostasis. The element plays an essential role in bone tissue, not only serving as a constituent but also facilitating the formation of the collagen matrix, the process of mineralization, and the turnover of bone. Zinc deficiency can lead to a gradual reduction in bone density. Bones that lack or have a severe deficiency of Zn are known to be thin, fragile and exhibit increased resorption.[4,5]

Copper is an essential element in numerous physiological processes. It plays a key role in the synthesis of hemoglobin, the formation of bones, the deposition of calcium (Ca) and phosphorus (P) in bones, the absorption of Fe, and the hardening of collagen. Additionally, Cu exhibits antiviral properties and aids in detoxification processes.[6,7] It functions as a coenzyme in a number of enzymes, such as cytochrome c oxidase, representing a crucial component in the process of cellular respiration. Furthermore, Cu participates in the process of keratinization of hair and coat. A deficiency in Cu results in the inhibition of melanin synthesis, a reduction in the immune response, an increased likelihood of infections, an elevated risk of cardiovascular disorders, and impaired cholesterol metabolism.[6,8]

Lipid metabolism is a complex process that involves various factors, one of which is Fe. Iron plays a crucial role in oxidation processes and serves as a constituent of important molecules such as cytochromes, hemoglobin and myoglobin.[9,10] One of its primary functions is to facilitate the transportation of oxygen, storage of transitional tissues and cellular utilization of oxygen. Additionally, it is equally significant in the cytochromes that are housed within the mitochondria. Iron is involved in the transfer of electrons in the electron transport chain.[6] It is stored in the liver, spleen and bone marrow through proteins called ferritin and hemosiderin, both of which have a strong affinity for the element. Additionally, Fe plays a role in erythropoiesis, the formation of leukocytes and immune reactions, impacting both cellular and humoral immunity. Furthermore, the presence of Fe is of the utmost importance for the optimal performance of osteoblasts, which are the cells responsible for bone development.[11,12]

Nevertheless, it is important to acknowledge the presence of heavy metals under specific circumstances. The elements that occur naturally within the Earth's crust play a role in various natural phenomena, including the erosion of rocks, oceanic evaporation, the formation of soil, and volcanic eruptions.[13] The human body can be exposed to heavy metals through various means, including air, water and soil. Soil contamination can occur due to the presence of fertilizers, plant protection products, industrial dust, and sewage. The absorption of water by plants from contaminated soil results in the uptake of toxic metals, which subsequently accumulate in their tissues. However, it is worth noting that the inhalation route provides the most accessible means of absorption. A number of industrial sectors serve as the primary sources of environmental contamination. These include metallurgy, electrotechnology and the chemical industry, as well as the production of fertilizers, paints and solvents. Metals are also heavily present in car exhaust emissions. Additionally, cigarette smoking is a significant non-industrial factor that exposes individuals to high levels of heavy metals. It is crucial to take into account the presence of toxic metals in amalgam fillings and the alloys utilized in prosthetics and orthodontics. Furthermore, the presence of heavy metals has been identified in a range of cosmetics and dietary supplements.[1–3]

Metal toxicity operates through various mechanisms, which can be classified into 3 primary categories. These classifications encompass the obstruction of vital functional groups within proteins, the displacement of metal ions that act as cofactors for enzymes and other functional proteins, and the modification of the spatial arrangement of proteins.[14] A wide array of illnesses can be attributed to the presence of heavy metals. The manifestation of the harmful impact of toxic metals on the human body is contingent upon a number of factors, including the specific type of metal, the dosage, the method of exposure, the duration of exposure, and the individual's personal susceptibility. Heavy metals have an impact on various cellular organelles and components within biological systems. These include the cell membrane, mitochondria, lysosomes, endoplasmic reticulum, nuclei, and certain enzymes that are critical for metabolic processes, detoxification and the repair of damage.[15] Toxic metals, in addition to their toxic properties, possess the capability to accumulate within human parenchymal tissues. Once they enter the body, these metals tend to accumulate in the kidneys, liver and pancreas.[16] Additionally, heavy metals accumulate in the hard tissues of teeth.[1–3] The gradual deterioration of physical, muscular and neurological capabilities, which resembles the symptoms of diseases such as multiple sclerosis, Parkinson's disease, Alzheimer's disease, and muscular dystrophy, can be attributed to extended contact with specific metals and their compounds. Some metals possess properties that have been linked to the development of birth defects and cancer. The progression of cancers can be influenced by certain heavy metals, which stimulate cancer cells through various pathogenic connections and may also decrease their receptiveness to treatment.[1,2,17]

The significance of biomonitoring in the context of health and the environment is gradually increasing. As a component of environmental dentistry, biomonitoring encompasses a range of actions aimed at evaluating the condition of the environment through the use of biomonitors. These biomonitors, which are utilized to assess the exposure to toxic metals in humans, are known as non-invasive matrices. Examples of such matrices include hair, nails, urine, saliva, and teeth. Among these, deciduous teeth are particularly convenient for obtaining biological material, resulting in a wealth of studies that analyze the levels of toxic metals present in their structure.[1,3] The teeth of an individual are an enduring testimony to their lifestyle within a specific environment and an indicator of the influence of environmental pollutants. During the process of mineralization, noxious metal cations become incorporated into the crystalline framework of hydroxyapatites. These detrimental substances gain access to the tooth tissues via the bloodstream.[18,19]

It is important to acknowledge that chelation therapy has been the main approach to the management of cases of heavy metal poisoning. This method employs the use of chelating agents to form chelates, which are complex ring-like structures that trap metal ions and facilitate their removal from the body. However, the use of metal chelators has its limitations. Metal chelators have the potential to facilitate the migration of heavy metals from other regions of the body to the brain, which can escalate the neurotoxicity of the individual in question. Moreover, the use of these chelators may lead to the depletion of important metals, including Zn and Cu, resulting in serious side effects, such as liver damage.[20,21]

The content of toxic metals in dental tissues is influenced by both industrial and non-industrial factors. The following paragraphs will present a comprehensive analysis of this dichotomy, offering a more exhaustive investigation and inquiry. Within the discourse, certain publications that explore the composition of heavy metals within teeth and the various factors that contribute to their presence will be discussed.

This review offers a novel perspective by examining the interplay between environmental and non-environmental factors in the accumulation of heavy metals in dental tissues. It integrates recent research to identify key sources of toxic metal exposure, elucidate the biological mechanisms of metal deposition in teeth, and assess the broader health implications of this accumulation. The aim of this review is to enhance understanding of the potential of teeth as biomarkers for environmental exposure, to assess the impact of environmental factors on both dental and systemic health, and to underscore the importance of developing diagnostic and preventive strategies. Furthermore, the study emphasizes the need for improved environmental safety measures.

Material and methods

Literature search strategy

A comprehensive literature search was conducted using the following databases: PubMed; Google Scholar; Polska Bibliografia Lekarska; and Web of Science. The search was limited to articles published between 1978 and 2023. The keywords and phrases used in the search included "heavy metals in teeth," "toxic metals and dental health" and "smoking impact on metal accumulation." Boolean operators AND and OR were employed to refine the search results. The search was limited to articles published in English and Polish.

Inclusion and exclusion criteria

The articles included in the review examined the impact of heavy metals on dental health or associated environmental factors, were peer-reviewed and focused on human subjects. Non-peer-reviewed articles, articles not available in full text, and studies with an unrelated focus were excluded from consideration.

Selection process

The articles were subjected to a screening process based on their titles and abstracts. Full-text articles were reviewed for relevance, resulting in the inclusion of 83 studies. Duplicate articles were excluded during the screening process. The methodology is presented in Fig. 1.

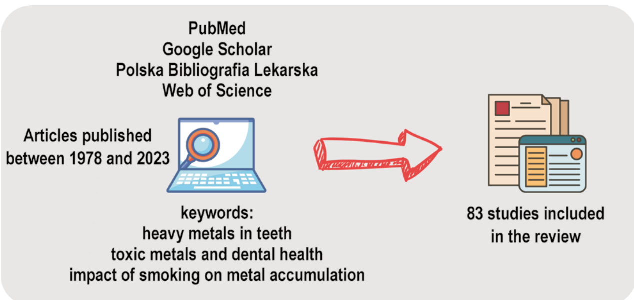

Fig. 1. Methodology of the selection process

Data extraction and analysis

Data extraction involved reviewing abstracts and full-text articles to identify relevant studies. The extracted information included study design, sample size, metal types examined, and key findings related to heavy metal impact on dental health. We employed a narrative synthesis approach to integrate and summarize findings across studies. This involved categorizing results according to themes such as types of metals, mechanisms of impact, and health outcomes.

Quality assessment

The quality of the included studies was evaluated based on the study design, the sample size and the methodological rigor. No specific assessment tools were employed; rather, a critical appraisal was conducted to evaluate the reliability and relevance of each study.

Results

Age and sex

The levels of toxic metals found within human teeth can be influenced by age. Fischer and Wiechuła conducted a study with the objective of analyzing the accumulation of Pb in the calcified tissue of permanent teeth.[22] Atomic absorption spectroscopy (AAS) was employed to measure the concentration of Pb in teeth samples from a particular group of Polish individuals. The research aimed to determine the accumulation of Pb in the human body based on the changes in Pb concentration in teeth from individuals aged 13–84 years. The results of the study showed that the concentration of Pb increased with age in calcified tooth tissues, and this was a statistically significant process. The research also revealed that subjects over the age of 60, born in the 1930s, had a lower concentration of Pb compared to those born in the 1950s. The concentration of Pb in the teeth of younger individuals (<60 years) was observed to increase. The analysis of changes in Pb levels revealed that even low exposure can result in a relatively high accumulation of Pb concentration in calcified tooth tissues. Fischer et al. have analyzed the changes in the concentrations of various elements, including manganese (Mn), Fe, magnesium (Mg), Cu, potassium (K), chromium (Cr), Pb, Cd, and Ca, in deciduous teeth.[23] The study aimed to investigate whether metal concentration changes with age and to describe changes in mineral composition. The researchers obtained deciduous teeth samples from children aged 5–14 years living in southern Poland through non-invasive physiological replacement. Metal concentration was determined via AAS. The results demonstrated a significant decrease in the concentration of the analyzed elements in the deciduous teeth of older children, when compared to those of younger children. However, no significant correlation was observed between the total metal concentration and the Ca content in relation to age.[23]

Chromium is a vital element for the human body. Although it has a significant physiological function, it can also be harmful, with potential carcinogenic, mutagenic, embryotoxic, and teratogenic effects, which depend on its valence state. Malara et al. conducted research into the various factors that influence the presence of Cr in teeth.[24] The objective of the research was to evaluate the influence of tooth type, age and sex on the level of Cr present in the tooth structure. The study sample consisted of permanent teeth sourced from individuals aged between 20 and 68 years residing in Ruda Śląska, Poland. Atomic absorption spectrometry was employed to measure the concentration of Cr in the teeth. The type of tooth and the sex of the donor had no significant influence on the level of Cr in the tooth tissue. Furthermore, there was no meaningful correlation between the Cr contents in teeth and the age of the donors.

Weight

Eating disorders represent a prevalent social phenomenon that may manifest in weight loss and weight gain. Malnutrition, whether due to an excess or deficiency of nutrients, can result in severe health consequences. The study conducted by Fischer and Wiechuła aimed to determine the levels of certain elements, including Cr, Ca, Cu, Fe, and Mn, in the deciduous teeth of children in relation to their body weight.[25] Although there were no significant differences in the concentration of the metals between children with normal and abnormal body weight, the correlation between the metals in teeth varied according to the children's weight.[25] This observation may indicate fluctuations in the mineral composition of tissues that are associated with metabolic disorders.

Systemic diseases, neurodevelopmental disorders and food allergies

The assessment of element levels in teeth, like in other bodily tissues, has the potential to be valuable in the diagnosis of diseases. The concentrations of heavy metals in both the tissue and serum have been linked to a wide range of illnesses, making them potentially valuable biomarkers for early disease detection.

Orzechowska-Wylęgała et al. investigated the presence of Cd and Pb in the teeth of children from the Upper Silesia region of Poland suffering from celiac disease or food allergies.[26] This particular region is known for its prevalent heavy industries, including coal mining, which have led to considerable environmental contamination. As all the children who participated in the study resided in the same location, their exposure to environmental pollution was consistent across the studied groups. While several factors can impact the levels of heavy metals in dental tissues, the focus of their research was on systemic illnesses that necessitate long-term adherence to restricted diets.[26] The most commonly observed illnesses in pediatric patients include celiac disease and food allergies. The prolonged implementation of restricted diets has been associated with mineral deficiencies in children, as well as quantitative and qualitative changes in the mineral composition of bones and dental tissues. Such alterations may lead to an increased accumulation of toxic metals within the body, such as Pb, Cd, strontium (Sr), Hg, and arsenic (As). The study revealed that children with celiac disease and food allergies have a higher tendency for the accumulation of certain metals in their deciduous teeth, in comparison to healthy individuals. Moreover, the Pb to Ca and Cd to Ca ratios were observed to be elevated in children with celiac disease and food allergies, providing additional evidence of the presence of these harmful metals in the body.[26] The results of this study suggest that children who undergo restrictive diets may require adjustments to their dietary intake, with a particular focus on increasing the consumption of proteins and sulfur amino acids.

In a study conducted by Yalçin et al., the teeth and blood samples of both healthy children and those with congenital heart disease (CHD) were examined.[27] The study included 39 children with CHD and 42 healthy children. The researchers used inductively coupled plasma mass spectrometry to evaluate the levels of 13 different elements, namely Mg, P, Ca, Cr, Mn, Fe, Cu, Zn, Sr, Cd, Pb, Hg, and molybdenum (Mo). After adjusting for potential confounding variables, it was observed that children with cyanotic and acyanotic CHD exhibited significantly lower levels of tooth Ca and a lower Ca:P ratio in comparison to the control group. Furthermore, children with acyanotic CHD exhibited markedly elevated levels of Cu in their teeth, along with increased levels of Mo in their blood and reduced levels of Mg in their blood, when compared to the control group, which consisted of healthy children.[27]

The research conducted by Sitarik et al. investigated the correlation between in utero and postnatal levels of Pb, which were measured using deciduous baby teeth, and the bacterial and fungal gut microbiota of infants in their first year of life.[28] The discovered associations between Pb exposure and gut microbiota could potentially affect the development of a child.[28] However, since there is a lack of research that investigates these correlations in humans, particularly with regards to fungal microbiota, further examination is necessary.

The study conducted by Abdullah et al. aimed to investigate the potential correlation between the presence of heavy metals in children's tooth enamel and the occurrence of autism and disruptive behaviors.[29] The researchers analyzed the concentrations of Pb, Hg and Mn in both prenatal and postnatal enamel regions of deciduous teeth from children diagnosed with Autism Spectrum Disorder (ASD), those exhibiting high levels of disruptive behavior, and typically developing children. The usage of laser ablation inductively coupled plasma mass spectrometry revealed no statistically significant disparities in the levels of these neurotoxicants between children with ASD and typically developing children.[29]

The variations in sex regarding ASD diagnosis and the mutagenic influence of toxic exposures suggest that these factors might have a significant impact on the causal relationships observed in any potential associations.[30]

In a study conducted by Adams et al., the levels of Hg, Pb and Zn in the baby teeth of children with autism were compared to those of a control group.[31] The results showed that children with autism exhibited significantly elevated levels of Hg, while the levels of Pb and Zn were comparable to those observed in the control group. This study indicates that children with autism may have had a greater accumulation of Hg in their bodies during fetal and infant development. The researchers also suggest that the increased use of oral antibiotics in children with autism could have hindered their ability to eliminate Hg, thereby contributing to the higher levels of the element observed in their baby teeth.[31]

The study conducted by Figueroa-Romero et al. aimed to investigate the potential dysregulation of metal uptake during childhood in individuals who were later diagnosed with amyotrophic lateral sclerosis.[32] By examining the co-exposure to different elements, the researchers found a strong association between childhood metal dysregulation and the development of amyotrophic lateral sclerosis.[32]

The impact of childhood exposure to low levels of Pb was assessed by Needleman et al.[33] The study revealed a strong correlation between elevated Pb levels during childhood and lower social standing during high school, increased rates of absenteeism, diminished language skills and logical thinking abilities, decreased hand-eye coordination, delayed reaction times, and reduced finger tapping speed.[33]

The study by Haavikko et al. investigated the concentrations of Zn and Cu in the deciduous teeth of Finnish children and adolescents.[34] The study focused on ways in which these mineral levels could serve as indicators of potential atherosclerosis precursors and systemic diseases. By analyzing the mineral content in dental tissues, the study aimed to understand the relationship between these trace elements and the risk of developing atherosclerosis and other systemic health issues.[34]

Although Mn is a necessary component for growth and development, elevated Mn levels have been associated with neurobehavioral impairment in children. The aim of the study conducted by Bauer et al. was to determine whether there is a correlation between visuospatial learning and memory test results and prenatal or postnatal Mn levels, as measured in deciduous teeth.[35] The deciduous teeth were collected from 142 participants who resided in areas with varying ferromanganese industries in Italy. The prenatal and postnatal tooth regions were analyzed for Mn concentrations using laser ablation inductively coupled plasma mass spectrometry. The results of the study indicate that the prenatal period could be a crucial timeframe for the influence of environmental Mn on executive function and visuospatial ability, particularly in females.[35]

In a study conducted by Gunier et al., the relationship between Mn levels in teeth and neurodevelopment was analyzed in young Mexican-American children.[36] The researchers used laser ablation inductively coupled plasma mass spectroscopy to measure Mn levels in both prenatal and postnatal dentin from children's shed teeth. The children's scores on the Mental Development Index (MDI) and Psychomotor Development Index (PDI) on the Bayley Scales of Infant Development at 6, 12 and 24 months were examined and compared to Mn levels. A unique biomarker was used to measure prenatal and early postnatal Mn levels in tooth dentin. The findings revealed a negative, temporary correlation between postnatal Mn levels and early neurodevelopment, with sex-specific modifications and prenatal hemoglobin interactions.[36]

Horton et al. investigated the relationships between dentin biomarkers of Pb, Zn and Mn and behaviors observed in later childhood.[37] The behaviors exhibited during childhood could reveal postnatal periods of vulnerability to both individual and combined metal levels found in deciduous teeth. While Mn present in prenatal dentin may offer protective effects, elevated levels of Mn during early postnatal development may increase the risk of negative behaviors. Furthermore, the simultaneous presence of higher concentrations of Mn, Zn and Pb may adversely affect behavior, with Pb specifically associated with an increase in anxiety symptoms.[37]

Mora et al. analyzed the levels of Mn in the dentin of shed teeth during both prenatal and early postnatal stages.[38] The teeth were collected from children residing near agricultural fields that had been treated with Mn-containing fungicides in California, USA. The study aimed to examine the relationship between Mn levels and various aspects of behavior, cognition, memory, and motor functioning in children. The results revealed a significant association between higher levels of prenatal and early postnatal Mn in deciduous teeth and adverse behavioral outcomes, including internalizing, externalizing and hyperactivity problems, in both boys and girls.[38]

By utilizing tooth-matrix biomarkers and examining detailed temporal patterns of exposure, scientists have identified specific periods of development during which Mn is linked to visual-spatial skills. The findings indicate that the associations between Mn and cognitive abilities are significantly influenced by the timing of exposure, with positive effects observed for prenatal levels and detrimental effects observed for postnatal levels.[39]

Environmental pollution

It is crucial to underscore the significant impact of environmental pollution, such as that of the air, soil and water, on the accumulation of toxic metals in the human body, including the teeth. There are a number of methods that can be used to evaluate the extent of heavy metal exposure in the environment. One of these methods is the analysis of various biological samples. Specifically, human teeth are a particularly suitable option because they possess a stable elemental composition. This stability allows for the comparison of the effects of long-term exposure to heavy metals among individuals inhabiting regions with varying degrees of pollution. It should be noted that the placenta is a crucial organ that receives considerable attention during pregnancy. Unfortunately, this organ provides an inadequate barrier against the transfer of dangerous heavy metals, especially Pb, to the developing fetus. The presence of Pb in this organ poses a serious environmental threat to the well-being of future generations. Hormonal fluctuations during pregnancy result in the release of Pb from long-term deposits in bones and teeth into the mother's bloodstream, which can have harmful effects due to exposure to a contaminated environment.[40]

In order to assess early-life metal exposure in a community that had expressed concerns about previous exposures, Friedman et al. conducted a study using deciduous teeth.[41] The teeth were collected from children who had lived in Holliston, Massachusetts (USA) from the time of conception. By analyzing naturally shed teeth, the researchers were able to obtain detailed information about the timing and dosage of metal exposure during early life. This study effectively demonstrates the usefulness of deciduous teeth in community-based research, particularly in cases with a history of water contamination.[41]

Anttila and Anttila investigated the absolute concentrations of Mn, Fe, Ni, Cu, Zn, Sr, and Pb in whole enamel, as well as in the labial and lingual surface enamel of deciduous incisors, using proton-induced X-ray emission.[42] The mean concentration values derived from the 19 samples collected in the urban area did not exhibit significant differences when compared to the 9 samples obtained from the rural region of Finland.[42]

The study by Anttila et al. focused on the Pb content in the enamel of deciduous teeth from an area with high radon (Rn) levels.[43] The study aimed to assess the concentration of Pb in the enamel of teeth from children living in a region with elevated Rn exposure and to investigate any potential relationship between Rn exposure and Pb levels in the teeth. The average Pb concentration in the enamel was comparable to previous measurements of Pb in other regions of Finland, indicating that Rn decay did not cause a notable rise in Pb levels in the teeth.[43]

In a study conducted by Järvinen et al., the levels of Pb in the enamel of deciduous molars were analyzed using proton-induced X-ray emission.[44] The results suggest that the general population of Finland is not currently subjected to significantly elevated levels of artificially introduced environmental Pb, whether in urban or rural settings. Naturally occurring environmental Pb remains a critical factor in cumulative long-term exposure experienced in Finland.[44]

Fosse and Justesen conducted an investigation into the levels of Pb present in the deciduous teeth of Norwegian children.[45] The study aimed to assess the concentration of Pb in dental tissues in order to understand the extent of Pb exposure among children in Norway. The research sought to identify potential sources of Pb exposure and to evaluate the impact of environmental factors on Pb accumulation in teeth. The deciduous teeth were obtained from a variety of Norwegian counties, including urban centers, industrial zones, and rural as well as fishing communities. The findings indicated that both urbanization and industrialization resulted in increased Pb absorption. However, the average level of Pb detected in Norway was significantly lower than the levels typically observed in other nations. Additionally, automobile exhaust was dismissed as a major contributor to excessive Pb absorption.[45]

The assessment conducted by Arora et al. focused on examining the distribution of Pb in primary teeth as a means of assessing Pb exposure during the pre- and neonatal stages.[46] The researchers employed laser ablation inductively coupled plasma mass spectrometry to measure the presence of Pb in both the enamel and dentin of 10 primary teeth. The study illustrates a valuable methodology for obtaining temporal data on environmental Pb exposure during the pre- and neonatal periods by analyzing the spatial distribution of Pb in the dentin of primary teeth.[46]

Modern human populations are increasingly exposed to chronic environmental heavy metal contamination due to rapid urbanization and extensive industrial activities. A bioindicator for elemental uptake is found in tooth dentin, where the absorption occurs during the processes of mineralization and formation, resulting in significant storage over many years. This uptake encompasses essential elements, primarily sourced from geogenic diets, along with non-essential elements introduced through environmental exposure. Asaduzzaman et al. examined 50 human teeth from different ethnic groups.[47] It has been noted that concentrations of heavy metals tend to increase with age. A comparison of ethnic groups revealed that the teeth of ethnic Chinese individuals exhibited slightly higher metal concentrations than those of Malays and Indians. Additionally, female dentin demonstrated greater levels of metal concentrations than male dentin. The molars contained higher concentrations of Hg, Cu and tin (Sn), whereas the incisors showed elevated levels of Pb, Sr, antimony (Sb), and Zn. The increased levels of heavy metals in tooth dentin indicate pollution originating from industrial emissions and urbanization. This demonstrates that human tooth dentin serves as a reliable bioindicator of environmental pollution and can provide chronological data on exposure.[47]

Wychowanski and Malkiewicz conducted a study on residents of Central Poland (Mazowieckie province) to measure the concentration of metal ions present in the hard tissues of their teeth.[48] They collected samples of enamel and dentin from participants living in urban and agricultural areas. The researchers used graphite furnace atomic absorption spectrometry to determine the concentration of Mn, Pb, Cd, and Cr in the enamel and dentin samples from retained teeth. A comparative analysis of the data revealed that the enamel and dentin of individuals living in industrialized areas exhibited significantly higher levels of Pb and Cd compared to those residing in agricultural areas. However, both groups exhibited comparable levels of Mn and Cr in hard tooth tissues. The results of this study confirm that the likelihood of exposure to heavy metals is dependent on both the place of residence and the extent of environmental pollution in that area.[48]

Nowak analyzed the presence of sodium (Na), Ca, K, and heavy metals in human teeth.[49] The analysis was conducted in Katowice and Istebna in Poland between 1992 and 1993. The measurements were taken to determine the levels of heavy metals and Na, Ca and K in the teeth of individuals. Notably, in the relative unpolluted southern region of Poland, namely Istebna, the concentration of heavy metals in the teeth of the local population was significantly lower than in Katowice, an industrial hub in Silesia. The ratios of Pb:Fe and Pb:Mn detected in teeth could potentially serve as a measure of pollution. The analysis of the metal concentration levels in the teeth of individuals residing in Katowice and Istebna revealed significant differences in the mean concentrations of Pb, nickel (Ni), Cd, Zn, cobalt (Co), Mn, Cr, Na, and Ca.[49]

The focus of the study conducted by Nowak and Chmielnicka was to examine the correlation between Pb and Cd and essential elements present in the hair, teeth and nails of individuals living in the environmentally exposed Katowice District in Poland.[50] The investigation aimed to assess the extent of environmental exposure to Pb and Cd between 1990 and 1997 in the residents of this district, which is known for its high levels of environmental exposure to these toxic metals. Additionally, the study examined the exposure to Fe, Zn, Cu, Mn, Ni, Cr, Ca, Na, and K based on the concentration levels found in hair, teeth and nails. The investigation aimed to ascertain whether the accumulation of Pb and Cd could have an impact on the concentration of essential metals, including Fe, Zn, Cu, and Ca. Additionally, a control group was included in the study, consisting of residents from the Beskid area in Poland. The aforementioned elements were analyzed with regard to sex, age and tooth type. Atomic absorption spectroscopy was utilized to determine the concentrations of the elements present in the media under examination. Notably, teeth samples obtained from individuals residing in the Katowice District exhibited elevated levels of Ni, Cr and Mn.

The purpose of the study conducted by Fischer et al. was to analyze the Pb content in various groups of teeth among inhabitants of the Silesian region in Poland.[51] The highest concentration of Pb was identified in canines, while molars exhibited the lowest concentration of Pb among all groups of teeth. The study demonstrated that the accumulation of Pb in the hard tissues of the body increases with age. Additionally, individuals exposed to Pb emissions from industrial sources were found to have significantly higher levels of Pb present in their teeth.[51]

In their study, Malara et al. aimed to establish whether impacted mandibular teeth and the adjacent bones could serve as a form of biomonitoring media for evaluating the exposure to heavy metals.[52] The research involved the utilization of impacted lower third molars and fragments of the cortical bone, which were extracted during the removal of wisdom teeth. The research participants were chosen from 2 locations: the relatively polluted Ruda Śląska region in Poland; and the Bielsko-Biała region in Poland. Atomic absorption spectrometry with flame atomization was used to determine the concentrations of Cd, Cr, Cu, Fe, Pb, Mn, and Zn in the samples. The inhabitants of the Ruda Śląska region exhibited significantly higher concentrations of Cd and Pb in their impacted third molars and the surrounding bones compared to those living in the Bielsko-Biała region. Furthermore, a significant positive correlation was observed between the concentrations of Cd in the impacted teeth and the surrounding bones. These findings suggest that the levels of Cd and Pb in the environment may be reflected in the impacted mandibular teeth and the surrounding bones of individuals.

A study that explored the concentration of toxic metals in impacted third molars and surrounding bone tissue was carried out by Bryła et al.[3] The study was similar to a previous investigation, but the samples were collected from 2 distinct groups: residents of the Wroclaw district; and residents of Wroclaw city in Poland. The objective of the study was to determine the concentrations of several elements, including Pb, Cd, Cr, Ni, Fe, Mn, Cu, and Zn, in a portion of bone covering the third molars and in the teeth themselves. The levels of Cd, Pb and Mn in the samples demonstrated an increase in correlation with the patient's age. The levels of Cd and Pb were found to be higher in the residents of Wroclaw, as compared to other locations. Additionally, the residents of Wroclaw exhibited higher levels of Cu in their teeth. It was observed that the concentrations of Cr in the teeth was 33% lower than those in the mandibular bone, and that the concentration of Ni decreased with age. In all hard tissues of the tooth and bone, Pb and Cd aggregates were identified, in contrast to bioelements, which demonstrated a greater tendency to aggregate, primarily within the dentin.

The primary source of Cr in the human body is dietary consumption. However, in cases where there is occupational exposure or residing in areas with high levels of Cr in the air, the absorption of Cr through the lungs may be greater than through ingestion. Fischer et al. conducted a study to investigate the correlation between Cr and other selected elements in impacted wisdom teeth.[53] The objective of the study was to determine the levels of Cr, as well as other elements such as Fe, boron (B), Co, Cu, Zn, selenium (Se), Mo, and barium (Ba) in the mineralized tissues of impacted teeth in a population exposed to elevated levels of Cr in the air. The level of exposure to Cr compounds can be determined through the use of impacted wisdom teeth as a diagnostic tool. A comparison between the Cr content in impacted third molars and erupted permanent teeth reveals a lower amount of Cr in the former, indicating that the primary source of Cr in embedded teeth is the bloodstream. The concentration of Cr in hydroxyapatites of impacted wisdom teeth was found to be mainly influenced by the levels of Fe, B, Cr, Co, Se, and Mo.[53]

Rayad et al. conducted an in vitro assessment to measure the concentration of toxic metals in third molars obtained from residents of the Legnica–Głogów Copper District in Poland.[1] The objective of the study was to identify the concentration of toxic metals, which included Mn, Cr, Ni, Cu, Fe, Cd, Pb, and Zn, in the extracted third molars of patients from the Legnica–Głogów Copper District, as well as to ascertain the risk factors that determine the accumulation of these metals. The patients were divided into 2 groups based on their place of residence: residents of the Legnica–Głogów Copper District; and a control group of residents from Wroclaw. The SpectraAA atomic absorption spectrometer was used to determine the concentrations of Pb, Cd, Cr, Ni, Fe, Mn, Cu, and Zn in an air-acetylene flame. The analysis of third molars among inhabitants of the Legnica–Głogów Copper District revealed elevated levels of Fe and Pb. The identification of major risk factors that could lead to the accumulation of harmful metals in the teeth has been established. The study demonstrated a strong correlation between the concentration levels of Cr, Cu and Zn and a person's age. Additionally, a correlation was identified between the amount of Cr present and the concentration of vitamin D3 found in the bloodstream.[1]

In their research, Rayad et al. conducted an analysis of the Hg levels in impacted wisdom teeth obtained from individuals residing in the Legnica–Głogów Copper District.[2] The primary objective of the study was to emphasize the impact of environmental pollution on the human body by determining the amount of Hg present in the impacted third molars of residents in this area. To ascertain the levels of Hg in the samples, AAS was employed. The accumulation of Hg in the teeth of individuals in the control group located in Wroclaw was also studied, with a focus on identifying the risk factors that contribute to this phenomenon. The final model examined a number of factors, including thyroid and parathyroid gland ailments, cardiac ailments, and interval-scale vitamin D3 concentration. Among these factors, the presence of cardiac diseases was found to be statistically significant in relation to an increase in Hg concentration in third molars. The concentration of Hg was found to increase with age and the duration of residence in the Legnica–Głogów Copper District.[2]

The impact of environmental pollution in the Legnica–Głogów Copper District on the incidence of tooth count disorders in adolescents residing in that region was investigated by Rzepnicka et al.[54] The primary objective of their research was to ascertain the degree of hypodontia in adolescents from educational institutions in Legnica (Poland). The dental health of students from secondary schools in Legnica was evaluated. Of the 1,000 students evaluated, 7.7% had hypodontia. This finding is higher than in other parts of Poland, which could be a result of environmental pollution in the Copper Basin of Lublin, the Głogów Region of Poland.[54]

The study conducted by Malara et al. aimed to investigate the impact of environmental exposure on the coexistence of Cd and Zn in teeth.[55] Specifically, the objective of their research was to determine whether the exposure to heavy metals in the environment affects the coexistence of Cd and Zn in human teeth. The study utilized a sample size of permanent teeth, with teeth sourced from residents of Bielsko-Biała and Ruda Śląska in Poland. The levels of Cd and Zn in the teeth were determined through the use of AAS. A negative correlation was observed between Cd and Zn levels in the teeth of residents from Bielsko-Biała, whereas a positive correlation was noted between the 2 metals in the teeth of residents from Ruda Śląska. The research findings indicated a notable discrepancy between the levels of Zn and Cd present in the teeth of residents from Ruda Śląska, in comparison to those from Bielsko-Biała. Additionally, the exposure to environmental sources of these metals influenced their interaction within the human body.[55]

The assessment of the exposure of a population to heavy metals from the environment can be determined through the analysis of biological samples. Teeth, in particular, can be utilized to estimate long-term environmental exposure due to the stability of their elemental composition. The objective of the study conducted by Malara and Kwapuliński was to identify the presence of physiological and toxic metals in the teeth of individuals residing in Ruda Ślaska and Bielsko-Biała in Poland.[56] Additionally, the study aimed to explore the potential impact of environmental exposure to heavy metals on the occurrence and coexistence of these metals in teeth. The research material for this study consisted of the permanent teeth obtained from individuals between the ages of 20 and 68 from both cities. The levels of Cd, Cr, Cu, Fe, Mn, Pb, Zn, K, Na, Ca, and Mg were measured using AAS with flame atomization. The teeth of individuals residing in Ruda Śląska showed a significant increase in the levels of Cd, Cr, Cu, Fe, Mn, Pb, and Zn, accompanied by a decrease in the levels of Ca and Mg and elevated Pb:Ca and Pb:Mn ratios. An examination of the correlation matrix revealed differences in the co-occurrence of metals in the teeth of the 2 studied groups. The study confirmed that the exposure to heavy metals in the environment has a significant impact on the presence and coexistence of these elements in individuals' teeth.[56] Malara et al. conducted similar research on the effect of Pb on the content of other metals to investigate their interdependence.[57] The permanent teeth extracted from the inhabitants of Ruda Śląska were analyzed. The content of Pb, Fe, Mn, K, Ca, and Mg was determined through the use of AAS with flame atomization. Furthermore, Pearson's product moment correlation analysis demonstrated that Pb exerted an adverse effect on the content of Ca, Mn, Fe, and Mg, while having no impact on the content of Na and K. The tooth structure showed a strong interdependence between Ca, Fe and Mn.[57] Studies have identified an inverse correlation between the presence of Pb and these 3 vital elements, which may be attributed to the process of substitution.

In their investigation, Fischer et al. conducted a detailed analysis of the concentration of Ba in the facial bones, deciduous teeth and impacted teeth of individuals residing in Poland.[58] The research involved both children and adults (aged from 6 to 78 years) from an industrialized region. The condition of the teeth was assessed holistically, without any distinction between the dentin and enamel. To prepare the facial bones and teeth, a sequence of procedures was undertaken, including rinsing, desiccation, crushing in a ceramic mortar, measurement of the sample (approx. 0.2 g), and microwave mineralization in pure nitric acid with a spectral dimension. The concentration of Ba increased with age in bone tissue and in impacted teeth. In contrast, the level of Ba was observed to decline with age in the deciduous teeth.[58]

The study conducted by Fischer et al. involved an evaluation of the Se content in retained wisdom teeth.[59] Specifically, the researchers analyzed the Se content in the teeth of 10 individuals residing in the Upper Silesian Industrial Region in Poland. The results indicated an average Se content of 0.77 ±0.25 μg/g in the teeth, which was notably lower in comparison to the concentration of Se found in other hard tissues, as reported by prior researchers. Notably, a high correlation was observed between the Se content and the content of other elements, such as As, Fe, Sb, and Hg, which suggests that the industrial emissions in the area were the primary source of these elements found in the teeth.[59]

Piekut et al. conducted an evaluation to determine the potential of primary teeth as an indicator of children's environmental exposure to heavy metals.[60] The objective of their study was to ascertain whether primary teeth could serve as an effective environmental indicator of Cd, Pb and Zn exposure in children residing in Bytom, Poland. The study involved an examination of the primary teeth of children between the ages of 2 and 14. Before the analysis, the teeth underwent a mineralization process. The concentration of Cd, Pb and Zn in the samples was determined using AAS. The results of the analysis demonstrated variability in the levels of Cd, Pb and Zn within the primary teeth. Younger children have been found to have higher levels of Cd and Pb than their older counterparts. This indicates that these metals may have been present during prenatal development. Additionally, the concentration of heavy metals identified in primary teeth differs between sexes, with boys showing higher levels of Pb and Zn than girls. The study conducted in Bytom analyzed primary teeth of children and found limited potential for these teeth to serve as an indicator of exposure to Cd, Pb and Zn in the environment. It appears that prenatal exposure may be a complicating factor in assessing the exposure to these metals in children.[60]

Wiechuła et al. performed a statistical examination of quantities of metals found in the teeth of individuals residing in the Silesian region of southern Poland.[61] Specifically, the concentrations of 11 different metals, namely Cd, Cr, Cu, Fe, Mn, Pb, Zn, Na, K, Ca, and Mg, were measured in the teeth of 2 different groups of people residing in the Silesian region. The first group consisted of individuals living in the town of Katowice-Szopienice, situated in the center of the Upper Silesian Industrial Region, in close proximity to a Pb plant. The second group was comprised of residents of Strumień, an agricultural community. The results of the analysis showed that the residents of Katowice-Szopienice exhibited higher levels of trace metals in their teeth.[61]

Fischer et al. evaluated metal concentrations (Cd, Pb, Mn, Cu, Cr, Fe, Zn, Na, K, Mg, and Ca) in deciduous and permanent human teeth, specifically in the maxilla and mandible.[62] The results showed significantly higher concentrations of metals in the deciduous teeth in comparison to the permanent teeth. Regression and principal component analyses revealed that the process of binding elements by hydroxyapatite in deciduous teeth is becoming more dynamic. Furthermore, the concentration of metals was observed to be higher in the permanent and deciduous teeth of the maxilla compared to those in the mandible.[62]

It is important to acknowledge that the exposure to toxic metals in the workplace leads to their accumulation in the human body. In a study conducted by Malara et al., the presence of certain metals in the teeth of coal miners was investigated in relation to age and duration of employment.[63] The research consisted of an evaluation of Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, K, Na, Ca, and Mg concentrations in different types of teeth, including incisors, canines, premolars, and molars. Atomic absorption spectroscopy was utilized to measure the concentration levels of these metals. The results showed that the concentration of Pb in molars had a positive correlation with the age of the coal miners, whereas the concentration of Na and K demonstrated a positive correlation with the duration of employment.[63] Kwapuliński et al. also conducted a study on the prevalence of selected metals in the teeth of coal miners.[64] The study aimed to investigate the metal concentrations in the teeth of coal miners and non-miners in the town of Ruda Śląska, which is situated in the Upper Silesian Industrial Region in Poland. The study subjects consisted of men between the ages of 20 and 50. The results of the study provide valuable insights into the prevalence of metals in the teeth of individuals exposed to environmental contaminants in both occupational and non-occupational settings. The findings revealed that the concentrations of Pb, Cr, Fe, Na, Ca, and Mg in the teeth varied depending on the tooth type. Although there were no statistically significant differences in the concentrations of elements present in the teeth of miners and non-miners, some isolated differences were observed in the context of specific elements and teeth, such as elevated levels of K in incisors, Pb in premolars, and Cr, Zn and Mg in molars.[64]

Poczatek et al. performed an investigation to assess the potential risks to workers in the industry by evaluating the levels of particular elements in their teeth and bodily fluids.[65] The study involved measuring the levels of certain elements in the teeth and body fluids of Polish workers across 3 distinct industries with established production profiles: Rail Rolling Stock Repair Workshops, which specializes in repairing rail vehicles; Philips Lighting Poland, which produces lighting systems; and Metal-Plast, a factory that is involved in building and furnishing. As part of the study, the teeth were extracted, and the samples of various bodily fluids, including urine, blood and saliva, were collected during routine health check-ups. The study evaluated the levels of various elements, including Ca, Mg, fluorine (F), P (in the form of phosphates), K, Na, Fe, Zn, Cu, Cd, and Pb. To measure the elements, AAS was utilized. The study demonstrated that the concentrations of these elements present in the teeth and body fluids of the subjects varied across the industries. Significant differences were identified in the levels of Mg, phosphates, Zn, Na, and K in the teeth.[65]

Johnston et al. evaluated Pb and As in the shed deciduous teeth of children living near a lead-acid battery smelter.[66] The assessment of prenatal and initial stage exposure to toxic elements can be achieved through the use of shed deciduous teeth to measure Pb and As. A community-based research approach was taken to analyze 50 shed deciduous teeth from 43 children who have lived within a 2-mile radius of a smelter for the entirety of their lives. Laser ablation inductively coupled plasma mass spectrometry was used for the evaluation of the concentrations of Pb and As in the teeth. In order to determine the concentration of Pb in the soil, the researchers utilized spatial kriging to analyze the soil's surface. Findings of the research imply that the exposure to toxic metals during the prenatal and early life stages is linked to soil contamination caused by past industrial activities in an urban community located near a smelter.[66]

The detonation of bombs, bullets and other forms of ammunition in areas of conflict results in the release of several neurotoxicants into the environment. Currently, the Middle East is facing a considerable degree of environmental degradation as a result of extensive bombardments. Savabieasfahani et al. developed a method for elemental bioimaging to examine trace elements in the deciduous teeth of children from Iraq who were born with defects.[67] Additionally, the authors analyzed naturally shed teeth from Lebanon and Iran for trace elements. The research confirmed that elevated levels of metal in deciduous teeth are correlated with increased levels of war activity.[67]

Haavikko et al. employed the proton-induced X-ray emission technique to assess Pb levels in the enamel and dentin of deciduous teeth of children from 2 Finnish towns.[68] It was presumed that Helsinki, the capital, would indicate high Pb exposure, while the rural town of Kuopio in central Finland would represent low to moderate Pb exposure. In nearly all cases, with the exception of 2 teeth, the enamel exhibited greater Pb concentrations than the dentin. The ratio of Pb concentration between enamel and dentin was not consistent and showed significant variability.[68]

The study conducted by Shishniashvili et al. focused on the use of primary teeth and hair as reliable markers for measuring environmental pollution.[69] The results of their study revealed that in areas with unfavorable environmental conditions, the levels of toxic elements such as Pb, Hg, Sn, and titanium (Ti) were significantly higher compared to areas with more favorable conditions. Furthermore, their research demonstrated that dental hard tissues in polluted regions of Tbilisi (Georgia) exhibited higher concentrations of toxic elements and lower levels of essential elements when compared to less polluted areas.[69]

Tsuji et al. reported increased levels of Pb in the dentin of deciduous teeth obtained from isolated First Nations communities in the western James Bay region of northern Ontario, Canada.[70] The research findings indicated that the average concentration of Pb in shed teeth from this remote area was comparable to the levels observed in children living in urban environments or in proximity to smelting facilities. Furthermore, a notable percentage of the children exhibited elevated Pb levels in their dentin.[70]

van Wyk and Grobler assessed the Pb concentrations in the shed deciduous teeth from children residing in 2 selected urban areas of the Cape Peninsula.[71] The children residing in proximity to 2 major industrial facilities exhibited the following average Pb levels: whole teeth at 20,419 ppm; enamel at 10,952 ppm; and dentin at 22,733 ppm. In contrast, the children living near light industrial facilities exhibited Pb levels of 16,556 ppm in whole teeth, 2,919 ppm in enamel and 19,926 ppm in dentin. These variations were statistically significant at the 1% level for teeth and enamel, and at the 5% level for dentin.[71]

Lyngbye et al. conducted a study of Pb exposure in children. In a low-exposure area, the researchers explored potential predictors of Pb burden in children.[72] A total of 1,302 first-form school children from the municipality of Aarhus, Denmark, donated their deciduous teeth for the measurement of Pb concentration in the circumpulpal dentin. Families were interviewed regarding possible sources of Pb exposure. Children with a high Pb burden resided significantly more often in heavily-travelled streets than those with a lower burden, but only during their first 3 years of life. The relationship between traffic intensity and the risk of a high Pb burden was evident in a dose-response manner.[72]

Gomes et al. assessed the levels of Pb present in the superficial enamel of deciduous teeth in children aged 4 to 5 years.[73] The findings revealed that children residing in industrial regions exhibited considerably higher concentrations of Pb in their enamel compared to those living in areas distant from industrial influences.[73]

It is important to highlight that the dental environment is becoming increasingly contaminated with metals from dental materials, primarily due to procedures that generate aerosols, which could compromise the long-term health of dentists, dental students and dental staff. The current pollution in dentistry includes metallic nanoparticles that are highly reactive and can easily become airborne, particularly those that detach from the bulk composition. Additionally, dental amalgam may release liquid Hg or Hg vapors. These issues give rise to concerns within the dental community.[74]

Smoking

The deposition of tar and toxic metals in the body represents a significant health concern associated with smoking. Any form of smoking, whether active or passive, can result in the accumulation of toxic metals in one's teeth. In their analysis, Malara et al.[75] evaluated the impact of smoking on the concentration of Pb and Cd in the hard tissue of teeth. The study was conducted on permanent teeth obtained from both smokers and non-smokers residing in Ruda Śląska in Poland. Atomic absorption spectrophotometry was used to determine the concentration of Cd and Pb. To gain further insight into the accumulation of these harmful metals, the Ca levels in the tooth samples were assessed, resulting in the calculation of the Cd:Ca and Pb:Ca quotients. The act of smoking cigarettes has been observed to affect the concentration of Pb and Cd present in the teeth of individuals. The teeth of smokers contained higher levels of these elements in comparison to non-smokers, with the discrepancy in Cd content being statistically significant.[75] Additionally, studies have shown that the extent of the increase in Cd and Pb levels due to smoking is more pronounced in males than in females.[75,76] Inhalation of cigarette smoke represents a significant source of exposure to heavy metals. Passive exposure to cigarette smoke can also result in the accumulation of these substances. Deciduous teeth, with their stable chemical composition, are often utilized as an indicator of heavy metal exposure in children. In a study conducted by Malara et al., the impact of passive smoking on the concentration of specific metals in deciduous teeth was examined.[76] The study utilized deciduous teeth as its primary research material, with some samples sourced from children exposed to cigarette smoke in their apartments. The levels of various elements, including Cd, Cu, Fe, Mn, Pb, Zn, Ca, and Mg, were measured using AAS with flame atomization. The results indicate that the exposure to cigarette smoke in children can lead to changes in the levels of both toxic and essential elements found in deciduous teeth. Specifically, higher levels of Cd, Cu, Pb, and Zn, which are permanent constituents of cigarette smoke, were observed, along with lower levels of Mn, Ca and Mg. Additionally, it was noted that children exposed to cigarette smoke in their apartments exhibited a disturbed gradient of Pb levels depending on the type of tooth.[76] Malara et al. also evaluated the occurrence of Pb and Cd in deciduous teeth of children exposed to cigarette smoke in apartments.[77] The study utilized shed deciduous teeth from children between the ages of 6 and 13 who had been exposed to tobacco smoke in their apartments, as well as from children in the same age group who lived in smoke-free apartments. The results showed that the deciduous teeth of children exposed to tobacco smoke in their apartments exhibited higher levels of Pb and Cd, accompanied by elevated Pb:Ca and Cd:Ca ratios, indicative of significant accumulation of these metals when compared to the teeth of children residing in smoke-free apartments.[77]

In several biological samples, there is an alteration in the coexistence pattern of elements (regardless of whether they are antagonistic or synergistic) when in the presence of toxic elements at harmful levels. The study conducted by Malara et al. aimed to determine whether there is a variation in the amount of Cd and Zn present in the hard tissues of retained wisdom teeth in smokers and non-smokers, and whether the exposure to cigarette smoke has an effect on the coexistence of these metals within the tissues.[78] The materials used for this study were retained wisdom teeth from both smokers and non-smokers, and the Cd and Zn levels were determined using AAS. The results showed that the retained wisdom teeth of smokers exhibited higher levels of Cd and Zn compared to the non-smokers' teeth. Furthermore, the coexistence pattern of Cd and Zn in teeth was influenced by exposure to heavy metals, exhibiting a strong synergistic relationship in smokers.[78]

The study conducted by Alhasmi et al. employed laser-induced breakdown spectroscopy to detect the presence of toxic elements in the teeth of smokers and non-smokers.[79] The research investigated periodontal parameters associated with differences in exposure to these toxic elements. The aim of the study was to ascertain the impact of smoking on the accumulation of toxic elements in teeth and the associated changes in periodontal health. The study revealed that the concentrations of Pb, Cd and As were significantly higher in the teeth of smokers compared to non-smokers and the control group. Specifically, smokers exhibited elevated levels of these toxic elements compared to non-smokers, who also demonstrated higher concentrations than the control group. Overall, the control group exhibited the lowest levels of these elements.[79]

One aspect investigated by Olovčić et al. was the impact of smoking on the accumulation of toxic metals.[80] The researchers analyzed the presence of 12 different metals in dental samples collected from 2 cities in Bosnia and Herzegovina, namely Bihać and Sarajevo. The research revealed statistically significant differences in the levels of Zn between the dentin samples of smokers and non-smokers.[80]

The study conducted by Fischer et al. evaluated the significance of passive smoking on the levels of Pb and Cr found in deciduous teeth.[81] The study presented the changes in Pb and Cr content in deciduous teeth of children exposed to environmental tobacco smoke. The control group consisted of children whose deciduous teeth were not exposed to tobacco smoke at home. The analysis revealed that the Pb content was higher in the non-exposed population (13.81 μg/g) than in the passive smoking population (12.28 μg/g). Passive smoking resulted in a reduction in Cr content in deciduous teeth. The quotient of Pb and Cr contents was higher in passive-smoking boys and girls and in different types of deciduous teeth.[81] Fischer et al. also evaluated the effect of passive smoking on the concentration of other metals in deciduous teeth.[82] The increased concentration of toxic elements may disrupt the balance of trace elements which are crucial for the body's physiology. The study involved the examination of deciduous teeth that were collected during the process of being replaced by permanent dentition. The content of various elements, including Mn, Fe, Cu, Ca, K, Na, Mg, Zn, Cr, Cd, and Pb, was determined through the use of AAS. The deciduous teeth of children who were not exposed to tobacco smoke exhibited higher levels of elements that are essential for the body's physiological functions, such as Fe, Zn, K, Na, and Ca. In contrast, the deciduous teeth of children exposed to passive smoking exhibited lower levels of these essential elements. Furthermore, the study demonstrated higher levels of toxic metals in the hard tissues of the teeth of children who were exposed to passive smoking at home.[82] It is possible that these toxic metals originate from tobacco smoke.

Toxic metals are a major contaminant in cigarette smoke, leading to the accumulation of these elements in calcified tissue of the teeth after entering the human body. This creates new conditions for the coexistence and occurrence of metals, which can be described using Czarnowski's model of the equilibrium between biological and chemical cations.[83] In their study, Malara et al. investigated the impact of smoking on the cationic equilibrium present in the teeth of men.[83] The study aimed to determine whether the consumption of cigarettes had any effect on the levels of Cd, Cr, Cu, Fe, Mn, Pb, Zn, K, Na, Ca, and Mg present in the hard tissues of men's teeth, as well as on the constant value of the cationic equilibrium model. The research material consisted of extracted permanent teeth from both smokers and non-smokers. The results indicated that smokers exhibited higher levels of Cd, Cr, Cu, Pb, and Zn in their teeth, whereas the concentrations of K, Na, Ca, Mg, Fe, and Mn were lower in smokers' teeth than those found in non-smokers. Additionally, the constant value of cationic equilibrium in the teeth of smokers was observed to be lower than that of non-smokers.[83] The abovementioned studies are summarized in Table 1.

Table 1. Summary of the studies included in the literature review

Study	Study design	Aim of the study/study results
Rayad et al. 2023[1]	in vitro pilot study	The study evaluates the concentration of toxic metals in third molars obtained from residents of the Legnica–Głogów Copper District and identifies risk factors for metal accumulation.
Rayad et al. 2023[2]	in vitro pilot study	The study investigates the mercury content in impacted wisdom teeth of patients from the Legnica–Głogów Copper District and evaluates environmental exposure.
Bryła et al. 2021[3]	research article	The study measures the concentration of toxic metals in impacted third molars and adjacent bone tissue across different patient groups, revealing significant variations.
Fukunaka and Fujitani 2018[4]	review	The study investigates the role of zinc homeostasis in the pathogenesis of diabetes and obesity.
Taniguchi et al. 2013[5]	experimental research	The zinc transporter ZIP9/SLC39A9 is a critical regulator of B-cell receptor signaling pathways.
Ciosek et al. 2023[6]	review	The study describes the interactions of iron, zinc, copper, cadmium, and mercury with bone tissue and discusses their potential health effects.
Brodziak-Dopierala et al. 2009[7]	research article	The study explores the interactions of copper and iron with other elements in the osseous tissue of the femoral head.
Kabata-Pendias and Mukherjee 2007[8]	book	The book traces the pathway of trace elements from soil to humans, emphasizing the environmental and health implications.
Otten et al. 2006[9]	book	The guide provides dietary reference intakes for essential nutrients, including trace elements.
World Health Organization 2007[10]	guidelines	The report offers guidelines for the assessment of the iron status in populations and emphasizes the implications for public health.
Wang et al. 2018[11]	experimental research	Iron-induced oxidative stress stimulates osteoclast differentiation via the NF-κB signaling pathway in a mouse model.
Angus et al. 1988[12]	research article	The study examines the relationship between dietary intake and bone mineral density.
Nriagu 1989[13]	research article	The study provides a global assessment of the natural sources of atmospheric trace metals.
Collins et al. 2010[14]	review	The article explores the metabolic crossroads of iron and copper, highlighting their interactions.
Wang and Shi 2001[15]	review	The study investigates the molecular mechanisms underlying metal toxicity and carcinogenesis.
Dudek-Adamska et al. 2018[16]	research article	The study analyzes chromium levels in postmortem material to assess environmental exposure.
Pietrzak et al. 2021[17]	research article	The study investigates the influence of arsenic, cadmium, mercury, and lead levels on the overall survival of patients with lung cancer.
Tvinnereim et al. 2000[18]	research article	The study identifies factors that influence the concentration of heavy metals in human primary teeth.
Alomary et al. 2013[19]	research article	The study measures lead, cadmium, copper, iron, and zinc levels in deciduous teeth of children in Jordan, identifying factors affecting their concentrations.
Amadi et al. 2019[20]	review	The review explores natural antidotes and management strategies for metal toxicity.
Andersen 2004[21]	review	The review discusses the chemical and biological aspects of treating metal intoxications with chelating agents, emphasizing their mechanisms of action and effectiveness.
Fischer and Wiechuła 2016[22]	research article	The study reveals age-dependent changes in lead concentration in human teeth, indicating an increased accumulation with advancing age.
Fischer et al. 2013[23]	research article	The concentrations of elements in deciduous teeth vary with age, reflecting alterations in metal exposure and mineral metabolism as individuals grow older.
Malara et al. 2005[24]	research article	The study identifies factors influencing the presence of chromium in teeth, including environmental and dietary influences.
Fischer and Wiechuła 2016[25]	research article	The study explores the potential relationship between a child's body weight and the concentration of metals in their deciduous teeth, revealing significant correlations.
Orzechowska-Wylęgała et al. 2011[26]	research article	The accumulation of cadmium and lead in deciduous teeth is higher in children with celiac disease or food allergies.
Yalçin et al. 2021[27]	research article	The study compares the element profiles in the blood and teeth of children with congenital heart disease to those of healthy children, demonstrating significant differences.
Sitarik et al. 2020[28]	research article	The exposure to lead in utero and in the early postnatal period is associated with changes in the infant gut microbiota.

Study	Study design	Aim of the study/study results
Abdullah et al. 2012[29]	research article	There is a potential correlation between the presence of heavy metals in children's tooth enamel and the occurrence of autism spectrum disorder and disruptive behaviors.
Dickerson et al. 2017[30]	review	There are potential sex differences in the relationship between metal exposure and autism spectrum disorder. Males and females may respond differently to the effects of metal toxicity.
Adams et al. 2007[31]	research article	The study compares the levels of mercury, lead and zinc in baby teeth between children with autism spectrum disorder and a control group, indicating higher levels in the former.
Figueroa-Romero et al. 2020[32]	research article	Early life metal dysregulation is correlated with the development of amyotrophic lateral sclerosis.
Needleman et al. 1990[33]	research article	The study examines the long-term effects of low-dose lead exposure during childhood, showing its lasting impact on cognitive function.
Haavikko et al. 1985[34]	research article	The concentrations of zinc and copper in the deciduous teeth of Finnish children and adolescents are associated with early indicators of atherosclerosis.
Bauer et al. 2017[35]	research article	The correlation between manganese levels in teeth and neurobehavioral outcomes highlights the existence of sex-specific windows of susceptibility.
Gunier et al. 2015[36]	research article	Manganese levels in teeth are associated with neurodevelopmental outcomes in young Mexican-American children.
Horton et al. 2018[37]	observational study	Dentin biomarkers of prenatal and early childhood exposure to manganese, zinc and lead are associated with variations in childhood behavior.
Mora et al. 2015[38]	research article	The prenatal and postnatal manganese levels in teeth are linked to neurodevelopmental performance in children aged 7–10.5 years.
Henn et al. 2018[39]	research article	Using dentin microspatial analyses, researchers identify critical windows of susceptibility to manganese exposure that affect neurodevelopment.
Rísová 2019[40]	review	The study identifies the pathway of lead transfer from the mother's body to the child, highlighting the risks associated with prenatal exposure.
Friedman et al. 2022[41]	research article	The study analyzes the presence of multiple metals in children's deciduous teeth, revealing significant environmental exposure.
Anttila and Anttila 1987[42]	cross-sectional study	The trace element content in the enamel of deciduous incisors varies between children from rural and urban Finnish areas, as evidenced by proton-induced X-ray emission analysis.
Anttila 1987[43]	cross-sectional study	The enamel of deciduous teeth from high-radon areas exhibits a significantly higher lead content in comparison to enamel from areas with lower radon levels.
Järvinen et al. 1984[44]	analytical study	The concentrations of lead in deciduous molar enamel in Finland vary, with elevated levels detected in certain regions, suggesting the potential for environmental or occupational exposure.
Fosse and Justesen 1978[45]	environmental study	The concentration of lead in the deciduous teeth of Norwegian children is elevated, indicating a significant environmental or occupational exposure to lead.
Arora et al. 2006[46]	research article	The study uses the spatial distribution of lead in human primary teeth as a biomarker for pre- and neonatal lead exposure.
Asaduzzaman et al. 2017[47]	analytical study	Dentin from human teeth can serve as a bioindicator for heavy metal exposure and environmental pollution.
Wychowanski and Malkiewicz 2017[48]	research article	The study evaluates the concentration of metal ions in the hard tissues of teeth among residents in central Poland, revealing patterns of environmental exposure.
Nowak 1995[49]	research article	The study evaluates the prevalence of heavy metals and essential elements, including sodium, potassium and calcium, in human teeth, demonstrating regional variations.
Nowak and Chmielnicka 2000[50]	research article	The study examines the relationship between lead and cadmium and essential elements in the hair, teeth and nails of individuals who have been exposed to environmental factors.
Fischer et al. 2004[51]	research article	The study analyzes the content of lead in the teeth of individuals residing in the Silesian region, revealing elevated levels of contamination.
Malara et al. 2016[52]	research article	The study analyzes the presence of selected toxic and essential heavy metals in impacted teeth and mandibular bones of individuals exposed to environmental heavy metals.
Fischer et al. 2008[53]	analytical study	The study analyzes the co-occurrence of chromium with selected elements in impacted wisdom teeth, revealing significant interrelationships and potential sources of exposure.
Rzepnicka et al. 2002[54]	observational study	Microintoxication with lead, copper and zinc causes disturbances in the number of teeth among the youth from Legnica, Poland.
Malara et al. 2005[55]	environmental exposure assessment study	Environmental exposure affects the coexistence of cadmium and zinc in teeth, reflecting the extent of environmental contamination.
Malara and Kwapuliński 2004[56]	environmental exposure assessment study	The presence of metals in teeth is influenced by environmental factors, which can be useful in assessing population exposure.

Study	Study design	Aim of the study/study results
Malara et al. 2004[57]	analytical study	Lead interferes with the occurrence of essential elements in teeth.
Fischer et al. 2014[58]	analytical study	The study evaluates the concentration of barium in deciduous teeth, impacted teeth and facial bones of Polish residents, identifying variations associated with environmental exposure.
Fischer et al. 2008[59]	analytical study	The study analyzes the content of selenium in retained wisdom teeth, demonstrating a range of values.
Piekut et al. 2018[60]	environmental exposure assessment study	Primary teeth can serve as indicators of the extent of environmental exposure of children to heavy metals.
Wiechuła et al. 2006[61]	analytical study	The study reveals significant variation in metal concentrations in the teeth of residents of the Silesian region, indicating environmental impact.
Fischer et al. 2009[62]	analytical study	The concentrations of metals in the maxilla and mandible of deciduous and permanent teeth vary significantly.
Malara et al. 2005[63]	occupational exposure assessment study	The occurrence of selected metals in the teeth of coal miners is contingent upon age and the duration of employment.
Kwapuliński et al. 2000[64]	occupational exposure assessment study	The prevalence of selected metals varies among different types of teeth in coal miners.
Poczatek et al. 2004[65]	occupational exposure assessment study	The analysis indicates an occupational risk for industrial employees based on the concentration of specific elements in their teeth and body fluids.
Johnston et al. 2019[66]	environmental exposure assessment study	The presence of lead and arsenic in the deciduous teeth of children living near a lead-acid battery smelter indicates a considerable degree of environmental exposure.
Savabieasfahani et al. 2016[67]	environmental exposure assessment study	Prenatal metal exposure in the Middle East, as evidenced by the presence of traces in the deciduous teeth of children, is likely attributable to war-related pollution.
Haavikko et al. 1984[68]	observational study	The study reveals that the concentration of lead in both the enamel and dentin of deciduous teeth varies among children from 2 Finnish towns, indicating regional differences in environmental lead exposure.
Shishniashvili et al. 2016[69]	environmental exposure assessment study	Primary teeth and hair are reliable indicators of environmental pollution.
Tsuji et al. 2001[70]	observational study	The elevated levels of lead in the dentin of deciduous teeth collected from remote First Nations communities in the western James Bay region of northern Ontario, Canada, indicate significant environmental lead exposure in these communities.
van Wyk et al. 1983[71]	cross-sectional study	The elevated levels of lead in the deciduous teeth of children from selected urban areas in the Cape Peninsula indicate a higher degree of environmental lead exposure in these regions.
Lyngbye et al. 1990[72]	epidemiological study	The levels of lead in children's teeth are significantly associated with traffic-related lead exposure.
Gomes et al. 2004[73]	in vivo study	The lead content in the superficial enamel of deciduous teeth in preschool children is measurable, indicating environmental exposure to lead.
Fernandes and França 2023[74]	review	Dental materials can contribute to nanometal and metal ion pollution in the dental environment, potentially impacting both patients and dental professionals.
Malara et al. 2004[75]	observational study	Cigarette smoking increases the presence of cadmium and lead in the hard tissues of the teeth.
Malara et al. 2006[76]	observational study	Passive smoking elevates the levels of selected metals in deciduous teeth.
Malara et al. 2004[77]	observational study	Lead and cadmium are present in the deciduous teeth of children exposed to cigarette smoke in their apartments.
Malara et al. 2005[78]	observational study	Cigarette smoking affects the coexistence of cadmium and zinc in retained wisdom teeth.
Alhasmi et al. 2015[79]	observational study	The concentration of toxic elements in the teeth of smokers is higher than in the teeth of non-smokers. These levels correlate with adverse periodontal parameters.
Olovčić et al. 2019[80]	cross-sectional study	The concentrations of metals in human enamel and dentin are influenced by sex, geographic location and smoking habits.
Fischer et al. 2009[81]	observational study	Passive smoking affects the levels of lead and chromium in deciduous teeth.
Fischer et al. 2011[82]	observational study	The study evaluates the impact of passive smoking on the metal content in deciduous teeth, showing a significant exposure risk.
Malara et al. 2004[83]	analytical study	Cigarette smoking affects the cationic equilibrium in men's teeth.

Limitations

It is important to consider a number of potential biases in this review. One potential source of bias is the use of language. The review included only articles written in English and Polish. Another potential source of bias is publication bias, which can occur when only studies with significant findings are published. It is important to note that this narrative review is limited to studies published between 1978 and 2023. Consequently, research published outside of the specified time frame may have been excluded.

Conclusions

The accumulation of toxic metals in the human body is an important issue due to its potential to result in a variety of adverse health outcomes. A visible sign of exposure to such substances is the development of oral complications. Human teeth can be negatively impacted by environmental chemicals, drugs, or physical agents during both embryonic development and after they emerge in the oral cavity. According to the presented publications, the concentration of heavy metals in human teeth is influenced by both environmental and non-environmental factors. The findings of these studies suggest that teeth can serve as a valuable tool for monitoring the presence of pollutants in the environment. It is crucial to emphasize the urgent need to minimize exposure to hazardous metals. The accumulation of toxic metals, such as Pb, Cd and Hg, can lead to structural damage to the teeth and gums. The presence of these metals can result in the weakening of tooth enamel, thereby increasing the susceptibility of the teeth to the development of cavities and mechanical damage. The presence of toxic metals in the body can disrupt the balance of the oral microbiota, fostering the growth of cavity-causing bacteria and increasing the risk of tooth decay. Toxins can affect tooth mineralization, resulting in weakened teeth that are more susceptible to erosion and fractures. In children, toxic metals can impede the normal development of the teeth, resulting in complications with tooth eruption and deformities. Additionally, heavy metals can affect inflammation and gum health, potentially leading to chronic periodontal diseases, such as gingivitis and periodontitis. Toxins present in the body can affect the effectiveness of dental treatments and interactions with medications used for oral health issues.[1–3] The presence of toxic metals in the body can lead to significant adverse consequences for overall health. Metals such as Pb and Hg are recognized for their detrimental effects on the nervous system. The exposure to Pb may result in cognitive impairments, developmental delays in children and various neurological disorders. On the other hand, the exposure to Hg can lead to tremors, memory loss and additional neurological complications. Heavy metals, including Cd and Hg, can accumulate in the kidneys, resulting in a gradual impairment of their functionality. This accumulation may cause kidney damage or disease, thereby compromising the body's capacity to filter waste and regulate fluid balance. Additionally, toxic metals can affect cardiovascular health. For example, Cd has been associated with hypertension and atherosclerosis, while Pb exposure has been shown to increase the risk of heart disease. Prolonged exposure to specific metals can weaken the immune system, increasing susceptibility to infections and illnesses. This decline in immune function can adversely affect the body's overall ability to combat diseases and sustain health. Certain toxic metals have the potential to interfere with endocrine function, impacting hormone equilibrium and resulting in complications such as thyroid dysfunction, reproductive issues and metabolic irregularities. Prolonged exposure to specific heavy metals has been associated with an increased risk of cancer development. Notably, As and Cd are recognized as carcinogens that play a role in the onset of different cancer types.[1–3,6] Reducing exposure to hazardous metals requires a multifaceted approach involving strict regulations, increased public awareness and enhanced monitoring systems. Governments and environmental agencies must implement and enforce rigorous guidelines to limit the release of toxic metals into the environment. Public education campaigns should raise awareness about the sources and risks of metal exposure, encouraging individuals to take preventive measures. Additionally, technological advances can improve the detection and analysis of metal concentrations in teeth, providing valuable data for environmental health studies. Furthermore, interdisciplinary collaboration among scientists, healthcare professionals and policymakers is crucial for the development of effective strategies to mitigate the impact of toxic metals on human health. By integrating findings from dental research with broader environmental health studies, we can gain a more comprehensive understanding of the impact of these metals on various aspects of health and well-being. This holistic approach will enable the formulation of targeted interventions to reduce metal exposure and the associated health risks.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Sadri Rayad https://orcid.org/0000-0003-3415-369X
Sylwia Klimas https://orcid.org/0009-0004-6861-9034
Maciej Janeczek https://orcid.org/0000-0003-4357-2271
Agata Małyszek https://orcid.org/0009-0007-5187-5324
Marta Bort https://orcid.org/0009-0002-7032-0379
Andrzej Małysa https://orcid.org/0000-0001-6328-9743
Marzena Dominiak https://orcid.org/0000-0001-8943-0549
Maciej Dobrzyński https://orcid.org/0000-0003-2368-1534

References

1. Rayad S, Dobrzyński M, Kuźniarski A, et al. An in-vitro evaluation of toxic metals concentration in the third molars from residents of the Legnica–Głogów Copper Area and risk factors determining the accumulation of those metals: A pilot study. *Appl Sci*. 2023;13(5):2904. doi:10.3390/app13052904
2. Rayad S, Dobrzyński M, Kuźniarski A, et al. Mercury content in impacted wisdom teeth from patients of the Legnica–Głogów Copper Area – an in vitro pilot study. *J Xenobiot*. 2023;13(3):463–478. doi:10.3390/jox13030029
3. Bryła E, Dobrzyński M, Konkol D, Kuropka P, Styczyńska M, Korczyński M. Toxic metals content in impacted third molars and adjacent bone tissue in different groups of patients. *Materials (Basel)*. 2021;14(4):793. doi:10.3390/ma14040793
4. Fukunaka A, Fujitani Y. Role of zinc homeostasis in the pathogenesis of diabetes and obesity. *Int J Mol Sci*. 2018;19(2):476. doi:10.3390/ijms19020476
5. Taniguchi M, Fukunaka A, Hagihara M, et al. Essential role of the zinc transporter ZIP9/SLC39A9 in regulating the activations of Akt and Erk in B-cell receptor signaling pathway in DT40 cells. *PLoS One*. 2013;8(3):e58022. doi:10.1371/journal.pone.0058022
6. Ciosek Ż, Kot K, Rotter I. Iron, zinc, copper, cadmium, mercury, and bone tissue. *Int J Environ Res Public Health*. 2023;20(3):2197. doi:10.3390/ijerph20032197
7. Brodziak-Dopierala B, Kwapuliński J, Paukszto A, Kowol J, Bogunia M, Ahnert B. Interactions of copper and iron with other elements in the osseous tissue of the femur head. *Fresenius Environ Bull*. 2009;18(10a):1963–1966. https://www.researchgate.net/publication/286570065_Interactions_of_copper_and_iron_with_other_elements_in_the_osseous_tissue_of_the_femur_head. Accessed September 11, 2024.
8. Kabata-Pendias A, Mukherjee AB. *Trace Elements from Soil to Human*. Berlin/Heidelberg, Germany: Springer; 2007. doi:10.1007/978-3-540-32714-1
9. Otten JJ, Hellwig JP, Meyers LD, eds. *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*. Washington, DC: National Academies Press; 2006:328–339.
10. World Health Organization. *Assessing the Iron Status of Populations*. 2nd ed. Geneva, Switzerland: WHO Press; 2007.
11. Wang X, Chen B, Sun J, et al. Iron-induced oxidative stress stimulates osteoclast differentiation via NF-κB signaling pathway in mouse model. *Metabolism*. 2018;83:167–176. doi:10.1016/j.metabol.2018.01.005
12. Angus RM, Sambrook PN, Pocock NA, Eisman JA. Dietary intake and bone mineral density. *Bone Miner*. 1988;4(3):265–277. PMID:3191284.
13. Nriagu JO. A global assessment of natural sources of atmospheric trace metals. *Nature*. 1989;338:47–49. doi:10.1038/338047a0
14. Collins JF, Prohaska JR, Knutson MD. Metabolic crossroads of iron and copper. *Nutr Rev*. 2010;68(3):133–147. doi:10.1111/j.1753-4887.2010.00271.x
15. Wang S, Shi X. Molecular mechanisms of metal toxicity and carcinogenesis. *Mol Cell Biochem*. 2001;222(1–2):3–9. PMID:11678608.
16. Dudek-Adamska D, Lech T, Konopka T, Kościelniak P. Chromium in postmortem material. *Biol Trace Elem Res*. 2018;186(2):370–378. doi:10.1007/s12011-018-1328-8
17. Pietrzak S, Wójcik J, Baszuk P, et al. Influence of the levels of arsenic, cadmium, mercury and lead on overall survival in lung cancer. *Biomolecules*. 2021;11(8):1160. doi:10.3390/biom11081160
18. Tvinnereim HM, Eide R, Riise T. Heavy metals in human primary teeth: Some factors influencing the metal concentrations. *Sci Total Environ*. 2000;255(1–3):21–27. doi:10.1016/s0048-9697(00)00436-8
19. Alomary A, Al-Momani IF, Obeidat SM, Massadeh AM. Levels of lead, cadmium, copper, iron, and zinc in deciduous teeth of children living in Irbid, Jordan by ICP-OES: Some factors affecting their concentrations. *Environ Monit Assess*. 2013;185(4):3283–3295. doi:10.1007/s10661-012-2790-y
20. Amadi CN, Offor SJ, Frazzoli C, Orisakwe OE. Natural antidotes and management of metal toxicity. *Environ Sci Pollut Res Int*. 2019;26(18):18032–18052. doi:10.1007/s11356-019-05104-2
21. Andersen O. Chemical and biological considerations in the treatment of metal intoxications by chelating agents. *Mini Rev Med Chem*. 2004;4(1):11–21. doi:10.2174/1389557043487583
22. Fischer A, Wiechuła D. Age-dependent changes in Pb concentration in human teeth. *Biol Trace Elem Res*. 2016;173(1):47–54. doi:10.1007/s12011-016-0643-1
23. Fischer A, Wiechuła D, Przybyła-Misztela C. Changes of concentrations of elements in deciduous teeth with age. *Biol Trace Elem Res*. 2013;154(3):427–432. doi:10.1007/s12011-013-9744-2
24. Malara P, Kwapuliński J, Drugacz J, Orzechowska-Wylęgała B. Factors influencing the presence of chromium in teeth. *J Elem*. 2005;10(4):951–957.
25. Fischer A, Wiechuła D. Is there a dependence between children's body weight and the concentration of metals in deciduous teeth? *Homo*. 2016;67(6):508–514. doi:10.1016/j.jchb.2016.09.002
26. Orzechowska-Wylęgała B, Obuchowicz A, Malara P, Fischer A, Kalita B. Cadmium and lead accumulate in the deciduous teeth of children with celiac disease or food allergies. *Int J Stomatol Occlusion Med*. 2011;4(1):28–31. doi:10.1007/s12548-011-0005-8
27. Yalçin SS, Dönmez Y, Aypar E, Yalçin S. Element profiles in blood and teeth samples of children with congenital heart diseases in comparison with healthy ones. *J Trace Elem Med Biol*. 2021;63:126662. doi:10.1016/j.jtemb.2020.126662
28. Sitarik AR, Arora M, Austin C, et al. Fetal and early postnatal lead exposure measured in teeth associates with infant gut microbiota. *Environ Int*. 2020;144:106062. doi:10.1016/j.envint.2020.106062
29. Abdullah MM, Ly AR, Goldberg WA, et al. Heavy metal in children's tooth enamel: Related to autism and disruptive behaviors? *J Autism Dev Disord*. 2012;42(6):929–936. doi:10.1007/s10803-011-1318-6
30. Dickerson AS, Rotem RS, Christian MA, Nguyen VT, Specht AJ. Potential sex differences relative to autism spectrum disorder and metals. *Curr Environ Health Rep*. 2017;4(4):405–414. doi:10.1007/s40572-017-0164-x
31. Adams JB, Romdalvik J, Ramanujam VMS, Legator MS. Mercury, lead, and zinc in baby teeth of children with autism versus controls. *J Toxicol Environ Health A*. 2007;70(12):1046–1051. doi:10.1080/15287390601172080
32. Figueroa-Romero C, Mikhail KA, Gennings C, et al. Early life metal dysregulation in amyotrophic lateral sclerosis. *Ann Clin Transl Neurol*. 2020;7(6):872–882. doi:10.1002/acn3.51006
33. Needleman HL, Schell A, Bellinger D, Leviton A, Allred EN. The long-term effects of exposure to low doses of lead in childhood. An 11-year follow-up report. *N Engl J Med*. 1990;322(2):83–88. doi:10.1056/NEJM199001113220203
34. Haavikko K, Anttila A, Helle A, Pesonen E. Atherosclerosis precursors in Finnish children and adolescents. XIV. Zinc and copper concentrations in deciduous teeth. *Acta Paediatr Scand Suppl*. 1985;318:213–219. doi:10.1111/j.1651-2227.1985.tb10096.x
35. Bauer JA, Henn BC, Austin C, et al. Manganese in teeth and neurobehavior: Sex-specific windows of susceptibility. *Environ Int*. 2017;108:299–308. doi:10.1016/j.envint.2017.08.013
36. Gunier RB, Arora M, Jerrett M, et al. Manganese in teeth and neurodevelopment in young Mexican-American children. *Environ Res*. 2015;142:688–695. doi:10.1016/j.envres.2015.09.003
37. Horton MK, Hsu L, Henn BC, et al. Dentine biomarkers of prenatal and early childhood exposure to manganese, zinc and lead and childhood behavior. *Environ Int*. 2018;121(Pt 1):148–158. doi:10.1016/j.envint.2018.08.045
38. Mora AM, Arora M, Harley KG, et al. Prenatal and postnatal manganese teeth levels and neurodevelopment at 7, 9, and 10.5 years in the CHAMACOS cohort. *Environ Int*. 2015;84:39–54. doi:10.1016/j.envint.2015.07.009
39. Henn BC, Austin C, Coull BA, et al. Uncovering neurodevelopmental windows of susceptibility to manganese exposure using dentine microspatial analyses. *Environ Res*. 2018;161:588–598. doi:10.1016/j.envres.2017.12.003

40. Rísová V. The pathway of lead through the mother's body to the child. *Interdiscip Toxicol*. 2019;12(1):1–6. doi:10.2478/intox-2019-0001
41. Friedman A, Bauer JA, Austin C, et al. Multiple metals in children's deciduous teeth: Results from a community-initiated pilot study. *J Expo Sci Environ Epidemiol*. 2022;32(3):408–417. doi:10.1038/s41370-021-00400-x
42. Anttila A, Anttila A. Trace-element content in the enamel surface and in whole enamel of deciduous incisors by proton-induced X-ray emission of children from rural and urban Finnish areas. *Arch Oral Biol*. 1987;32(10):713–717. doi:10.1016/0003-9969(87)90114-2
43. Anttila A. Lead content of deciduous tooth enamel from a high-radon area. *Acta Odontol Scand*. 1987;45(4):283–288. doi:10.3109/00016358709097540
44. Järvinen V, Anttila A, Lappalainen R, Rytömaa I. Lead content of deciduous molar enamel in Finland, as measured by proton-induced X-ray emission. *Scand J Work Environ Health*. 1984;10(2):103–107. doi:10.5271/sjweh.2360
45. Fosse G, Justesen NP. Lead in deciduous teeth of Norwegian children. *Arch Environ Health*. 1978;33(4):166–175. doi:10.1080/00039896.1978.10667329
46. Arora M, Kennedy BJ, Elhlou S, et al. Spatial distribution of lead in human primary teeth as a biomarker of pre- and neonatal lead exposure. *Sci Total Environ*. 2006;371(1–3):55–62. doi:10.1016/j.scitotenv.2006.07.035
47. Asaduzzaman K, Khandaker MU, Binti Baharudin NA, et al. Heavy metals in human teeth dentine: A bio-indicator of metals exposure and environmental pollution. *Chemosphere*. 2017;176:221–230. doi:10.1016/j.chemosphere.2017.02.114
48. Wychowanski P, Malkiewicz K. Evaluation of metal ion concentration in hard tissues of teeth in residents of Central Poland. *Biomed Res Int*. 2017;2017:6419709. doi:10.1155/2017/6419709
49. Nowak B. Occurrence of heavy metals and sodium, potassium and calcium in human teeth. *Analyst*. 1995;120(3):747–750. doi:10.1039/an9952000747
50. Nowak B, Chmielnicka J. Relationship of lead and cadmium to essential elements in hair, teeth, and nails of environmentally exposed people. *Ecotoxicol Environ Saf*. 2000;46(3):265–274. doi:10.1006/eesa.2000.1921
51. Fischer A, Wiechuła D, Kwapuliński J. The lead content of the teeth in inhabitants of the Silesian region. *Czas Stomatol*. 2004;57(3):177–182.
52. Malara P, Fischer A, Malara B. Selected toxic and essential heavy metals in impacted teeth and the surrounding mandibular bones of people exposed to heavy metals in the environment. *J Occup Med Toxicol*. 2016;11:56. doi:10.1186/s12995-016-0146-1
53. Fischer A, Wiechuła D, Malara P, et al. The study of co-occurrence of chromium with selected elements in impacted wisdom teeth. *Czas Stomatol*. 2008;61(1):48–54.
54. Rzepnicka M, Wicher K, Szymaniak M. Disturbances in the number of teeth in youth from Legnica, in the context of microintoxication with Pb, Cu, Zn. *Stomatol Współcz*. 2002;9(4):50–54.
55. Malara P, Kwapuliński J, Drugacz J, Malara B. Impact of environmental exposure on coexistence of cadmium and zinc in teeth. *J Elem*. 2005;10(4):959–965.
56. Malara P, Kwapuliński J. Environmental conditioning of metals occurrence in teeth. *Med Śr*. 2004;7(2):113–119.
57. Malara P, Kwapuliński J, Malara B. The influence of lead on occurrence of some essential elements in teeth. *Acta Toxicol*. 2004;12(1):47–53. https://www.researchgate.net/publication/286551127_The_influence_of_lead_on_occurrence_of_some_essential_elements_in_teeth,%20access. Accessed September 10, 2024.
58. Fischer A, Malara P, Wiechuła D. The study of barium concentration in deciduous teeth, impacted teeth, and facial bones of Polish residents. *Biol Trace Elem Res*. 2014;161(1):32–37. doi:10.1007/s12011-014-0061-1
59. Fischer A, Wiechuła D, Kwapuliński J, Malara P, Łazarczyk-Henke J. Content of selenium in retained wisdom teeth [in Polish]. *Bromatol Chem Toksykol*. 2008;41(4):981–986. https://ptfarm.pl/pub/File/wydawnictwa/bromatologia/4_08/Bromat%204%20s.%200981-0986.pdf. Accessed September 10, 2024.
60. Piekut A, Moskalenko O, Gut K. Can primary teeth be an indicator of the environmental exposure of children to heavy metals? [in Polish] *Med Srod*. 2018;21(4):18–23. https://www.environmed.pl/pdf-113108-42887?filename=Can%20primary%20teeth%20be%20an.pdf. Accessed September 10, 2024.
61. Wiechuła D, Fischer A, Kwapuliński J, Loska K, Fischer T, Kurpas P. Multivariate statistical analysis of metal concentrations in teeth of residents of Silesian region, southern Poland. *Arch Environ Contam Toxicol*. 2006;51(2):314–320. doi:10.1007/s00244-004-0202-1
62. Fischer A, Wiechuła D, Postek-Stefańska L, Kwapuliński J. Concentrations of metals in maxilla and mandible deciduous and permanent human teeth. *Biol Trace Elem Res*. 2009;132(1–3):19–26. doi:10.1007/s12011-009-8383-0
63. Malara P, Łangowska-Adamczyk H, Kwapuliński J, Kyrcz-Florek Z. Occurrence of selected metals in teeth of coal-miners in the function of age and years of employment [in Polish]. *Stomatol Współcz*. 2005;12(5):25–32.
64. Kwapuliński J, Malara B, Malara P. Occurrence of selected metals in various teeth of coal miners. *Med Śr*. 2000;3(2):55–64.
65. Poczatek M, Machoy Z, Gutowska I, Chlubek D. A comparative analysis of occupational risk in industry employees based on concentrations of some elements in teeth and body fluids [in Polish]. *Ann Acad Med Stetin*. 2004;50(1):107–114. https://eurekamag.com/research/012/371/012371878.php?srsltid=AfmBOortr6G8T4l_q_Pba-1Tr1SJaTGe3FsuBEjq573FXpbbtRrMMEW7e. Accessed September 10, 2024.
66. Johnston JE, Franklin M, Roh H, Austin C, Arora M. Lead and arsenic in shed deciduous teeth of children living near a lead-acid battery smelter. *Environ Sci Technol*. 2019;53(10):6000–6006. doi:10.1021/acs.est.9b00429
67. Savabieasfahani M, Ali SS, Bacho R, Savabi O, Alsabbak M. Prenatal metal exposure in the Middle East: Imprint of war in deciduous teeth of children. *Environ Monit Assess*. 2016;188(9):505. doi:10.1007/s10661-016-5491-0
68. Haavikko K, Anttila A, Helle A, Vuori E. Lead concentrations of enamel and dentine of deciduous teeth of children from two Finnish towns. *Arch Environ Health*. 1984;39(2):78–84. doi:10.1080/00039896.1984.10545839
69. Shishniashvili TE, Suladze NN, Margvelashvili VV. Primary teeth and hair as indicators of environmental pollution. *J Clin Pediatr Dent*. 2016;40(2):152–155. doi:10.17796/1053-4628-40.2.152
70. Tsuji LJ, Karagatzides JD, Katapatuk B, et al. Elevated dentine-lead levels in deciduous teeth collected from remote first nation communities located in the western James Bay region of northern Ontario, Canada. *J Environ Monit*. 2001;3(6):702–705. doi:10.1039/b106163c
71. van Wyk CW, Grobler SR. Lead levels in deciduous teeth of children from selected urban areas in the Cape Peninsula. *S Afr Med J*. 1983;63(15):559–562. PMID:6845044.
72. Lyngbye T, Hansen ON, Grandjean P. Predictors of tooth-lead level with special reference to traffic. A study of lead-exposure in children. *Int Arch Occup Environ Health*. 1990;62(6):417–422. doi:10.1007/BF00379057
73. Gomes VE, da Luz Rosário de Sousa M, Barbosa F Jr, et al. In vivo studies on lead content of deciduous teeth superficial enamel of preschool children. *Sci Total Environ*. 2004;320(1):25–35. doi:10.1016/j.scitotenv.2003.08.013
74. Fernandes ACBCJ, França R. Nanometals and metal ion pollution from dental materials in dental environment. *Micro*. 2023;3(2):471–483. doi:10.3390/micro3020031
75. Malara P, Łangowska-Adamczyk H, Kwapuliński J, Malara B. The effect of cigarette smoking on the presence of cadmium and lead in the hard tissues of the teeth [in Polish]. *Czas Stomatol*. 2004;57(2):118–124.
76. Malara P, Kwapuliński J, Malara B, Drugacz J. Effect of passive smoking on the level of selected metals in deciduous teeth [in Polish]. *Przegl Lek*. 2006;63(10):1002–1006. PMID:17288202.
77. Malara P, Kwapuliński J, Drugacz J. Lead and cadmium occurrence in deciduous teeth of children exposed to cigarette smoke in apartments [in Poland]. *Przegl Lek*. 2004;61(10):1122–1125. PMID:15794269.
78. Malara P, Kwapuliński J, Drugacz J, Malara B. Effect of cigarette smoking on coexistence of cadmium and zinc in retained wisdom teeth [in Polish]. *Przegl Lek*. 2005;62(10):1072–1074. PMID:16521956.
79. Alhasmi AM, Gondal MA, Nasr MM, Shafik S, Habibullah YB. Detection of toxic elements using laser-induced breakdown spectroscopy in smokers' and nonsmokers' teeth and investigation of periodontal parameters. *Appl Opt*. 2015;54(24):7342–7349. doi:10.1364/AO.54.007342

80. Olovčić A, Ramić E, Memić M. Human enamel and dentin: Effect of gender, geographic location, and smoking upon metal concentrations. *Anal Lett*. 2019;53(2):245–261. doi:10.1080/00032719.2019.1646753
81. Fischer A, Kwapuliński J, Nogaj E, Ahnert B, Rzepka J. The important mean of passive smoking for lead and chromium content in deciduous teeth [in Polish]. *Przegl Lek*. 2009;66(10):669–672. PMID:20301907.
82. Fischer A, Wiechuła D, Kwapuliński J. Evaluation of the passive smoking effect on metal content in deciduous teeth. *J Stomatol*. 2011;64(8):620–630.
83. Malara P, Kwapuliński J, Malara B. The influence of smoking on cationic equilibrium in men's teeth. *J Elem*. 2004;9(3):413–419.

applied sciences

MDPI

Article

An In-Vitro Evaluation of Toxic Metals Concentration in the Third Molars from Residents of the Legnica-Głogów Copper Area and Risk Factors Determining the Accumulation of Those Metals: A Pilot Study

Sadri Rayad [1,*], Maciej Dobrzyński [2,*], Amadeusz Kuźniarski [3], Marzena Styczyńska [4], Dorota Diakowska [5], Tomasz Gedrange [6], Sylwia Klimas [2], Tomasz Gębarowski [7] and Marzena Dominiak [6]

1 Academic Dental Polyclinic of Dental Center of Technology Transfer Ltd., Krakowska 26, 50-425 Wroclaw, Poland
2 Department of Pediatric Dentistry and Preclinical Dentistry, Wroclaw Medical University, Krakowska 26, 50-425 Wroclaw, Poland
3 Department of Prosthetic Dentistry, Wroclaw Medical University, Krakowska 26, 50-425 Wroclaw, Poland
4 Department of Human Nutrition, Wroclaw University of Environmental and Life Sciences, Chelmonskiego 37/41, 51-630 Wroclaw, Poland
5 Department of Basic Sciences, Wroclaw Medical University, Chalubinskiego 3, 50-368 Wroclaw, Poland
6 Department of Dental Surgery, Wroclaw Medical University, Krakowska 26, 50-425 Wroclaw, Poland
7 Department of Biostructure and Animal Physiology, Wrocław University of Environmental and Life Sciences, Kożuchowska 1/3, 51-631 Wroclaw, Poland
* Correspondence: sadri.rayad@gmail.com (S.R.); maciej.dobrzynski@umw.edu.pl (M.D.); Tel.: +48-71-7186-292 (S.R.); +48-71-7840-378 (M.D.)

Abstract: The purpose of this study was to determine tissue concentration of toxic metals, namely Mn, Cr, Ni, Cu, Fe, Cd, Pb, and Zn, in the removed third molars in patients from the Legnica-Głogów copper district. A group of 69 patients with an average age of 27.3 ± 6.9 years was enrolled into the study. There were 16 (23.2%) men and 53 (76.8%) women. Patients were divided into two groups according to the place of residence—residents of the Legnica-Głogów Copper Area (n = 49) and the control group, residents of Wrocław ($n = 20$). Determination of the Pb, Cd, Cr, Ni, Fe, Mn, Cu, Zn content was performed by atomic absorption spectrometry in an air–acetylene flame using the SpectraAA atomic absorption spectrometer with a V2 AA240FS flame attachment. The content of Fe and Pb in the third molars was higher among residents of the Legnica-Głogów Copper Area ($p = 0.016$ and $p = 0.002$, respectively). The significant risk factors that may contribute to the accumulation of toxic metals in teeth were identified. We showed a significant correlation between the level of Cr, Cu, and Zn and age, and between chromium and vitamin D3 concentration in the blood ($p < 0.05$ for all).

Keywords: toxic metals; third molars; vitamin D3; biomonitoring; elements

Citation: Rayad, S.; Dobrzyński, M.; Kuźniarski, A.; Styczyńska, M.; Diakowska, D.; Gedrange, T.; Klimas, S.; Gębarowski, T.; Dominiak, M. An In-Vitro Evaluation of Toxic Metals Concentration in the Third Molars from Residents of the Legnica-Głogów Copper Area and Risk Factors Determining the Accumulation of Those Metals: A Pilot Study. *Appl. Sci.* **2023**, *13*, 2904. https://doi.org/10.3390/app13052904

Academic Editors: Luca Sbricoli, Riccardo Guazzo and Eriberto A. Bressan

Received: 27 January 2023
Revised: 19 February 2023
Accepted: 21 February 2023
Published: 24 February 2023

1. Introduction

Biomonitoring, as part of environmental dentistry, is defined as a set of activities to assess the state of the environment using biomonitors. The study of the toxic metal content forms the basis of environmental policy making, which affects the health of the human population living in a specific area. Biomonitors of toxic metal exposure in humans are so-called non-invasive matrices, which include teeth, saliva, hair, nails, or urine [1,2]. Because of the ease of obtaining biological material in the form of deciduous teeth, there are many studies and research available that assess the toxic metal content of their structure [3–6]. Teeth are a permanent record of an individual's lifestyle in a specific environment, reflecting the impact of environmental contaminants [7,8]. In the process of teeth mineralization, toxic metal cations are incorporated into the xystalline structure of hydroxyapatites. They enter the tooth tissues via the bloodstream.

Vitamin D3 is an offshoot of cholesterol and constitutes a part of secosteroid compounds. Humans may obtain vitamin D3 from a wholesome diet; however, the greatest part is produced by organisms (~90%). In addition, vitamin D may reduce bone absorption by increasing gut Ca absorption and its reabsorption in the distal renal tubule. It can also affect the secretion of calcitonin (acceleration) and it reduces the proliferation of parathyroid cells, and then inhibits the synthesis and activity of PTH [9]. Vitamin D3 stimulates the co-absorption of toxic metals [10], which disrupt calcium–phosphate metabolism (Figure 1).

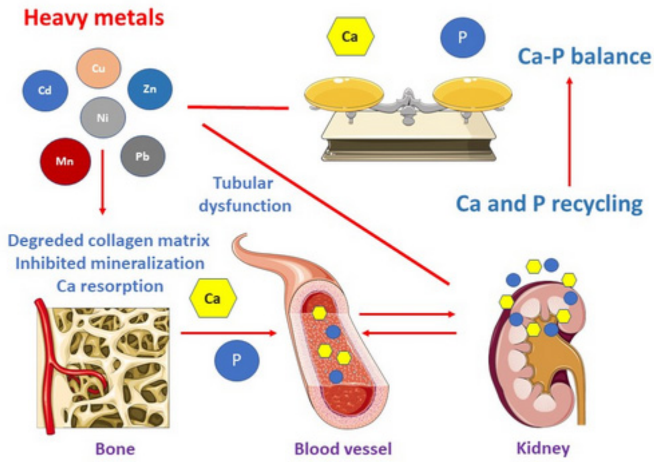

Figure 1. The effects of toxic metals.

On the other hand, it plays a key role in the development and mineralization of tooth germs and also participates in the formation and maturation of enamel and dentine [11].

Extracting teeth for biomonitoring purposes is ethically unacceptable. However, the relatively high frequency of extraction procedures of completely retained third molars due to orthodontic or surgical reasons creates opportunities for their use for this purpose [12].

The Legnica-Głogów Copper District is an urban–industrial area and a copper ore basin in the northern part of the lower Silesia Province (Poland), with an area of approximately 2200 km^2, inhabited by about half a million inhabitants. It is one of the largest in the world, and its development dates back to the 1960s, when deep copper deposits were first discovered there. Currently, the copper ore mining sector comprises three mines—"Lubin", "Rudna", and "Polkowice-Sieroszowice", supported by the "Ore Enrichment Plant" and the "Hydrotechnical Plant". In turn, the processing sector includes three steelworks—"Legnica", "Głogów", and "Cedynia". Copper ore tailings are stored in a huge engineering facility "Żelazny Most", covering an area of approximately 1400 ha [13].

The exploitation of deposits is associated with the emission of sulfur, nitrogen, and carbon compounds into the environment, as well as dust and waste containing toxic metals [14]. The Legnica-Głogów Copper Area in Poland is a district where the risk of heavy metal contamination is increased due to the industrial profile and structure.

There are many sources of toxic metals: polluted soil and atmosphere, contaminated food or drinking water, and cigarettes (Figure 2). Heavy metals may cause various diseases including the stomatognathic system disorders [15,16]. To the best of our knowledge, our study is the first to assess the accumulation of toxic metals: Pb, Cd, Cr, Ni, Fe, Mn, Cu, and Zn in third molars from residents of the Legnica-Głogów Copper Area. The relationship between toxic metals and demographics, clinical data, diet, vitamin supplementation, and the concentration of vitamin D3 in the blood of patients was studied.

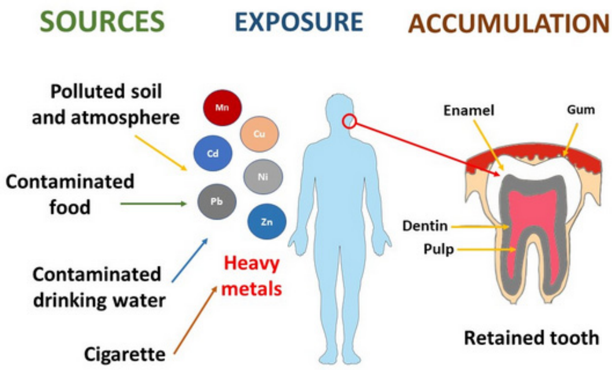

Figure 2. The sources of toxic metals.

2. Materials and Methods

2.1. Material

The study was conducted in full compliance with the Declaration of Helsinki, in a group of 69 patients in whom the third molars were extracted as the main study material after giving informed consent for the study and medical procedure. In addition to the extracted teeth, blood was drawn from the patients and responses were elicited in a proprietary questionnaire.

The authors obtained the consent of the Bioethics Committee of the Wroclaw Medical University (consent number: KB-246/2019) prior to the study.

Patients were divided into two subgroups—the research group of 49 patients who were residents of the Legnica-Głogów Copper Area, and the control group of 20 patients who were residents of the city of Wrocław. The research material was collected in the period from June 2020 to June 2021, during the COVID-19 epidemic; therefore, the number of patients in the control group, which was matched according age and gender to the study group, was small.

The authors collected lower completely retained molars from the patients of both groups, namely the control and study. Data on gender, age, permanent residency, and absence of dietary supplements were gathered as part of the study. The most important criterion for inclusion and exclusion was that the surveyed patients had lived since birth in the Legnica-Głogów Copper Area or in Wrocław. In addition, patients needed to be healthy and not take any dietary supplements or medications.

The sample consisted of the whole extracted tooth. To avoid chemical treatment of the material prior to analytical testing, the material was stored at −20 °C in sterile boxes and then evaluated for the content of Mn, Cr, Ni, Cu, Fe, Cd, Pb, and Zn.

2.2. Determination of Toxic Metals in the Study Material

Atomic absorption spectrometry was used for the multi-element analysis of the dental material. The information collected by means of the author's questionnaire included: gender, age, place of residence, occupational exposure, smoking habits, incidence of allergies, general diseases, and nutritional supplementation used.

The content of selected minerals and toxic trace elements in the bone tissue was determined at the certified AAS laboratory of the Department of Human Nutrition, Wrocław University of Environmental and Life Sciences.

The data obtained from the author's questionnaires were cross-referenced with the specific toxic metal content of the individual tooth samples, the vitamin D concentration in capillary blood, and the results of the clinical assessment of the post-extraction wound healing, and were then statistically analyzed.

2.3. Mineralization of Examined Material

Sample mineralization was performed wet in a closed-loop microwave system. First, 5 cm^3 of concentrated nitric acid (V) ACS and 1 cm^3 of concentrated hydrogen peroxide

ACS were added to the sample weight of homogeneous samples (from 0.1 g to 0.5 g). After that, the samples were mineralized in a MARS 5 microwave sample preparation system. According to the Polish Standard PN-EN 13805: 2003 Food products, for the determination of trace elements, using pressure mineralization [17], we quantitatively transferred the minerals to a 10 cm^3 measuring container filled with distilled water.

2.4. Examination of Toxic Metals in the Samples

The concentration of Pb, Cd, Cr, Ni, Fe, Mn, Cu, and Zn was accomplished with a use of atomic absorption spectrometry in an air–acetylene flame using the SpectraAA atomic absorption spectrometer with a V2 AA240FS flame attachment under a special hollow cathode lamp (Table 1). The accuracy of the method was confirmed using certified reference material ERM-BD151 skim milk powder (Sigma-Aldrich, Saint Louis, MO, USA), with an estimated uncertainty of 5% [8]. The content of toxic metals was established in accordance with the following criteria:

- Determination of trace elements—Determination of lead, cadmium, zinc, copper, iron, and chromium by atomic absorption spectrometry (AAS) after dry mineralization [18]—Pb, Cd, Zn, Cu, Fe, and Cr—PN-EN 14082: 2004 Food products.
- Determination of heavy metal content by atomic emission spectrometry—Determination of nickel content [19]—Ni—PN-A-86939-6: 1998 Vegetable and animal oils and fats.
- Determination of calcium, copper, iron, magnesium, manganese, potassium, sodium, and zinc content—Atomic absorption spectrometry method [20]—Mn—PN-EN ISO 6869: 2002 Feed

Table 1. Measurement parameters applied according to the authors' previous study [8].

Determined Element	Wavelength (nm)	Gap (nm)	Air-Acetylene Flow (L/min)	Background Correlation	Characteristic Concentration c (mg/L)	r
Ni	341.5	0.2	13.50/2.00	on	0.005	0.9997
Fe	372.0	0.2	13.50/2.00	on	0.011	1.0000
Cu	327.4	0.2	13.50/2.00	on	0.015	0.9999
Mn	279.5	0.2	13.50/2.00	on	0.009	0.9996
Cd	228.8	0.5	13.50/2.00	on	0.005	0.9998
Pb	217.0	1.0	13.50/2.00	on	0.005	0.9997
Zn	213.9	1.0	13.50/2.00	on	0.012	1.0000

2.5. Methodology of Blood Collection and Determination of Vitamin D3

Vitamin D levels were measured from a capillary blood sample using the capillary method in the Vitality Health Check (VHC) Vitamind-D TEST system.

After thoroughly washing, disinfecting, and drying the blood collection site and obtaining the blood circulation, a sterile disposable lancet was used to puncture the fingertip, after which 10 µL of blood was collected using heparinized capillary tubes (BloodCollector—UniSampler device).

The blood was then placed in the CollectionTube, which had previously been used to obtain the buffer. After thorough mixing, the sample was placed using a micropipette on the Test Divice. After 15 min, the vitamin D value was read using the VHC Reader.

2.6. Statistical Analysis

Analysis of data was performed using Statistica 13.3 software (Tibco Software Inc., Palo Alto, CA, USA). Testing of the data distribution was performed with the Shapiro–Wilk normality test. Descriptive data were shown as number of observation (percent) or as median (quartile 1–quartile 3, Q1–Q3) and 95% CI (95% confidence interval). Qualitative data were analyzed using the chi-square test or Fisher exact test. As the distribution of the quantitative variable in one of the compared groups was significantly different from the normal distribution in all cases, non-parametric tests were used to analyze the data. The Mann–Whitney test was used to compare two independent study groups, and Kruskal–Wallis analysis was performed for the comparison of more than two groups. Dunn's test was used as a post hoc test for intragroup comparisons. The Spearman test and correlation coefficient (rho) were calculated to evaluate the associations between pairs of variables. Values $p < 0.05$ were assumed as statistically significant, and $0.05 < p < 0.1$ signified a tendency to statistical significance.

3. Results

3.1. Basic Characteristic of Patients

As demonstrated in Table 2, the largest proportion of study group were young non-smokers in the 16–26 age range, generally healthy people with a female predominance. There were insignificant differences in gender distribution, age, smoking, occupation, diseases, dietary supplementation, and vitamin D3 level between study and control group ($p > 0.05$ for all parameters). The reason for performing dental extractions for surgical reasons proved to be significantly statistical ($p = 0.020$).

Table 2. Demographical, clinical, and laboratory characteristic of total study group and two subgroups of patients, divided according to place of residence. Descriptive data were presented as number of observation (percent) or median (Q1–Q3) and [95% CI]. Chi-square test, Fisher exact test, or Mann–Whitney test were used for the analysis of differences between two subgroups of patients.

Variable	Total (n = 69)	LG Copper Area—No (n = 20)	LG Copper Area—Yes (n = 49)	p-Value
Gender:				
male	16 (23.2)	4 (20.0)	12 (24.5)	0.763
female	53 (76.8)	16 (80.0)	37 (75.5)	
Age (years old)	27.0 (22.0–31.0) [25.6–30.9]	25.0 (24.0–27.5) [23.7–27.6]	29.0 (22.0–32.0) [25.8–32.0]	0.305
Age (years old):				
16–26	34 (49.3)	12 (60.0)	22 (44.9)	0.092
27–37	29 (42.0)	8 (40.0)	21 (42.9)	
38–45	6 (8.7)	0 (0.0)	6 (12.2)	
Smoking				
no	61 (88.4)	17 (85.0)	44 (89.8)	0.682
yes	8 (11.6)	3 (15.0)	5 (10.2)	
Occupation:				
student	20 (28.9)	5 (25.0)	15 (30.6)	0.245
worker	10 (14.5)	1 (5.0)	9 (18.4)	
white-collar worker	39 (56.5)	14 (70.0)	25 (51.0)	
Years of residence in the LG copper district (n = 49):				
≤20 years old	17 (34.7)	-	17 (34.7)	-
21–30 years old	19 (38.8)		19 (38.8)	
≥31 years old	13 (26.5)		13 (26.5)	
Thyroid and parathyroid glands diseases:				1.000
no	62 (89.9)	18 (90.0)	44 (89.8)	
yes	7 (10.1)	2 (10.0)	5 (10.2)	
Cardiac diseases:				
no	67 (97.1)	19 (95.0)	48 (98.0)	0.498
yes	2 (2.9)	1 (5.0)	1 (2.0)	

Table 2. *Cont.*

Variable	Total (n = 69)	LG Copper Area—No (n = 20)	LG Copper Area—Yes (n = 49)	p-Value
Dietary supplements:				
no	46 (66.7)	12 (60.0)	34 (69.4)	0.453
yes	23 (33.3)	8 (40.0)	15 (30.6)	
Reason of extraction:				
surgical	54 (78.3)	20 (100.0)	34 (69.4)	0.020 *
orthodontic	8 (11.6)	0 (0.0)	8 (16.3)	
inflammation	7 (10.1)	0 (0.0)	7 (14.3)	
Vit. D3 (ng/mL)	27.0 (19.8–29.5) [22.5–32.1]	27.8 (25.5–32.2) [24.9–37.6]	26.4 (18.5–28.4) [20.9–31.8]	0.135
Vit. D3 (ng/mL):				
<20—big deficiency	18 (26.1)	3 (15.0)	15 (30.6)	0.173
20–29—deficiency	34 (49.3)	9 (45.0)	25 (51.0)	
30–50—norm	11 (15.9)	6 (30.0)	5 (10.2)	
51–100—above the norm	6 (8.7)	2 (10.0)	4 (8.2)	

*: statistically significant.

3.2. The Content of Toxic Metals in the Removed Teeth in Patients from the LG Copper Area and in Control Group

As shown in Table 2, the concentrations of Fe and Pb were significantly higher in the teeth removed from patients from the LG Copper Area than in patients from the control group ($p = 0.016$ and $p = 0.002$, respectively).

3.3. Risk Factors Determining the Accumulation of Toxic Metals in the Teeth of Patients Residing in the LG District

The significant risk factors determining the accumulation of toxic metals, such as Mn, Cr, Ni, and Cu, in the teeth of patients residing in the LG Cooper Area are presented in Table 3.

Table 3. Tissue concentrations of toxic metals in the removed teeth in the total study group and two subgroups of patients, divided according to place of residence. Descriptive data are presented as median (Q1–Q3) and [95% CI]. Mann–Whitney test was used for analysis of differences between two subgroups of patients.

Variable	Total (n = 69)	LG Copper Area —No (n = 20)	LG Copper Area —Yes (n = 49)	p-Value
Mn (µg/g)	0.19 (0.13–0.28) [0.19–0.28]	0.17 (0.14–0.25) [0.14–0.34]	0.22 (0.13–0.30) [0.19–0.28]	0.796
Cr (µg/g)	0.0 (0.00–0.00) [0.03–0.16]	0.00 (0.00–0.00) [0.00–0.29]	0.00 (0.00–0.00) [0.03–0.14]	0.667
Ni (µg/g)	0.0 (0.00–0.00) [0.00–0.32]	0.0 (0.00–0.00) [0.00–0.13]	0.00 (0.00–0.04) [0.00–0.43]	0.183
Cu (µg/g)	0.12 (0.05–0.24) [0.09–0.25]	0.08 (0.01–0.15) [0.01–0.17]	0.13 (0.06–0.28) [0.10–0.26]	0.103
Fe (µg/g)	3.59 (1.82–5.88) [3.56–6.14]	1.81 (1.23–4.79) [1.23–6.03]	3.86 (2.51–6.23) [3.82–6.28]	0.016 *
Cd (µg/g)	0.06 (0.05–0.09) [0.05–0.09]	0.06 (0.05–0.08) [0.05–0.08]	0.07 (0.05–0.09) [0.05–0.09]	0.117
Pb (µg/g)	0.47 (0.30–0.66) [0.34–0.68]	0.31 (0.22–0.48) [0.25–0.48]	0.53 (0.37–0.75) [0.39–0.76]	0.002 *
Zn (µg/g)	100.36 (90.24–150.69) [93.29–141.26]	99.70 (81.40–114.95) [88.63–118.51]	103.80 (90.31–155.17) [94.06–151.67]	0.311

*: statistically significant.

The manganese content significantly depended on the occupation of the patients (p = 0.043). In the group of students, the median was 0.14 µg/g, while among the white-collar workers, the median was 0.25 µg/g—both of the results were statistically significant in the pair of subgroups (post hoc test).

The high manganese content was determined as statistically significant in relation to increase of vitamin D3 concentration in capillary blood (p = 0.012). In the group of patients with 20–29 ng/mL of vitamin D3 concentration, the median of manganese content in the third molars was 0.13 µg/g, in the group of patients with 30–50 ng/mL of vitamin D3 concentration the median of manganese content in the third molars was 0.28 µg/g—both results were classified as statistically significant in the pair of subgroups (post hoc test).

In the case of chromium and nickel concentration in the removed third molars, the occupation showed a tendency to statistical significance (p = 0.085 and p = 0.072, respectively).

A statistically significant relationship between vitamin D3 concentration in the capillary blood and chromium content was shown (p = 0.034).

The content of copper in the examined samples increased with the age of patients—values showed a tendency to statistical significance (p = 0.061).

3.4. Content of Manganese

No statistically significant effect of patients' sex on the tooth manganese content was observed (p-value = 0.633).

In the 27–37 age group, the manganese content in the tooth was higher than in the other age groups; however, the differences were not considered to be statistically significant (p-value = 0.328).

No statistically significant difference was observed between smoking and non-smoking patients (p-value = 0.436).

The manganese content in the tested samples significantly depended on the occupation of the patients (p-value = 0.043). In the group of students, the median was 0.14 (µg/g), and among the white-collar workers, the median was 0.25 (µg/g)—both of the results were statistically significant in the pairs of subgroups (post hoc test).

In the group of patients that lived for 21–30 years in the L-G Copper Area, the median content of the manganese was the highest (0.26 (µg/g)); however, it was not determined as statistically significant.

Among the patients with thyroid and parathyroid gland diseases, a slightly higher concentration of manganese was observed (0.28 (µg/g), p-value = 0.381).

Dietary supplements and the reason for extraction were also considered as not statistically significant (p-value = 0.182, p-value = 0.563).

The vitamin D3 concentration in the capillary blood was determined as statistically significant (p-value = 0.012). In the group of patients with 20–29 (ng/mL) vitamin D3 concentration, the median manganese content in the third molars was 0.13 (µg/g), in the group of patients with 30–50 (ng/mL) vitamin D3 concentration, the median of manganese content in the third molars was 0.28 (µg/g)—both of the results were classified as statistically significant in the pairs of subgroups (post hoc test).

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results and their interpretation, as well as the experimental conclusions that can be drawn.

3.5. Content of Chromium and Nickel

In the case of chromium and nickel concentration in the removed third molars, the occupation showed a tendency to statistical significance (p-value = 0.085, p-value = 0.072).

The vitamin D3 concentration (ng/mL) in the capillary blood and chromium content in the samples were classified as statistically significant (p-value = 0.034).

3.6. Content of Copper

No statistically significant effect for patients' sex on the tooth copper content was observed (p-value = 0.463).

The content of copper in the examined samples increased with the age of the patients—the values showed a tendency to statistical significance (p-value = 0.061).

Smoking was not classified as statistically significant (p-value = 0.778).

In the group of white-collar workers, the level of copper was slightly higher than among the other occupations (0.15 (μg/g)); however, it was not considered to be statistically significant (p-value = 0.569).

The highest value of copper was noticed in the patients who have lived in the L-G copper district for over 31 years (0.22 (μg/g)—p-value = 0.389).

In Table 4B, we demonstrate associations between content of Fe, Cd, Pb, Zn and risk factors which have an influence on accumulation of these toxic metals.

No statistically significant effect for all of the tested parameters on the content of iron in the removed teeth was observed ($p > 0.05$ for all).

A statistically significant effect of patient sex on the cadmium content was observed ($p = 0.037$), in the female group the cadmium concentration was 0.08 μg/g and in the male group it was 0.05 μg/g.

The concentration of lead in the removed teeth significantly increased with the age of patients ($p = 0.032$). In the 38–45 age group, the lead content was significantly higher than in the 16–26 age group (post hoc test).

The content of zinc in the examined samples increased with the age of the patients, the results showed a tendency to statistical significance ($p = 0.088$). The occupation of the patients showed a tendency to statistical significance ($p = 0.100$). The highest concentration of zinc was detected in the group of workers (132.22 μg/g).

The content of zinc in the studied teeth increased with the years of residence in the L-G copper district, and the results showed a tendency to statistical significance ($p = 0.088$).

Significant differences in zinc content depending on vitamin D3 concentration ranges were observed ($p = 0.008$). The greatest value for zinc (158.62 μg/g) was noted in a group of patients with <20 ng/mL vitamin D3 in the capillary blood.

3.7. Content of Iron

No statistically significant effect of patients' sex on the content of iron in removed teeth was observed (p-value = 0.311); however, in the male group, the iron content was 1.81 times higher than in the female group.

The content of iron in the third molars increased with the age of the patients (p-value = 0.497).

The iron content did not depend on the smoking habits (p-value = 0.888).

The highest value of Fe concentration in the samples was found in the worker group (4.90 (μg/g), p-value = 0.504).

The iron content did not depend on the years of residence in the L-G Copper Area district (p-value = 0.956).

The concentration of iron in the samples from patients with thyroid and parathyroid gland diseases was comparable to the samples from patients without these diseases (p-value = 0.346).

Dietary supplements did not affect the iron concentration in the samples (p-value = 0.580).

The highest concentration of iron was observed in the teeth removed for surgical reason and it was 1.85 times higher than for those from orthodontic and inflammation reasons, but it was not statistically significant (p-value = 0.152).

The highest content of iron was noticed in patients with the concentration of vitamin D3 of 20–29 (ng/mL), but it was not statistically significant (p-value = 0.693).

Table 4. A. Risk factors determining the accumulation of Mn, Cr, Ni, Cu in the teeth of patients residing in the LG Copper Area (*n* = 49). **B.** Risk factors determining the accumulation of Fe, Cd, Pb, Zn in the teeth of patients residing in the LG district (*n* = 49). Descriptive data are presented as median (Q1–Q3). Mann–Whitney test or Kruskal–Wallis analyses are used for analysis of differences between two or more than two categories of patients.

(A)

Variable	Mn (µg/g)	*p*-Value	Cr (µg/g)	*p*-Value	Ni (µg/g)	*p*-Value	Cu (µg/g)	*p*-Value
Gender:								
male (n = 12)	0.21 (0.15–0.29)	0.633	0.00 (0.00–0.00)	0.485	0.00 (0.00–0.00)	0.192	0.15 (0.07–0.32)	0.463
female (n = 37)	0.22 (0.12–0.30)		0.00 (0.00–0.00)		0.00 (0.00–0.10)		0.12 (0.06–0.24)	
Age (years old):								
16–26 (n = 22)	0.15 (0.12–0.23)	0.328	0.00 (0.00–0.00)	0.669	0.00 (0.00–0.00)	0.655	0.07 (0.05–0.15)	0.061 #
27–37 (n = 21)	0.25 (0.13–0.31)		0.00 (0.00–0.39)		0.00 (0.00–0.55)		0.16 (0.10–0.36)	
38–45 (n = 6)	0.24 (0.13–0.33)		0.00 (0.00–0.00)		0.00 (0.00–0.00)		0.26 (0.03–0.40)	
Smoking								
no (n = 44)	0.22 (0.13–0.31)	0.436	0.00 (0.00–0.00)	0.940	0.00 (0.00–0.05)	0.785	0.12 (0.06–0.28)	0.778
yes (n = 5)	0.13 (0.12–0.22)		0.00 (0.00–0.00)		0.00 (0.00–0.00)		0.14 (0.07–0.20)	
Occupation:								
student (n = 15)	0.14 (0.10–0.16) [1]	0.043 *	0.00 (0.00–0.00)	0.085 #	0.00 (0.00–0.00)	0.072 #	0.07 (0.05–0.42)	0.569
worker (n = 9)	0.27 (0.19–0.31)		0.00 (0.00–0.27)		0.00 (0.00–0.39)		0.12 (0.01–0.16)	
white-collar worker (n = 25)	0.25 (0.13–0.37) [1]		0.00 (0.00–0.00)		0.00 (0.00–0.45)		0.15 (0.07–0.28)	
Years of residence in the LG Copper Area:								
≤20 years (n = 17)	0.19 (0.11–0.23)	0.624	0.00 (0.00–0.00)	0.233	0.00 (0.00–0.00)	0.221	0.08 (0.06–0.28)	0.389
21–30 years old (n = 19)	0.26 (0.13–0.31)		0.00 (0.00–0.00)		0.00 (0.00–0.39)		0.13 (0.04–0.20)	
≥31 years old (n = 13)	0.21 (0.13–0.28)		0.00 (0.00–0.27)		0.00 (0.00–0.45)		0.22 (0.07–0.40)	
Thyroid and parathyroid glands diseases:								
no (n = 44)	0.21 (0.13–0.29)	0.381	0.00 (0.00–0.00)	0.509	0.00 (0.00–0.02)	0.620	0.12 (0.05–0.28)	0.655
yes (n = 5)	0.28 (0.13–0.33)		0.00 (0.00–0.27)		0.00 (0.00–0.45)		0.15 (0.03–0.22)	
Dietary supplements:								
no (n = 34)	0.17 (0.10–0.28)	0.182	0.0 (0.00–0.00)	0.189	0.0 (0.00–0.00)	0.250	0.12 (0.05–0.29)	0.887
yes (n = 15)	0.26 (0.13–0.33)		0.00 (0.00–0.44)		0.00 (0.00–0.52)		0.15 (0.06–0.23)	
Reason of extraction:								
surgical (n = 34)	0.19 (0.13–0.28)	0.563	0.00 (0.00–0.00)	0.797	0.00 (0.00–0.06)	0.694	0.15 (0.06–0.36)	0.121
orthodontic (n = 8)	0.21 (0.10–0.34)		0.00 (0.00–0.00)		0.00 (0.00–0.00)		0.06 (0.02–0.13)	
inflammation (n = 7)	0.27 (0.12–0.38)		0.00 (0.00–0.27)		0.00 (0.00–0.45)		0.08 (0.05–0.24)	
Vit. D3 (ng/mL):								
<20 (n = 15)	0.23 (0.16–0.30)	0.012 *	0.00 (0.00–0.42)	0.034 *	0.00 (0.00–0.55)	0.112	0.08 (0.04–0.29)	0.818
20–29 (n = 25)	0.13 (0.09–0.23) [1]		0.00 (0.00–0.00)		0.00 (0.00–0.00)		0.15 (0.07–0.28)	
30–50 (n = 5)	0.28 (0.25–0.40) [1]		0.00 (0.00–0.00)		0.00 (0.00–0.03)		0.06 (0.05–0.23)	
51–100 (n = 4)	0.27 (0.19–0.32)		0.00 (0.00–0.13)		0.00 (0.00–0.31)		0.09 (0.04–0.28)	

Table 4. *Cont.*

(B)								
Variable	**Fe (µg/g)**	***p*-Value**	**Cd (µg/g)**	***p*-Value**	**Pb (µg/g)**	***p*-Value**	**Zn (µg/g)**	***p*-Value**
Gender:								
male (n = 12)	6.72 (1.93–8.11)	0.311	0.05 (0.04–0.07)	0.037 *	0.52 (0.29–0.75)	0.843	122.27 (87.10–170.66)	0.492
female (n = 37)	3.72 (2.57–5.67)		0.08 (0.05–0.10)		0.53 (0.38–0.68)		102.57 (92.31–150.72)	
Age (years old):								
16–26 (n = 22)	3.41 (1.94–5.77)	0.497	0.07 (0.04–0.09)	0.736	0.51 (0.33–0.59) [1]	0.032 *	96.79 (76.67–129.16)	0.088 #
27–37 (n = 21)	3.90 (2.97–6.23)		0.08 (0.07–0.10)		0.47 (0.33–0.76)		120.94 (93.68–157.41)	
38–45 (n = 6)	4.87 (2.57–7.28)		0.06 (0.04–0.09)		0.77 (0.56–1.03) [1]		149.99 (96.73–179.69)	
Smoking								
no (n = 44)	3.88 (2.51–6.54)	0.888	0.07 (0.05–0.09)	0.714	0.54 (0.35–0.75)	0.453	103.82 (91.31–158.02)	0.459
yes (n = 5)	3.72 (3.59–4.04)		0.06 (0.03–0.09)		0.47 (0.47–0.53)		93.68 (84.49–114.73)	
Occupation:								
student (n = 15)	3.72 (2.33–7.34)	0.504	0.06 (0.05–0.10)	0.116	0.41 (0.33–0.66)	0.203	94.70 (75.72–108.77)	0.100 #
worker (n = 9)	4.90 (3.20–5.88)		0.09 (0.07–0.10)		0.56 (0.51–0.90)		132.22 (95.18–163.22)	
white-collar worker (n = 25)	3.62 (2.51–5.98)		0.06 (0.04–0.08)		0.53 (0.30–0.68)		129.16 (93.68–158.62)	
Years of residence in the LG Copper Area:								
≤20 years (n = 17)	3.91 (1.94–5.98)	0.956	0.09 (0.06–0.10)	0.719	0.54 (0.30–0.66)	0.740	95.18 (84.49–103.79)	0.088 #
21–30 years old (n = 19)	3.62 (2.51–6.35)		0.07 (0.06–0.09)		0.51 (0.33–0.68)		108.77 (92.31–155.17)	
≥31 years old (n = 13)	3.86 (2.61–5.88)		0.07 (0.05–0.09)		0.53 (0.41–0.92)		146.21 (96.73–167.76)	
Thyroid and parathyroid glands diseases:								
no (n = 44)	3.81 (2.54–6.54)	0.346	0.07 (0.04–0.10)	0.631	0.52 (0.35–0.71)	0.457	103.82 (87.37–156.29)	0.934
yes (n = 5)	3.86 (1.63–4.56)		0.08 (0.06–0.09)		0.62 (0.47–0.92)		96.73 (95.69–132.22)	
Dietary supplements:								
no (n = 34)	3.58 (2.33–6.23)	0.580	0.06 (0.04–0.09)	0.196	0.49 (0.30–0.74)	0.273	100.72 (84.49–163.22)	0.887
yes (n = 15)	3.90 (2.88–6.35)		0.08 (0.06–0.10)		0.59 (0.42–0.76)		114.73 (92.87–150.69)	
Reason of extraction:								
surgical (n = 34)	4.73 (2.99–6.72)	0.152	0.07 (0.05–0.09)	0.984	0.52 (0.38–0.74)	0.899	106.28 (90.24–163.22)	0.455
orthodontic (n = 8)	2.56 (1.69–7.05)		0.07 (0.04–0.09)		0.52 (0.26–0.73)		96.79 (75.40–127.28)	
inflammation (n = 7)	2.57 (1.82–3.90)		0.09 (0.03–0.10)		0.59 (0.37–0.92)		132.22 (95.69–158.62)	
Vit. D3 (ng/mL):								
<20 (n = 15)	3.62 (2.33–7.22)	0.693	0.06 (0.04–0.09)	0.783	0.51 (0.30–0.74)	0.722	158.62 (99.13–174.00) [1]	0.008 *
20–29 (n = 25)	4.90 (2.61–6.23)		0.08 (0.04–0.10)		0.54 (0.33–0.77)		95.18 (76.67–108.77) [1]	
30–50 (n = 5)	2.88 (2.57–2.99)		0.07 (0.07–0.10)		0.63 (0.57–0.68)		146.21 (90.24–155.17)	
51–100 (n = 4)	4.09 (1.80–5.78)		0.06 (0.06–0.08)		0.47 (0.44–0.51)		142.45 (108.36–165.77)	

*: statistically significant; #: tendency to statistical significance; [1]: statistically significant in pairs of the subgroups (post-hoc test).

3.8. Content of Cadmium

A statistically significant effect of patient sex on the cadmium content in the samples was observed (*p*-value = 0.037), where in the female group, the cadmium concentration was 0.08 (μg/g) and in the male group it was 0.05 (μg/g).

The cadmium content did not depend on the age of patients (*p*-value = 0.736).

The smoking habit did not affect the accumulation of cadmium in the samples (*p*-value = 0.714).

The concentration of cadmium was slightly higher in a worker group (*p*-value = 0.09 (μg/g), *p*-value = 0.116).

The highest level of cadmium was noticed in patients who lived in L-G cooper district ≤20 years (0.09 (μg/g), *p*-value = 0.719).

Thyroid and parathyroid glands diseases, intake of dietary supplements, and reasons for extraction were considered to not statistically significant (*p*-values in order: 0.631; 0.196; 0.984).

3.9. Content of Lead

In both the male and female groups, the lead content was comparable, it was considered not statistically significant (*p*-value = 0.843).

In the 38–45 age group, the lead content in the removed tooth was significantly higher than in the other age groups (0.77 (μg/g)). In the age groups of 16–26 and 38–45, the results were statistically significant in the pairs of subgroups (post hoc test).

Smoking habits and the occupation of the patients did not affect the concentration of lead in the samples (*p*-value= 0.453, 0.203).

The concentration of lead in the removed teeth did not depend on the years of residence in the L-G Copper Area (*p*-value = 0.740).

Thyroid and parathyroid gland diseases, intake of dietary supplements, and reasons for extraction were considered not statistically significant (*p*-values in order: 0.457; 0.273; 0.899).

The highest content of lead (0.63(μg/g)) was noted in a group of patients with 30–50 (ng/mL) vitamin D3 in the capillary blood, but it was not statistically significant (*p*-value = 0.722).

3.10. Content of Zinc

No statistically significant effect of patients' sex on zinc content was noted (*p*-value = 0.492).

The content of zinc in the examined samples increased with the age of patients, and the results showed a tendency to statistical significance (*p*-value = 0.088).

The occupation of patients showed a tendency to statistical significance (*p*-value = 0.100). The highest concentration of zinc was detected in the group of workers (132.22 (μg/g)).

The content of zinc in the studied teeth increased with the years of residence in the L-G Copper Area district, and the results showed a tendency to statistical significance (*p*-value = 0.088).

The highest concentration of zinc was identified in the samples extracted as a result of inflammation processes, but it was not statistically significant (*p*-value = 0.455).

The content of zinc in patients with <20 (ng/mL) and 20–29 (ng/mL) vitamin D3 was considered statistically significant in the pairs of subgroups (post hoc test). The greatest value for zinc was noted in a group of patients with <20 (ng/mL) vitamin D3 in the capillary blood (*p*-value = 0.008).

We observed a statistically significant positive correlation between Cr, Cu, and Zn content and the age of patients residing in the L-G Copper Area ($p < 0.05$ for all) (Table 5). The positive correlation between manganese and age showed a tendency to statistical significance ($p = 0.097$).

Correlations between vitamin D3 concentration in the capillary blood and toxic metals were both positive and negative. A statistically significant negative correlation between the level of vitamin D3 and chromium was shown (rho = −0.29; p = 0.038). A negative correlation between nickel content and vitamin D3 concentration demonstrated a tendency towards statistical significance (p = 0.071).

Table 5. Correlations between levels of toxic metals and continuous variables such as age and vitamin D3 concentration in patients residing in the LG Cooper Area (n = 49). Spearman correlation analysis was used for testing the data.

Toxic Metal (μg/g)	Age (Years)		Vit. D3 (ng/mL)	
	Correlation Coefficient (rho)	*p*-Value	Correlation Coefficient (rho)	*p*-Value
Mn	0.23	0.097 #	−0.04	0.791
Cr	0.29	0.044 *	−0.29	0.038 *
Ni	0.17	0.240	−0.25	0.071 #
Cu	0.31	0.027 *	0.02	0.840
Fe	0.15	0.303	−0.07	0.638
Cd	0.04	0.768	0.16	0.271
Pb	0.19	0.181	0.04	0.760
Zn	0.46	<0.001 *	−0.13	0.377

*: statistically significant; #: tendency to statistical significance.

4. Discussion

Biomonitoring studies within the Legnica-Głogów Copper Area in south-western Poland (impact of the "Głogów" and "Legnica" smelters and the "Żelazny Most" tailings pond) have focused, so far, on determining the level of toxic metals in the blood [21]. In contrast with blood, the use of extracted third molars for orthodontic or surgical indications does not raise ethical concerns.

Completely impacted teeth are a useful biomonitor of environmental exposure to many toxic metals [12]. They basically exclude exposure to toxic metals contained in cigarette smoke or contaminated food.

In the conducted research, the content of manganese, chromium, nickel, copper, cadmium, and zinc did not depend on the place of residence. The results from L-G Copper Area patients were comparable to our control group. However, the concentration of iron and lead was significantly higher in the removed teeth from the L-G Copper Area patients (3.86 vs. 1.81 and 0.53 vs. 0.31). This may indicate environmental pollution of lead and zinc in the L-G Copper Area. Human exposure to lead can cause numerous adverse effects [22], such as hypertension, slow nerve conduction, mood swings, fatigue, and impaired concentration. In addition, it contributes to fertility disorders and decreased sex drive [23,24].

Rzymski et al. [25] described the effects of cadmium, lead, and mercury on the female reproductive system. A high concentration of toxic metals may contribute to endometrial dysfunctions, implantation failure, premature delivery, subfertility, spontaneous abortions, and preeclampsia.

In our study, a positive Spearman's correlation was observed between the content of all toxic metals and the age of patients. The correlation between the concentration of vitamin D3 in the capillary blood and toxic metals was mainly negative, apart from copper, cadmium, and lead (rho 0.02; 0.16; 0.04). These results may be related to the stimulation of co-absorption by vitamin D [10].

Biomonitoring studies using teeth in industrialized areas have also been conducted in other parts of Poland [5,8,12,26–28].

Bryła et al. [8] examined the content of toxic metals in impacted third molars and adjacent bone tissue in patients from the Wrocław district and residents of the city of Wrocław. They observed that the cadmium contents in both the tooth and bone tissue were statistically significantly higher in patients living in Wroclaw ($p < 0.05$). In our study, the level of cadmium was comparable in patients from the L-G Copper Area and from Wrocław (0.07 vs. 0.06).

According to Maciejewska et al. [26], the concentration of zinc in the bone of rats decreased with age. In the research of Bryła et al. [8], the concentration of zinc did not depend on the patient age. The results obtained from our own study showed a positive correlation between age and the content of zinc in the removed teeth (p-value < 0.001).

Wychowański and Małkiewicz [27] evaluated trace elements in the hard dental tissues of third retained molars in patients from urban and rural areas of Mazovia. The concentration of lead, cadmium, manganese, and chromium was higher in the patients from the city.

In turn, Malara et al. [12] proved that the cadmium and lead concentrations in the impacted third molars were significantly higher for habitants of the polluted Ruda Slaska region than those living in the unindustrialized Bielsko-Biala region.

In our study, we compared the results obtained from patients living in the L-G Copper Area to the patients from Wrocław city, which is the third largest city in terms of agglomeration size in Poland, and it is relatively polluted. The authors' research showed that working in the copper mining industry is associated with a higher accumulation of manganese, chromium, and nickel. It has also been confirmed by other researchers [12].

It has been shown that Pb accumulates in the body with age and the mineralized tissues constitute a reservoir of approximately 90% of the lead reserve [28]. We indicated that the female gender may also have an effect on teeth levels of copper, as in the case of blood and brain tissue [29].

Impacted third molars of the Legnica-Głogów Copper Area inhabitants seem to be a useful biomonitor of environmental exposure to toxic metals. However, this requires confirmation in further research.

5. Conclusions

Based on the obtained results, it can be concluded that the concentration of all of the tested toxic metals increased with the age of the patients; however only chromium, copper, and zinc were considered statistically significant. The content of iron and lead was statistically significantly higher in patients living in the L-G Copper Area than in residents of Wrocław, which may indicate greater environmental pollution in the L-G Cooper Area during the mineralization process. Further studies concerning the affect of the environment on the content of toxic metals in the human body should be conducted.

6. The Limitations of the Study

The material was collected in the period from June 2020 to June 2021, during the COVID-19 pandemic, thus the number of patients in the control group, which was matched according age and gender to the study group, was small. We performed analyses of test power for both groups and used statistical tests. Based on the calculations, we concluded that the number of people in the study group was correct, while the size of the control group was slightly lower than required. The authors of the study are aware that the volume of the study was small; however, we will perform further examinations.

Author Contributions: Conceptualization, S.R., M.D. (Maciej Dobrzyński) and M.D. (Marzena Dominiak); methodology, S.R. and M.S.; software, S.R. and A.K.; validation, S.R. and M.S.; formal analysis, D.D.; investigation, S.R. and M.S.; resources, S.R. and A.K.; data curation, S.R. and M.S.; writing—original draft preparation, S.R., S.K., D.D. and A.K.; writing—review and editing, M.D. (Maciej Dobrzyński), M.D. (Marzena Dominiak), T.G. (Tomasz Gębarowski), T.G. (Tomasz Gedrange) and D.D.; visualization, S.R. and A.K.; supervision, M.D. (Maciej Dobrzyński) and M.D. (Marzena Dominiak); project administration, M.D. (Maciej Dobrzyński), M.D. (Marzena Dominiak) and T.G. (Tomasz Gedrange); funding acquisition, M.D. (Maciej Dobrzyński)., M.D. (Marzena Dominiak), T.G. (Tomasz Gedrange) and T.G. (Tomasz Gębarowski). All authors have read and agreed to the published version of the manuscript.

Funding: This article was co-financed by a subsidy from Wroclaw Medical University, number SUBZ.B180.23.054, SUBZ.B040.23.048 and SUBZ.B020.23.051. The APC costs were also supported by statutory research and development activity funds assigned to the Faculty of Veterinary Medicine and the Faculty of Biotechnology and Food Science, Wrocław University of Environmental and Life Sciences.

Institutional Review Board Statement: Institutional Review Board Statement: Consent of the Bioethics Committee of the Wroclaw Medical University: KB-246/2019.

Informed Consent Statement: Informed consent was obtained from all of the subjects involved in the study.

Data Availability Statement: Not applicable.

Acknowledgments: The figures were partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tsz-Shan Lum, J.; Chan, Y.-N.; Sze-Yin Leung, K. Current applications and future perspectives on elemental analysis of non-invasive samples for human biomonitoring. *Talanta* **2021**, *234*, 122683. [CrossRef]
2. Esteban, M.; Castaño, A. Non-invasive matrices in human biomonitoring: A review. *Environ. Int.* **2009**, *35*, 438–449. [CrossRef] [PubMed]
3. Barton, H.J. Advantages of the use of deciduous teeth, hair, and blood analysis for lead and cadmium bio-monitoring in children. A study of 6-year-old children from Krakow (Poland). *Biol. Trace Elem. Res.* **2011**, *143*, 637–658. [CrossRef]
4. Alomary, A.; Al-Momani, I.F.; Obeidat, S.M.; Massadeh, A.M. Levels of lead, cadmium, copper, iron, and zinc in deciduous teeth of children living in Irbid, Jordan by ICP-OES: Some factors affecting their concentrations. *Environ. Monit. Assess.* **2013**, *185*, 3283–3295. [CrossRef] [PubMed]
5. Fischer, A.; Wiechuła, D.; Przybyła-Misztela, C. Changes of concentrations of elements in deciduous teeth with age. *Biol. Trace Elem. Res.* **2013**, *154*, 427–432. [CrossRef]
6. Tvinnereim, H.M.; Eide, R.; Riise, T. Heavy metals in human primary teeth: Some factors influencing the metal concentration. *Sci. Total Environ.* **2000**, *255*, 21–27. [CrossRef]
7. Leventouri, T.; Antonakos, A.; Kyriacou, A.; Venturelli, R.; Liarokapis, E.; Perdikatsis, V. Crystal structure studies of human dental apatite as a function of age. *Int. J. Biol. Mater.* **2009**, *2009*, 1–17. [CrossRef]
8. Bryła, E.; Dobrzyński, M.; Konkol, D.; Kuropka, P.; Styczyńska, M.; Korczyński, M. Toxic Metals Content in Impacted Third Molars and Adjacent Bone Tissue in Different Groups of Patients. *Materials* **2021**, *14*, 793. [CrossRef]
9. Krawiec, M.; Dominiak, M. Prospective evaluation of vitamin D levels in dental treated patients: A screening study. *Dent. Med. Probl.* **2021**, *58*, 321–326. [CrossRef]
10. Moon, J. The role of vitamin D in toxic metal absorption: A review. *J. Am. CollNutr.* **1994**, *13*, 559–564. [CrossRef]
11. Ślebioda, Z.; Szponar, E.; Dorocka-Bobkowska, B. Vitamin D and its relevance in the etiopathogenesis of oral cavity diseases. *Arch. Immunol. Ther. Exp. (Warsz)* **2016**, *64*, 385–397. [CrossRef] [PubMed]
12. Malara, P.; Fischer, A.; Malara, B. Selected toxic and essential heavy metals in impacted teeth and the surrounding mandibular bones of people exposed to heavy metals in the environment. *J. Occup. Med. Toxicol.* **2016**, *11*, 56. [CrossRef]
13. Kołacz, R.; Dobrzański, Z.; Kupczyński, R.; Cwynar, P.; Opaliński, S.; Pogoda-Sewerniak, K. Impact of the copper industry on the content of selected heavy metals and biochemical indicators in the blood of dairy cows. *Med. Weter* **2017**, *73*, 171–175. [CrossRef]
14. Strzelec, Ł.; Niedźwiecka, W. Natural environment in the area of copper smelter plants. *Trend Chang. Med. Sr. Environ. Med.* **2012**, *15*, 21–31.
15. Tsushima, F.; Sakurai, J.; Shimizu, R.; Kayamori, K.; Harada, H. Oral lichenoid contact lesions related to dental metal allergy may resolve after allergen removal. *J. Dent. Sci.* **2022**, *17*, 1300–1306. [CrossRef] [PubMed]
16. Lucchese, A.; Dolci, A.; Minervini, G.; Salerno, C.; DIStasio, D.; Minervini, G.; Laino, L.; Silvestre, F.; Serpico, R. Vulvovaginal gingival lichen planus: Report of two cases and review of literature. *Oral. Implantol.* **2016**, *9*, 54–60. [CrossRef]
17. *PN-EN 13805:2003*; Food Products-Determination of Trace Elements-Pressure Mineralization. European Commission: Brussels, Belgium, 2003.
18. *PN-EN 14082:2004*; Food Products-Determination of Trace Elements-Determination of Lead, Cadmium, Zinc, Copper, Iron and Chromium by Atomic Absorption Spectrometry (AAS) after Dry Mineralization. European Commission: Brussels, Belgium, 2004.
19. *PN-A-86939-6:1998*; Vegetable and Animal Oils and Fats-Determination of Heavy Metal Content by Atomic Emission Spectrometry-Determination of Nickel Content. European Commission: Brussels, Belgium, 1998.
20. *PN-EN-ISO 6869:2002*; Determination of Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Sodium and Zinc Content-Atomic Absorption Spectrometry Method. European Commission: Brussels, Belgium, 2002.

21. Sławińska-Ochla, T.; Ignasiak, Z.; Fugiel, J.; Pokrywka, J. Lead concentration in blood of school children from copper mining area and the level of somatic development at birth. *Med. Environ. Med.* **2011**, *14*, 42–48.
22. Dembicka, D.; Pastuszek, B.; Strugała-Stawik, H.; Zaręba, A. Monitoring biologiczny stopnia narażenia na ołów i skutków zdrowotnych u dzieci z byłego województwa legnickiego (1996–1999). [W:] Z. Rudkowski (red.) Materiały Konferencji Naukowej nt. Dziecko w środowisku zagrożonym ekologicznie- profilaktyka i problemy zdrowotne. *Legnica* **1999**, 42–44.
23. Charkiewicz, A.E.; Backstrand, J.R. Lead toxicity and pollution in Poland. *Int. J. Environ. Res. Public Health* **2020**, *17*, 4385. [CrossRef]
24. Krzywy, I.; Krzywy, E.; Pastuszak-Gabinowska, M.; Brodkiewicz, A. Lead—Is there something to be afraid of? *Ann. Acad. Med. Stetin.* **2010**, *56*, 118–128.
25. Rzymski, P.; Tomczyk, K.; Rzymski, P.; Poniedziałek, B.; Opala, T.; Wilczak, M. Impact of heavy metals on the female reproductive system. *Ann. Agric. Environ. Med.* **2015**, *22*, 259–264. [CrossRef] [PubMed]
26. Maciejewska, K.; Drzazga, Z.; Kaszuba, M. Role of trace elements (Zn, Sr, Fe) in bone development: Energy dispersive X-ray fluorescence study of rat bone and tooth tissue. *Biofactors* **2014**, *40*, 425–435. [CrossRef] [PubMed]
27. Wychowański, P.; Małkiewicz, K. Evaluation of metal ion concentration in hard tissues of teeth in residents of central Poland. *BioMed. Res. Int.* **2017**, *2017*, 6419709. [CrossRef] [PubMed]
28. Fischer, A.; DanutaWiechuła, D. Age-Dependent Changes in Pb Concentration in Human Teeth. *Biol. Trace Elem. Res.* **2016**, *173*, 47–54. [CrossRef] [PubMed]
29. Quinn, J.F.; Harris, C.; Kaye, J.A.; Lind, B.; Carter, R.; Anekonda, T.; Ralle, M. Gender effects on plasma and brain copper. *Int. J. Alzheimers Dis.* **2011**, *2011*, 150916. [CrossRef] [PubMed]

Journal of *Xenobiotics*

MDPI

Article

Mercury Content in Impacted Wisdom Teeth from Patients of the Legnica–Głogów Copper Area—An In Vitro Pilot Study

Sadri Rayad [1,*], Maciej Dobrzyński [2,*], Amadeusz Kuźniarski [3], Marzena Styczyńska [4], Dorota Diakowska [5], Tomasz Gedrange [6], Sylwia Klimas [2], Tomasz Gębarowski [7] and Marzena Dominiak [6]

1 Academic Dental Polyclinic of Dental Center, Technology Transfer Ltd., Krakowska 26, 50-425 Wroclaw, Poland
2 Department of Pediatric Dentistry and Preclinical Dentistry, Wroclaw Medical University, Krakowska 26, 50-425 Wroclaw, Poland; sylwia.klimas@umw.edu.pl
3 Department of Prosthetic Dentistry, Wroclaw Medical University, Krakowska 26, 50-425 Wroclaw, Poland; amadeusz.kuzniarski@umw.edu.pl
4 Department of Human Nutrition, Wroclaw University of Environmental and Life Sciences, Chelmonskiego 37/41, 51-630 Wroclaw, Poland; marzena.styczynska@upwr.edu.pl
5 Department of Basic Sciences, Wroclaw Medical University, Chalubinskiego 3, 50-368 Wroclaw, Poland; dorota.diakowska@umw.edu.pl
6 Department of Dental Surgery, Wroclaw Medical University, Krakowska 26, 50-425 Wroclaw, Poland; tomasz.gedrange@umw.edu.pl (T.G.); marzena.dominiak@umw.edu.pl (M.D.)
7 Department of Biostructure and Animal Physiology, Wrocław University of Environmental and Life Sciences, Kożuchowska 1/3, 51-631 Wroclaw, Poland; tomasz.gebarowski@upwr.edu.pl
* Correspondence: sadri.rayad@gmail.com (S.R.); maciej.dobrzynski@umw.edu.pl (M.D.); Tel.:+48-71-7186-292 (S.R.); +48-71-7840-378 (M.D.)

Abstract: The aim of this study was to determine the content of mercury in impacted third molars from Legnica–Głogów Copper Area residents to emphasize the effects of environmental pollution on the human body. A group of 72 patients with an average age of 27.3 ± 6.9 years participated in the study. Within this study, the research group (Legnica–Głogów Copper Area residents) comprised 51 individuals, while the control group (residents of Wrocław) consisted of 21 participants. A higher number of female individuals participated in the research (55). The amount of mercury present in the samples was determined through atomic absorption spectrometry with the use of a SpectraAA atomic absorption spectrometer and a V2 AA240FS flame attachment that utilized an air–acetylene flame. The accumulation of Hg in the teeth of members of the control group residing in Wrocław was studied, with a focus on identifying the risk factors that contribute to this phenomenon. The final model analyzed the presence of various factors, including thyroid and parathyroid gland diseases, cardiac diseases, and interval-scale Vit. D3 concentration. Among these factors, the presence of cardiac diseases was deemed statistically significant in relation to an increase in Hg concentration in third molars (rate ratio = 2.27, $p < 0.0001$). The concentration of mercury increased with the age and time of residence in the L-G Copper District.

Keywords: mercury; toxic metals; third molars; biomonitoring

Citation: Rayad, S.; Dobrzyński, M.; Kuźniarski, A.; Styczyńska, M.; Diakowska, D.; Gedrange, T.; Klimas, S.; Gębarowski, T.; Dominiak, M. Mercury Content in Impacted Wisdom Teeth from Patients of the Legnica–Głogów Copper Area—An In Vitro Pilot Study. *J. Xenobiot.* **2023**, *13*, 463–478. https://doi.org/10.3390/jox13030029

Academic Editor: Nikolaos Stamatis

Received: 24 July 2023
Revised: 18 August 2023
Accepted: 24 August 2023
Published: 27 August 2023

1. Introduction

The conditions and changes in the environment have a huge impact on human health. They also influence the development and emergence of many diseases of civilization, including cancer. Biomonitoring is taking on greater importance in the context of health and the environment. The interests of environmental medicine focus on two aspects. The first covers the physiological, mental and emotional responses of the body to environmental factors. The second examines the causes of disease in an environmental context and develops methods for the detection, prevention, and control of diseases whose development is influenced by the target population's environment. These aspects are very much linked to epidemiology. The combination of all factors gives an accurate picture of the health status of a region.

Knowledge of the health effects of environmental exposure provides a basis for specific health actions not only for individuals, but also for the population as a whole. The development of methods for detecting environment-related diseases is linked to the concept of biomonitoring. The concept of biomonitoring encompasses a range of actions aimed at evaluating the condition of the environment. These actions utilize bio-identifiers, which serve as indicators of pollution levels, in order to gauge the extent of environmental contamination [1].

In dentistry, a breakthrough occurred in the 1970s that resulted in an appreciation of the impact of external factors on the state of the oral cavity. In the 1970s and 1980s, an antibiotic called tetracycline was widely used, which showed similar staining properties on actively mineralizing tissues in both model animals and humans, enabling the determination of the dynamics of bone tissue synthesis. This was due to the formation of tetracycline–calcium–phosphate complexes, which had a brown color [2].

The oral cavity and the teeth are particularly vulnerable to metal accumulations. Apart from the metals present in the environment, metals are also utilized for the reconstruction of defects, for treatment, or for the filling of cavities within the oral cavity. According to the existing body of literature, there is compelling evidence to support the notion that mineralized tissues, such as teeth and bones, serve as an enduring testament to an individual's way of life within a particular environment. This is due to the fact that these tissues effectively capture and retain the long-term effects of environmental contaminants [3]. Since their development has been well defined, they can serve as a basis on which to determine an organism's exposure to toxic compounds (xenobiotics). This has enabled environmental monitoring using archeozoological and anthropological material, the biostructural analysis of human skeletons in forensic medicine, and animal experiments [4–10].

Environmental pollution by metals and their derivatives (compounds), and the resulting health risks, is an important area of interest for environmental medicine. Toxic metals (previously called heavy metals) can enter the human body by inhalation, by ingestion, or by absorption through the skin [11,12]. Metal and metal-alloy-based implants and prostheses have been utilized for over a century, and there have been reports of rejections, revisions, and toxicity being caused by metal particles. In recent years, concerns have grown regarding the complications arising from the use of metal ions, debris, and organo-metallic particles in orthopedic patients. Despite the well-established literature on environmental metal toxicants and safety limits for human exposure, efforts have not been sufficient in the case of implant-based metal toxicology. Serum metal ion concentration can serve as an indicator of systemic toxicity [13].

Typically, dental implants are constructed from alloys containing titanium. While this type of therapy for implants is currently perceived as having a positive outcome with no detrimental health effects, its success is contingent upon several different factors. To ensure the biocompatibility of implantable devices, it is crucial to discuss and define the characteristics of metals used for medical purposes. The presence of metals such as nickel, aluminum, and titanium in dental fillings, bridges, and implants can make them potential sensitizers. The benefits of endosseous prosthetics have garnered the attention of numerous dental professionals, leading to a rise in the implementation of implant-based procedures. With the prolonging of human life expectancy, there is a need for implant biomaterials that exhibit minimal adverse effects on the host's tissues [14].

The movement of toxic metals throughout the ecosystem is primarily linked to the interconnectedness of plant–animal–human food chains [12,15,16]. Soil contamination with these substances is caused by industrial emissions, vehicular transportation, municipal waste management and their ingress from bedrock [16,17]. Among the most significant sources of emissions are the chemical, metallurgical, and mining industries, as well as soil fertilization and surface runoff from roadways with heavy vehicular traffic [12,18–21].

Toxic metals are absorbed by plants through water uptake from the soil and are subsequently stored within their tissues [22]. For most plants in our geographical area, these are mostly roots and leaves [23], readily eaten by animals and humans. Plants that strongly accumulate metals and their derivatives include, e.g., beetroot, potato, lettuce, and cabbage [24–32].

Thus, the most common route of entry of toxic metals into the body is the oral route; nevertheless, the extent of metal absorption is often influenced by various factors, including the pH level, the chemical composition the metals, the presence of other substances that might alter its absorption, and the rate at which it passes through the gastrointestinal tract. In contrast, the inhalation route allows the easiest absorption and distribution throughout the body via the circulatory system [12,33]. Skin appendages, such as glands or hair follicles, allow the incorporation of toxic metals, provided they are highly concentrated in the external environment [34].

The Legnica–Głogów Copper District is a highly developed and urbanized region, situated in the Province of Lower Silesia—specifically in its northern region. It is also a significant copper ore basin, spanning around 2.2 thousand square kilometers and accommodating approximately 500,000 residents. The district's history dates back to the 1960s, during which the discovery of vast copper deposits spurred its growth. At present, the copper ore mining industry comprises three quarries: "Lubin", "Rudna", and "Polkowice-Sieroszowice". This industry includes two essential components: ore enrichment plants and hydrotechnical plants. Additionally, the processing sector encompasses three distinct steelworks: "Legnica", "Głogów", and "Cedynia". Lastly, the "Żelazny Most" engineering facility, which spans an area of 1400 hectares, serves as a storage site for copper ore tailings [35]. After analyzing the information provided, it can be inferred that the Legnica–Głogów Copper District would serve as an intriguing subject of study due to its significant issues with environmental contamination.

It is important to acknowledge that, in cases of brief and severe exposure, the quantity of poisonous substances that infiltrate the body is directly proportional to the degree of apparent impairment. In the case of prolonged moderate or weak exposure, the changes may be imperceptible, while non-specific disorders of organism functioning may appear, which are mostly not associated with toxic metal intoxication. The degree of toxicity of metals also depends on other factors, which include the chemical form in which they occur, their solubility in fats and in physiological fluids, the level of immunity of the organism concerned, and, finally, the duration of exposure [36,37].

An important factor modulating the toxic effects of metals is a person's lifestyle and mass [38]. People with higher mass accumulate toxic metals more easily in soft tissues compared to slim people, in whom the same dose can lead to liver or kidney lesions. Conversely, people with high levels of adipose tissue, where more toxicity has accumulated, are at greater risk of the so-called reintoxification phenomenon, which occurs as a result of fat loss with age [39].

There are three primary classifications for the mechanisms by which metal toxicity occurs. The first is the obstruction of crucial functional groups found in proteins. The second is the displacement of metal ions that serve as cofactors for enzymes and other functional proteins. In addition, finally, the third classification involves the modification of the spatial configuration of proteins [40]. When metals bind to proteins, not only is the elimination of these metals from the body hindered, but their bioaccumulation levels are also increased [34]. This leads to the dysfunction of proteins in the cell. Such interactions can cause death upon exposure to high concentrations. When exposure levels do not exceed a lethal dose, poisoning manifests as structural, functional, or biochemical damage [36]. The most common electrodonor groups to which metal cations attach are the amine, carboxyl and thiol groups. Especially when attached to thiol groups, many enzymatic active centers are blocked. In addition, the use of antioxidative enzymes can serve as an early indicator of copper toxicity, even before the symptoms become visible [41].

Toxic metals tend to accumulate in specific organs, with the liver and kidneys being the primary centers for the detoxification and elimination of these metals. Additionally, other organs that are commonly affected include adipose tissue, muscle, nervous tissue, and bone [42–44]. Prolonged exposure to certain metals and their compounds can result in a gradual decline in physical, muscular, and neurological function that mimics the characteristics of illnesses like multiple sclerosis, Parkinson's disease, Alzheimer's disease, and muscular dystrophy. Furthermore, continued long-term exposure to these same metals and their compounds can even result in the development of cancer [12,45].

The movement of metals within the human body is somewhat restricted by biological barriers. However, when an excessive amount of toxic elements is present, the effectiveness of these barriers becomes limited. Metals can be categorized into different groups based on their varying levels of toxicity [46]:

- Minimal risk of harm: strontium (Sr), zirconium (Zr);
- Medium degree of potential harm: cobalt (Co), nickel (Ni);
- High degree of potential harm: iron (Fe), manganese (Mn), molybdenum (Mo),
- Exceptionally elevated degree of potential harm: zinc (Zn), chromium (Cr), cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg).

The toxicity as well as the high levels of bioaccumulation of mercury have been well established. Mercury disrupts the tertiary and quaternary protein structure and modifies cellular functions by binding to selenohydryl and sulfhydryl groups, which react with methyl mercury and impair the cellular structure. Anthropogenic activities are the primary sources of mercury pollution, including agriculture, mining, municipal wastewater discharges, incineration, and industrial wastewater discharges (Figure 1). In nature, mercury exists in various forms, such as elemental or metallic forms, inorganic salts, and organomercurial compounds, each of which has different bioavailabilities and toxicities associated with them. The inhalation of mercury vapors can lead to bronchitis, asthma, and short-term respiratory issues. The following symptoms are associated with high-level exposure to metallic mercury: diarrhea, nausea, skin rashes, hypertension, and nephrotoxicity [12].

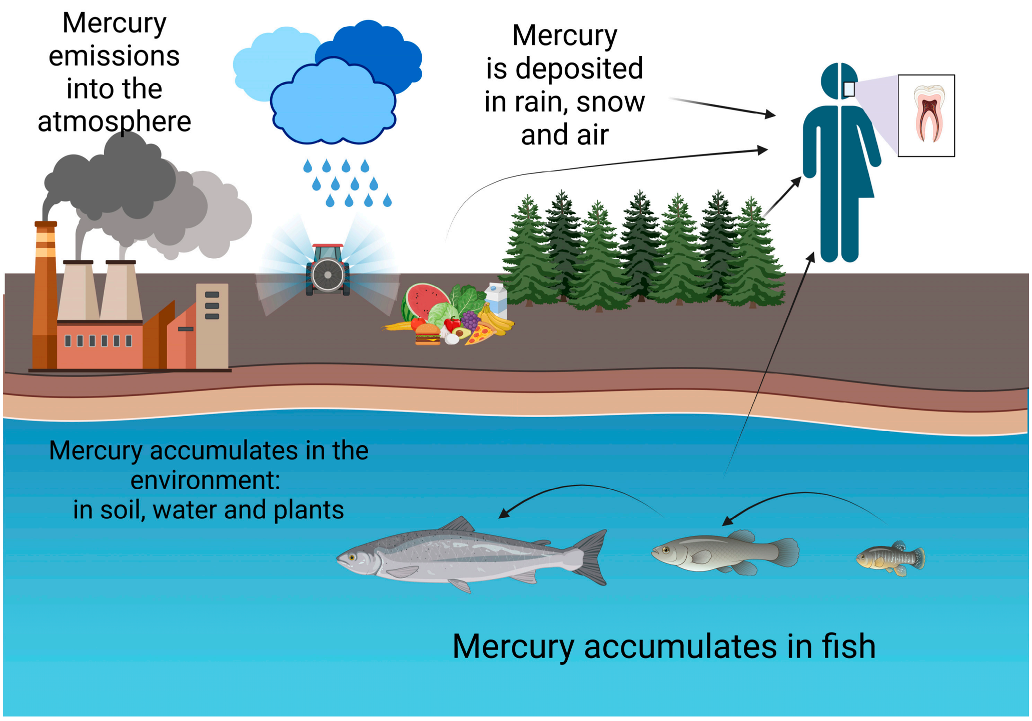

Figure 1. Sources of mercury.

An important point to consider is that mercury has the ability to accumulate in cartilage and bone tissue. During the development of molars, substitution occurs—mercury ions remain incorporated in the place of calcium ions in carbonates or hydroxyapatites [47] (Figure 2). In addition, there is intense metabolism of the developing bud, and an increased contact surface with the blood vessels, which can be used to transport Hg [48].

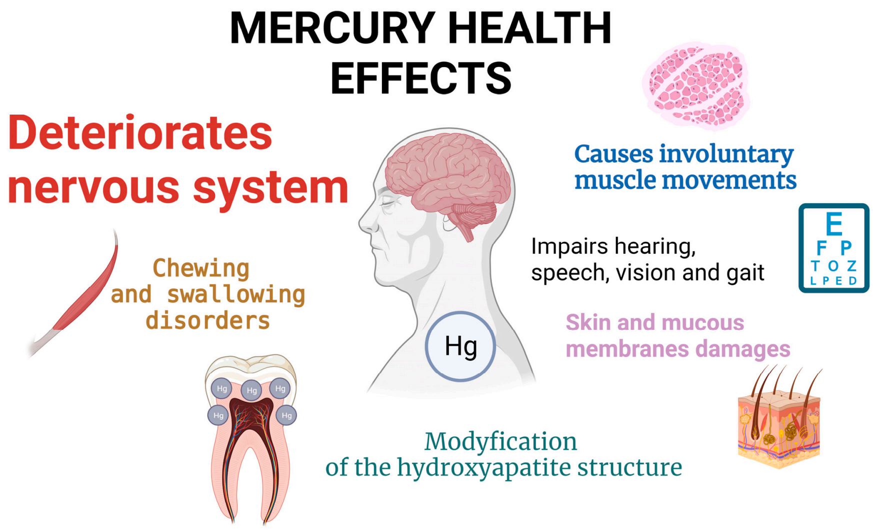

Figure 2. Mercury health effects.

Vitamin D is a term that encompasses both vitamin D2 and D3. Vitamin D3 is produced by exposing lanolin's 7-dehydrocholesterol to ultraviolet radiation. This results in the biological activity of cholecalciferol, which is also known as vitamin D3, and is naturally synthesized by the human skin [49]. However, it can also be obtained through dietary means, such as through foods or supplements. Unfortunately, not many natural sources of vitamin D exist, with the exception of certain oily fish, like mackerel, salmon, and herring. Vitamin D plays a significant role in the mineralization of teeth, and its levels have been garnering more attention in regard to oral health. During both growth and adulthood, a lack of vitamin D has been linked to various oral health issues. In children, a severe deficiency of vitamin D can lead to defects in mineralization, which can result in enamel and dentin defects. This could increase the risk of dental caries, and in adults, a lack of vitamin D has been connected with a higher prevalence of gingival inflammation and periodontitis [50,51].

In their study, Albawi et al. [52] investigated the occurrence of vitamin and mineral deficiencies, as well as excessive levels of non-essential heavy metals, among adults in Saudi Arabia. The incidence of vitamin D deficiency exhibited variations based on age. The prevalence of vitamin D deficiency was found to be higher in younger adults and men compared to older participants and women. Cho et al. [53] evaluated the relationship between blood mercury levels and osteoporosis in postmenopausal women. Elevated levels of Hg in the blood have been linked to a decreased risk of osteoporosis, as well as higher bone mineral density (BMD).

The aim of this study was to determine the amount of mercury found in the third molars removed from individuals living in the Legnica–Głogów Copper Area. This particular region poses an increased risk of heavy metal exposure due to its industrial and structural characteristics. The study also aimed to detect variations in mercury content in teeth among different groups of patients, as well as to evaluate the connection between the quantity of mercury found in the extracted third molars and the level of vitamin D detected in capillary blood.

2. Materials and Methods

2.1. Material

The authors were granted permission by the Bioethics Committee of Wrocław Medical University (consent number: KB-246/2019) before commencing this investigation. Following the principles outlined in the Declaration of Helsinki, the study was conducted on a cohort of 72 patients who had undergone the extraction of their third molars. These patients had provided informed consent for both the research and the medical procedure. Alongside the tooth extractions, blood samples were obtained and responses to a survey were collected. Patients were segregated into two groups—the research group had 51 patients, and the control group had 20 patients. The collection of research material took place between June 2020 and June 2021, coinciding with the COVID-19 pandemic, resulting in a limited number of patients.

For this study, the authors obtained lower impacted third molars from participants in both the control and study groups. The sample included the entire tooth's extracted portion. To preserve the integrity of the samples, they were stored in aseptic containers at a temperature of -20 °C, without undergoing any chemical treatment, and were subsequently analyzed for mercury concentration.

Data regarding residency status and lack of dietary supplements as well as gender and age were gathered as part of the investigation. The patients had been living in either the Legnica–Głogów Copper Area or Wrocław since their birth, which was the primary inclusion criterion of the study. Additionally, the participants were in good health and had not taken any medication or supplements. Furthermore, capillary blood was collected for the determination of Vit. D3 level to appraise the correlation between the level of mercury and Vit. D3 concentration in patients residing in the L-G district.

2.2. Determination of Mercury in the Studied Materials

The mercury concentration was measured at the Department of Human Nutrition, Wrocław University of Environmental and Life Sciences. The dental material was subjected to multi-element analysis using atomic absorption spectrometry. The authors' questionnaire yielded valuable data, encompassing a variety of personal details, including occupational exposure, history of allergies, age, gender, place of residence, smoking habits, general medical conditions, and any dietary supplements used by the individuals.

2.3. Mineralization of the Studied Materials

The process of mineralization of the samples occurred in a closed microwave system using a wet method. Homogeneous samples with weights ranging from 0.1 g to 0.5 g were taken, and 5 cm^3 of nitric acid (V) A.C.S. along with 1 cm^3 of concentrated hydrogen peroxide A.C.S. were added. The MARS 5 microwave sample preparation system was then used to mineralize the samples. Following mineralization, the minerals were transferred completely to measuring vessels with a capacity of 10 cm^3, using redistilled water [54].

2.4. Examination of Mercury Content in the Samples

The estimation of mercury concentration in the samples was accomplished by atomic absorption spectrometry in an air–acetylene flame using a SpectraAA atomic absorption spectrometer; the flame was attached to a V2 AA240FS atomic absorption spectrometer, and dedicated cathode lamps were employed. The precision of the method was confirmed by utilizing the certified reference material—ERM-BD151 skimmed milk powder (Sigma-Aldrich, Saint Louis, MO, USA). This material has a measurement uncertainty of 5%. According to the specification provided by the manufacturer of the mercury analyzer, the limit of quantification (LOQ), defined as the smallest amount or lowest concentration of a substance that can be quantified using a given analytical procedure with the assumed accuracy and precision, is 0.0005 mg/kg for a sample of approximately 100 mg (0.05 ng Hg). The upper limit of the instrument range is 5 mg/kg, which for a 100 mg sample is equivalent to 500 ng Hg in the sample. Higher concentrations can be determined provided that the weight is reduced so as not to exceed 500 ng Hg per sample.

2.5. Methodology of Blood Collection and Determination of Vit. D3

The vitamin-D test system in the Vitality Health Check (VHC) was used to assess vitamin D levels from a capillary blood sample using the capillary method. To begin this process, the blood collection site was thoroughly cleansed, disinfected, and dried, and blood circulation was established. To obtain a blood sample, a lancet that was intended for a single use and was sterile was utilized to puncture the fingertip. Following the puncture, 10 µL of blood was collected with the aid of capillary tubes that had been treated with heparin. This collection process was facilitated by the use of a UniSampler blood collection device. The blood sample was then placed in the collection tube, which had previously been employed for buffer collection. After thorough mixing, the specimen was transferred to the test device using a micropipette. The VHC reader was used to read the vitamin D value 15 min later.

2.6. Statistical Analysis

Data analysis was conducted using the Statistica 13.3 software package (Tibco Software Inc., Palo Alto, CA, USA). The Shapiro–Wilk normality test was employed to evaluate data distribution. Descriptive data are presented as median (quartile 1–quartile 3, Q1–Q3). Chi-square test or Fisher's exact test were utilized to analyze qualitative data. The non-parametric Mann–Whitney test was employed to compare quantitative data between two independent study groups. Techniques for the modeling of multivariable Poisson regression were used to identify the predictors of the Hg accumulation in third molars. A significance level of $p \leq 0.05$ was adopted to determine statistical significance.

3. Results

3.1. Basic Characteristics of Patients

The demographic, clinical, and laboratory characteristics of both subgroups are presented in Table 1. There were 21 people in the control group. The control group consisted of 17 women and 4 men. Most patients in the control group were in the age group of 16–26 years (13 patients). There were a total of eight individuals whose ages fell within the span of 27 to 37 years. The absence of any statistical relevance regarding age within the control group could be attributed to the absence of a reference category. In the control group, no individuals were included with ages between 38 and 45. Only three people in the control group were smokers. The control group was primarily composed of individuals in white-collar professions, corresponding to a total of 15 patients. Of all the patients in the control group, only a solitary individual had heart disease. A meager two patients were found to have afflictions relating to their thyroid and parathyroid glands.

The data in Table 1 reveal that the majority of participants in the study group were young people between the ages of 16 and 26, who did not smoke and were generally in good health. In terms of gender distribution, age, smoking habits, occupation, illnesses, dietary supplements, and vitamin D3 levels, there were no significant distinctions between the study group and the control group ($p > 0.05$ for all variables). Additionally, there was a higher number of female participants than male ones. Notably, the extraction of teeth for surgical reasons was statistically significant ($p = 0.003$).

Table 1. Demographic, clinical and laboratory characteristics of the total study group and the two subgroups of patients, divided according to place of residence. Descriptive data are presented as number of observation (percent) or median (Q1–Q3).

Variable	Total (n = 72)	L-G Copper District—No (n = 21)	L-G Copper District—Yes (n = 51)	*p*-Value
Gender:				
male	17 (23.6)	4 (19.1)	13 (25.5)	0.558
female	55 (76.4)	17 (80.9)	38 (74.5)	
Age (years old)	26.5 (16.0–45.0)	25.0 (24.0–27.0)	29.0 (22.0–32.0)	0.261
Age (years old):				
16–26	36 (50.0)	13 (61.9)	23 (45.1)	0.080
27–37	30 (41.7)	8 (38.1)	22 (43.1)	
38–45	6 (8.3)	0 (0.0)	6 (11.8)	
Smoking				
no	64 (88.9)	18 (85.7)	46 (90.2)	0.590
yes	8 (11.1)	3 (14.3)	5 (9.8)	
Occupation:				
student	21 (29.2)	5 (23.8)	16 (31.4)	0.172
worker	10 (13.9)	1 (4.8)	9 (17.6)	
white collar worker	41 (56.9)	15 (71.4)	26 (51.0)	
Years of living in the L-G Copper Area (n = 49):				
≤20 years old	18 (35.3)	-	18 (35.3)	1.000
21–30 years old	20 (39.2)	-	20 (39.2)	
≥31 years old	13 (25.5)	-	13 (25.5)	
Thyroid and parathyroid glands diseases:				0.971
no	65 (90.3)	19 (90.5)	46 (90.2)	
yes	7 (9.7)	2 (9.5)	5 (9.8)	
Cardiac diseases:				
no	70 (97.2)	20 (95.2)	50 (98.0)	0.531
yes	2 (2.8)	1 (4.8)	1 (2.0)	
Dietary supplements:				
no	49 (68.1)	13 (61.9)	36 (70.6)	0.472
yes	23 (31.9)	8 (38.1)	15 (29.4)	
Reason for extraction:				
surgical	57 (79.2)	21 (100.0)	36 (70.6)	0.003 *
orthodontic	8 (11.1)	0 (0.0)	8 (15.7)	
inflammation	7 (9.7)	0 (0.0)	7 (13.7)	
Vit. D3 (ng/mL)	27.0 (9.9–72.0)	27.8 (25.5–32.2)	26.4 (18.5–28.4)	0.134
Vit. D3 (ng/mL):				
<20—large deficiency	19 (26.4)	3 (14.3)	16 (31.4)	
20–29—deficiency	36 (50.0)	10 (47.6)	26 (51.0)	0.189
30–50—norm	11 (15.3)	6 (28.6)	5 (9.8)	
51–100—above the norm	6 (8.3)	2 (9.5)	4 (7.8)	

* Statistically significant.

3.2. The Concentration of Mercury in the Extracted Teeth in Residents from the L-G Copper District and in the Control Group

The results presented in Table 2 indicate that patients from the L-G Copper District had slightly higher concentrations of Hg in their extracted teeth compared to the control group (0.389 and 0.341 (μg/g)); however, this was not statistically significant (*p*-value = 0.655).

Table 2. Tissue concentrations of Hg in the extracted teeth in the total study group and in both subgroups of patients, divided according to place of residence. Descriptive data are given as median (Q1–Q3).

Variable	Total (n = 72)	L-G Copper District—No (n = 21)	L-G Copper District—Yes (n = 51)	*p*-Value
Hg (μg/g)	0.367 (0.277–0.557)	0.341 (0.287–0.480)	0.389 (0.274–0.557)	0.655

3.3. The Risk Factors Contributing to the Build-Up of Mercury (Hg) in the Teeth of Individuals Living in the L-G District and in the Control Group

Multivariable Poisson regression revealed that the following factors constituted an optimal set of independent predictors of accumulation of Hg in people living in the L-G area: sex, age in interval scale, residence in the L-G Copper District, and reason for extraction. Table 3 provides a comprehensive overview of the primary factors that contribute to the build-up of mercury in the teeth of residents residing in the L-G district.

Table 3. Poisson regression multivariable model of risk factors determining the accumulation of Hg in people living in the L-G area (n = 51).

Predictor	Risk Estimate	SE	95% Confidence Limits		Wald Test	*p*-Value
			Lower	Upper		
Intercept	3.64	0.87	1.92	5.35	17.33	<0.0001 *
Gender (for female)	−0.13	0.13	−0.39	0.14	0.96	0.325
Age (for 16–26 years old)	−0.45	0.32	−1.10	0.18	1.93	0.163
Age (for 27–37 years old)	0.77	0.23	0.31	1.23	10.67	0.001 *
Residence in the L-G Copper District (years)	−0.14	0.03	−0.21	−0.08	19.10	<0.0001 *
Residence in the L-G Copper District (for ≤20 years)	−2.49	0.56	−3.60	−1.38	19.32	<0.0001 *
Residence in the L-G Copper District (for 21–30 years)	−0.64	0.25	−1.15	−0.14	6.31	0.012 *
Residence in the L-G Copper District (for >30 years)	3.14	0.40	2.34	3.93	60.14	<0.0001 *
Reason for extraction (for orthodontic)	1.21	0.26	0.68	1.73	20.50	<0.0001 *

* Statistically significant.

The risk of accumulation of Hg in the teeth was significantly decreased in younger subjects compared to in the oldest people (rate ratio = 0.77, $p = 0.001$). Decreased risk of the build-up of mercury (Hg) in the teeth was significantly related to shorter length of residence in the L-G area (rate ratio = −2.49, $p < 0.0001$ for time below 20 years and rate ratio = −0.64, $p = 0.012$ for time of residence 21–30 years). Increased risk of accumulation of Hg in third molars was significantly associated with times of residence in the L-G Copper District greater than 30 years (rate ratio = 3.14, $p < 0.0001$) and extraction for orthodontic reasons (rate ratio = 1.21, $p < 0.0001$) (Table 3).

We analyzed risk factors contributing to the build-up of Hg in the teeth of people in the control group, living in the Wrocław area, and the results are provided in Table 4. The following factors were analyzed in the final model: the presence of thyroid and parathyroid gland diseases, the presence of cardiac diseases, and Vit. D3 concentration in an interval scale. The presence of cardiac diseases was considered to be statistically significant for increased Hg concentration in third molars (rate ratio = 2.27, $p < 0.0001$). In contrast to the L-G Copper District group, there were no people aged 38–45 years old in the control group. This age range is a reference category for other age ranges in the multivariable Poisson regression, where the median concentration of Hg was 0.326 (0.285–0.493) μg/g for the 16–26-year-old subgroup vs. 0.346 (0.290–1.690) μg/g for 27–37-year-old subgroup.

Therefore, no significant differences in concentrations of Hg were found in the control group in relation to age.

Table 4. Poisson regression multivariable model of risk factors determining the accumulation of Hg in the control group (n = 21).

Predictor	Risk Estimate	SE	95% Confidence Limits		Wald Test	*p*-Value
			Lower	Upper		
Intercept	2.65	0.63	1.41	3.90	17.60	<0.0001
Thyroid and parathyroid glands diseases	0.71	0.41	−0.11	1.53	2.87	0.090
Cardiac diseases	2.27	0.32	1.62	2.91	47.35	<0.0001 *
Vit. D3 (for <20 ng/mL—large deficiency)	−0.60	0.73	−2.04	0.83	0.68	0.408
Vit. D3: (for 20–29 ng/mL—deficiency)	−0.57	0.56	−1.68	0.53	1.03	0.310
Vit. D3: (for 30–50 ng/mL—norm)	−0.81	0.61	−2.01	0.38	1.76	0.184

* Statistically significant.

4. Discussion

The analysis of the aforementioned biological material for the presence of xenobiotics or their metabolites, as well as enzymes in inappropriate concentrations, makes it possible to assess the exposure of a given organism to harmful agents present in the environment [55]. The monitoring of xenobiotics, referring to toxic metals and persistent organic compounds (POPs), serves as the foundation for shaping environmental policies that impact the health of individuals residing in specific regions [56,57].

It is important to acknowledge that toxic metals are not biologically degradable. The process of detoxification involves either the concealment of active ions by metallothioneins or the deposition of these ions in insoluble forms, which are then either stored within the body or expelled from it afterwards [40,58]. Reducing the labile fraction of heavy metals in soil is crucial in managing plant toxicity, since it is the most significant factor. In order to mitigate the harmful effects of heavy metals present in contaminated soils, one effective chemical remediation method involves immobilizing the heavy metals in a form that is inaccessible to plants. This reduces their presence in the food chain, resulting in a lower risk of negative health and environmental impacts [59,60].

In addition, it is important to underscore that the form in which a metal is dissolved in lipids—i.e., whether it is a cation or an element—plays a significant role in determining which organs will be affected. For instance, when mercury is present in its cationic form, it tends to accumulate in the central or peripheral nervous system, while the elemental form has a strong affinity for the kidneys. Additionally, studies have shown that certain toxic metals have the ability to accumulate in specific organs. Lead tends to accumulate in bone tissues, while cadmium and mercury have a tendency to accumulate in parenchymal organs [61,62].

When present in high amounts, trace elements like copper, iron, manganese, and calcium can become hazardous, despite being necessary building blocks for cellular homeostasis. Dusek et al. [63] explored the manner in which discordance in metal balance leads to tissue accumulation and associated medical conditions. While certain disorders trigger the buildup of metals in specific areas of the brain, such as the globus pallidus, which is highly susceptible to divalent metal ion accumulation, other disorders generate widespread metal accumulation that can affect the whole brain, the liver, and other internal organs.

Certain elements that fall under the category of toxic metals are crucial for a variety of uses. Living organisms and elements with unknown physiological functions share a common trait: when consumed beyond their permissible limits, even those that are required in significant or trace quantities can have a harmful effect on all living organisms, including plants, animals, and humans. Even essential elements that are required in large or trace amounts can become hazardous once their threshold is surpassed. Among the most noxious metals are cadmium, mercury, and lead, which are not deemed necessary for living organisms. These metals have been known to cause a plethora of illnesses [13,16,45].

The assessment of the impact of chemical elements on living organisms in the environment has recently been approved as obligatory by law, and is known as biomonitoring. For human biomonitoring, non-invasive matrices like urine, saliva, and hair are the only options. Hair samples are ideal for evaluating prolonged exposure, as opposed to short-term exposure. The use of hair mineral analysis (HMA) has become an intriguing diagnostic tool for assessing toxic element exposure and evaluating health and nutritional status in the context of biomonitoring [64–67].

It is worth noting that, throughout the course of orthodontic treatment, the fixed appliance undergoes a variety of transformations. One such transformation occurs through changes in the surface of the appliance, which can lead to the emergence of metal elution or coverage on the surface due to the process of corrosion or passivation. Mikulewicz et al. [68] also examined the release of metal ions from orthodontic appliances and their sites of accumulation in pigs. The aorta, cheek, and hair sampled after three months showed the largest discrepancies in toxic metal content, with the experimental group exhibiting Ni levels 4.8 and 3.5 times higher, respectively, and Cr levels 3.4 times higher, respectively, than the control group.

The overall impact is greatly influenced by environmental conditions. Currently, the analysis of both dental health and overall physical well-being can be conducted using a combination of hair and tooth samples. The examination of bioindicators is a common practice for assessing environmental pollution levels. The examination of tooth and hair tissues can reveal the presence of different chemical elements. Shishniashvili et al. [69] evaluated primary teeth and hair as indicators of environmental pollution. Toxic elements were found to be at higher levels in polluted areas compared to areas with less pollution. The prevalence and severity of caries were found to be significantly related to regions with high levels of pollution. Furthermore, there was a clear correlation between the amount of pollution present and the extent of caries in affected populations.

Using atomic absorption spectrometry, Alomary et al. [70] conducted a study to measure the lead and cadmium levels in human teeth from Jordan. The results showed that the concentrations of Pb and Cd in the teeth of smokers were significantly higher than those of non-smokers. Meanwhile, Rayad et al. [71] examined the concentration of toxic metals, including Pb and Cd, in the third molars of people living in the Legnica–Głogów Copper Area. The study found that the content of all examined heavy metals increased with the age of the participants, but this was only found to be statistically significant for zinc, copper and chromium. Statistical analysis revealed that there were significantly higher levels of iron and lead in teeth extracted from patients residing in the L-G Copper Area than from those living in Wrocław, suggesting a possible link to increased environmental pollution in the former region during mineralization.

In a study conducted by Strugała-Stawik et al. [72], the average levels of lead in children's blood were assessed, taking into account gender and proximity to the Legnica smelter. The study was conducted between 1991 and 2009. The findings of the research indicate a gradual decline in the concentration of lead in children's blood. However, the presence of even small amounts of lead can have detrimental effects on cognitive abilities, learning aptitude, and memory retention, and have even been linked to the development of ADHD or antisocial behavior [73,74].

Mercury is categorized as a toxic metal, having earned the nickname "death metal" due to its extreme toxicity. Of all the potential sources of exposure to mercury, dental amalgam and mercury vapor from the production of chlorine are the most significant environmental sources, while occupational exposure is the most significant among all sources. Mercury dissolves in fats with ease, allowing it to infiltrate biological membranes. Mercury exposure has been linked with numerous harmful effects on various systems in the human body, such as the nervous system, the cardiovascular system, the endocrine system, and the kidneys.

The detrimental impact of mercury on organ structure and function is well documented [75]. Mercury's effects on the reproductive system should not be underestimated, as it can lead to endometrial dysfunction, implantation failure, premature delivery, subfertility, and spontaneous abortion [47,76].

Eide et al. [77] evaluated the content of mercury in primary teeth. Their research aimed to measure the amount of mercury present in primary teeth remaining from preindustrial times and compare it with a sample of primary teeth from modern-day Norway. According to the authors, the values obtained for mercury in the preindustrial primary teeth were indicative of minimal mercury levels being found in teeth, and were likely the result of natural environmental sources. Additionally, these values may serve as a benchmark for research on primary teeth from both preindustrial and contemporary time periods. Tvinnereim et al. [78] also analyzed the level of mercury in the primary teeth of humans, taking into account various factors that could influence the concentration of this metal. The research discovered variances in the concentrations of metal among the groups of teeth for lead, mercury, and zinc. Notably, positive correlations were found between lead and the other three metals, as well as between mercury and zinc.

Eide et al. [79] observed that rat teeth are prone to absorbing mercury once it has been introduced into the system, whether the mercury is in an organic or inorganic form. This finding is particularly significant, due to the prevalence of organic mercury in marine food sources. Conversely, inorganic mercury is the dominant form of mercury found in drinking water, and this can have significant ramifications in industrial contexts.

In the present study, it was found that the mercury content in extracted wisdom teeth was slightly higher in patients from the Legnica–Głogów Copper Area compared to patients from other areas (0.389 μg/g vs. 0.341 μg/g). However, this difference was not considered to be statistically significant (p-value = 0.655). It is possible that this may indicate an improvement in environmental conditions with respect to mercury. The lack of statistical significance with respect to age in the control group may be due to a missing reference category. There were no people aged 38–45 in the control group. Upon analysis of the data and the observations gathered, it was revealed that the concentration of mercury in the L-G Copper District escalated in conjunction with both age and duration of residency. In the control group, a noticeable correlation existed between the presence of cardiac ailments and the increase in Hg concentration.

Regular monitoring is deemed necessary, as anthropogenic pollution is found not only in industrialized and agricultural regions, but also in natural ecosystems, leading to the conclusion that it is widespread. In order to assess the extent of heavy metal pollution in the Warmia and Mazury region of Poland, Giżejewska et al. [80] analyzed the concentrations of mercury (Hg), lead (Pb) and cadmium (Cd) in the liver, muscles, and kidneys of European beavers (Castor fiber). The concentration of the elements was determined by means of atomic absorption spectrometry, and the metals were found to be present in all samples of individual tissues. Although the average concentrations of Pb and Hg were relatively low, the concentration of Cd was found to be high, particularly in the kidneys (7.933 mg/kg) and liver (0.880 mg/kg).

It is worth mentioning that dietary supplements containing shark liver oils, cod liver, and vegetables may potentially contain mercury. Brodziak-Dopierała et al. [81] examined mercury concentration in these supplements. The acceptable standard for the amount of Hg in dietary supplements (0.10 mg/kg) was not surpassed in any of the tested samples. In order to ensure consumer safety, it is also crucial to understand the levels of heavy metals present in crayfish tissues used for food due to the potential risk of contamination. Stanek et al. [82] conducted a study on the levels of mercury and other toxic metals detected in both the exoskeleton and abdominal muscles of spiny-cheek crayfish (Orconectes limosus) found in Lake Gopło. The heavy metal content in spiny-cheek muscles from Lake Gopło did not surpass the legal limits for fish and crayfish meant for human consumption, with the exception of lead.

In summary, biomonitoring enables comparative research on how environmental differences affect human health. This is accomplished by analyzing bone material taken from significant joint replacements or permanent teeth extracted for orthodontic or periodontal reasons. Teeth are a prime choice for biomaterials due to their accessibility and reliability as markers of exposure to environmental pollution. Furthermore, they provide a permanent, cumulative, and stable record of this exposure [70]. The third molars of individuals residing in the Legnica–Głogów Copper Area have proven to be a valuable biomonitor for determining environmental exposure to toxic metals.

5. Conclusions

The obtained results and observations showed that the concentration of mercury increased with the age and time of residence in the L-G Copper District. The presence of cardiac diseases was significantly associated with increased Hg concentration in the control group. Further investigation is needed to examine the influence of the habitat on the concentration of toxic metals in living organisms.

6. Study Limitations

The study was performed during the COVID-19 pandemic, between June 2020 and June 2021. Due to these circumstances, the number of patients in both groups was limited. Statistical tests were conducted, and test power was analyzed for both groups. After analyzing the concentration levels of Hg, we carried out a power examination that revealed the test power to be 0.65. The results indicated that the size of the study group was too small. While the authors acknowledge the limited scope of the study, they have plans to perform further examinations in future.

Author Contributions: Conceptualization, S.R., M.D. (Maciej Dobrzyński) and M.D. (Marzena Dominiak); methodology, S.R. and M.S.; software, S.R. and A.K.; validation, S.R. and M.S.; formal analysis, D.D.; investigation, M.S. and S.R.; resources, S.R. and A.K.; data curation, M.S. and S.R.; writing—original draft preparation, S.R., D.D., S.K. and A.K.; writing—review and editing, M.D. (Maciej Dobrzyński), M.D. (Marzena Dominiak), D.D., T.G. (Tomasz Gedrange) and T.G. (Tomasz Gębarowski); visualization, A.K. and S.R.; supervision, M.D. (Maciej Dobrzyński) and M.D. (Marzena Dominiak); project administration, M.D. (Maciej Dobrzyński), M.D. (Marzena Dominiak) and T.G.; funding acquisition, M.D. (Maciej Dobrzyński), M.D. (Marzena Dominiak), T.G. (Tomasz Gedrange) and T.G. (Tomasz Gębarowski). All authors have read and agreed to the published version of the manuscript.

Funding: This article was co-financed by a subsidy from Wroclaw Medical University, number SUBZ.B180.23.054, SUBZ.B040.23.048 and SUBZ.B020.23.051. The APC costs were also supported by statutory research and development activity funds assigned to the Faculty of Veterinary Medicine and the Faculty of Biotechnology and Food Science, Wrocław University of Environmental and Life Sciences.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. Approval was obtained from the Bioethics Committee of Wroclaw Medical University: KB-246/2019.

Informed Consent Statement: Informed consent was obtained from the patients involved in the study.

Data Availability Statement: Not applicable.

Acknowledgments: The figures were partly generated using BioRender.com (accessed on 17 August 2023).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gil, F.; Hernández, A.F. Toxicological importance of human biomonitoring of metallic and metalloid elements in different biological samples. *Food Chem. Toxicol.* **2015**, *80*, 287–297. [CrossRef] [PubMed]
2. Jańczuk, Z.; Kaczmarek, U.; Lipski, M. *Stomatologia Zachowawcza z Endodoncją. Zarys Kliniczny, Etiologia Próchnicy*; PZWL: Warszawa, Poland, 2014.

3. Asaduzzaman, K.; Khandaker, M.U.; Binti Baharudin, N.A.; Amin, Y.B.M.; Farook, M.S.; Bradley, D.A.; Mahmoud, O. Heavy metals in human teeth dentine: A bio-indicator of metals exposure and environmental pollution. *Chemosphere* **2017**, *176*, 221–230. [CrossRef]
4. Buddhachat, K.; Klinhom, S.; Siengdee, P.; Brown, J.L.; Nomsiri, R.; Kaewmong, P.; Thitaram, C.; Mahakkanukrauh, P.; Nganvongpanit, K. Elemental Analysis of Bone, Teeth, Horn and Antler in Different Animal Species Using Non-Invasive Handheld X-Ray Fluorescence. *PLoS ONE* **2016**, *11*, e0155458. [CrossRef] [PubMed]
5. Chudzińska, E.; Celiński, K.; Pawlaczyk, E.M.; Wojnicka-Półtorak, A.; Diatta, J.B. Trace element contamination differentiates the natural population of Scots pine: Evidence from DNA microsatellites and needle morphology. *Environ. Sci. Pollut. Res. Int.* **2016**, *23*, 22151–22162. [CrossRef]
6. Martínez-García, M.J.; Moreno, J.M.; Moreno-Clavel, J.; Vergara, N.; García-Sánchez, A.; Guillamón, A.; Portí, M.; Moreno-Grau, S. Heavy metals in human bones in different historical epochs. *Sci. Total Environ.* **2005**, *348*, 51–72. [CrossRef] [PubMed]
7. Kosugi, H.; Hanihara, K.; Suzuki, T.; Himeno, S.; Kawabe, T.; Hongo, T.; Morita, M. Elemental composition of ancient Japanese bones. *Sci. Total Environ.* **1986**, *52*, 93–107. [CrossRef]
8. Pawlicki, R.; Bolechała, P. X-ray microanalysis of fossil dinosaur bone: Age differences in lead, iron, and magnesium content. *Folia Histochem. Cytobiol.* **1991**, *29*, 81–83.
9. Piccioli, A.; Donati, F.; Giacomo, G.D.; Ziranu, A.; Careri, S.; Spinelli, M.S.; Giannini, S.; Giannicola, G.; Perisano, C.; Maccauro, G. Infective complications in tumour endoprostheses implanted after pathological fracture of the limbs. *Injury* **2016**, *47* (Suppl. 4), S22–S28. [CrossRef]
10. Smrčka, V.; Jambor, J. Trace elements and the European skeleton through 5000 years. *Acta Univ. Carol. Med.* **2000**, *41*, 59–68.
11. Herman, K.; Korczyński, M.; Janeczek, M.; Wełmiński, P.; Kowalczyk-Zając, M.; Leśków, A.; Całkosiński, I.; Dobrzyński, M. Effect of toxic metals on oral tissues. *J. Educ. Health Sport* **2017**, *7*, 209–220.
12. Jaishankar, M.; Tseten, T.; Anbalagan, N.; Mathew, B.B.; Beeregowda, K.N. Toxicity, mechanism and health effects of some heavy metals. *Interdiscip. Toxicol.* **2014**, *7*, 60–72. [CrossRef] [PubMed]
13. Badhe, R.V.; Akinfosile, O.; Bijukumar, D.; Barba, M.; Mathew, M.T. Systemic toxicity eliciting metal ion levels from metallic implants and orthopedic devices—A mini review. *Toxicol. Lett.* **2021**, *350*, 213–224. [CrossRef]
14. Přikrylová, J.; Procházková, J.; Podzimek, Š. Side Effects of Dental Metal Implants: Impact on Human Health (Metal as a Risk Factor of Implantologic Treatment). *BioMed Res. Int.* **2019**, *2019*, 2519205. [CrossRef]
15. Waseem, A.; Arshad, J.; Iqbal, F.; Sajjad, A.; Mehmood, Z.; Murtaza, G. Pollution status of Pakistan: A retrospective review on heavy metal contamination of water, soil, and vegetables. *BioMed Res. Int.* **2014**, *2014*, 813206. [CrossRef]
16. Cheng, S. Effects of heavy metals on plants and resistance mechanisms. A state-of-the-art report with special reference to literature published in Chinese journals. *Environ. Sci. Pollut. Res. Int.* **2003**, *10*, 256–264. [CrossRef] [PubMed]
17. He, Z.L.; Yang, X.E.; Stoffella, P.J. Trace elements in agroecosystems and impacts on the environment. *J. Trace Elem. Med. Biol.* **2005**, *19*, 125–140. [CrossRef] [PubMed]
18. Mao, X.; Sun, J.; Shaghaleh, H.; Jiang, X.; Yu, H.; Zhai, S.; Hamoud, Y.A. Environmental Assessment of Soils and Crops Based on Heavy Metal Risk Analysis in Southeastern China. *Agronomy* **2023**, *13*, 1107. [CrossRef]
19. Rachwał, K.; Waśko, A.; Gustaw, K.; Polak-Berecka, M. Utilization of brewery wastes in food industry. *PeerJ* **2020**, *8*, e9427. [CrossRef]
20. Zhao, B.; Yan, Y.; Chen, S. How could haloalkaliphilic microorganisms contribute to biotechnology? *Can. J. Microbiol.* **2014**, *60*, 717–727. [CrossRef]
21. Qureshi, A.S.; Hussain, M.I.; Ismail, S.; Khan, Q.M. Evaluating heavy metal accumulation and potential health risks in vegetables irrigated with treated wastewater. *Chemosphere* **2016**, *163*, 54–61. [CrossRef]
22. Khan, I.; Awan, S.A.; Rizwan, M.; Ali, S.; Hassan, M.J.; Brestic, M.; Zhang, X.; Huang, L. Effects of silicon on heavy metal uptake at the soil-plant interphase: A review. *Ecotoxicol. Environ. Saf.* **2021**, *222*, 112510. [CrossRef] [PubMed]
23. Bešter, P.K.; Lobnik, F.; Eržen, I.; Kastelec, D.; Zupan, M. Prediction of cadmium concentration in selected home-produced vegetables. *Ecotoxicol. Environ. Saf.* **2013**, *96*, 182–190. [CrossRef] [PubMed]
24. Chen, C.; Zhou, Q.; Cai, Z. Effect of soil HHCB on cadmium accumulation and phytotoxicity in wheat seedlings. *Ecotoxicology* **2014**, *23*, 1996–2004. [CrossRef]
25. Dziubanek, G.; Piekut, A.; Rusin, M.; Baranowska, R.; Hajok, I. Contamination of food crops grown on soils with elevated heavy metals content. *Ecotoxicol. Environ. Saf.* **2015**, *118*, 183–189. [CrossRef] [PubMed]
26. Dziubanek, G.; Baranowska, R.; Ćwieląg-Drabek, M.; Spychała, A.; Piekut, A.; Rusin, M.; Hajok, I. Cadmium in edible plants from Silesia, Poland, and its implications for health risk in populations. *Ecotoxicol. Environ. Saf.* **2017**, *142*, 8–13. [CrossRef] [PubMed]
27. Gattullo, C.E.; Mininni, C.; Parente, A.; Montesano, F.F.; Allegretta, I.; Terzano, R. Effects of municipal solid waste- and sewage sludge-compost-based growing media on the yield and heavy metal content of four lettuce cultivars. *Environ. Sci. Pollut. Res. Int.* **2017**, *24*, 25406–25415. [CrossRef]
28. Paltseva, A.; Cheng, Z.; Deeb, M.; Groffman, P.M.; Shaw, R.K.; Maddaloni, M. Accumulation of arsenic and lead in garden-grown vegetables: Factors and mitigation strategies. *Sci. Total Environ.* **2018**, *640–641*, 273–283. [CrossRef]
29. Song, Y.; Jin, L.; Wang, X. Cadmium absorption and transportation pathways in plants. *Int. J. Phytoremed.* **2017**, *19*, 133–141. [CrossRef]

30. Wang, B.; Zhou, Y.; Li, L.; Xu, H.; Sun, Y.; Wang, Y. Novel synthesis of cyano-functionalized mesoporous silica nanospheres (MSN) from coal fly ash for removal of toxic metals from wastewater. *J. Hazard. Mater.* **2018**, *345*, 76–86. [CrossRef]
31. Zhou, Y.; Liu, J. Emissions, environmental levels, sources, formation pathways, and analysis of polybrominated dibenzo-p-dioxins and dibenzofurans: A review. *Environ. Sci. Pollut. Res. Int.* **2018**, *25*, 33082–33102. [CrossRef]
32. Grzegorczyk, L.; Stawiński, K. *Zarys Stomatologii Przemysłowej*; PZWL: Warszawa, Poland, 1975.
33. Pan, T.L.; Wang, P.W.; Aljuffali, I.A.; Huang, C.T.; Lee, C.W.; Fang, J.Y. The impact of urban particulate pollution on skin barrier function and the subsequent drug absorption. *J. Dermatol. Sci.* **2015**, *78*, 51–60. [CrossRef]
34. Całkosiński, I.; Dobrzyński, M.; Całkosińska, M.; Seweryn, E.; Bronowicka-Szydełko, A.; Dzierzba, K.; Ceremuga, I.; Gamian, A. Characterization of an inflammatory response. *Postepy Hig Med Dosw (Online)* **2009**, *63*, 395–408.
35. Kołacz, R.; Dobrzański, Z.; Kupczyński, R.; Cwynar, P.; Opaliński, S.; Pogoda-Sewerniak, K. Impact of the copper industry on the content of selected heavy metals and biochemical indicators in the blood of dairy cows. *Med. Weter* **2017**, *73*, 171–175. [CrossRef]
36. Pandey, G.; Sharma, M. Heavy metals causing toxicity in animal and fishes. *Res. J. Anim. Vet. Fish. Sci.* **2014**, *2*, 17–23.
37. Tchounwou, P.B.; Yedjou, C.G.; Patlolla, A.K.; Sutton, D.J. Heavy metal toxicity and the environment. In *Molecular, Clinical and Environmental Toxicology*; Springer: Basel, Switzerland, 2012; Volume 3, pp. 133–164. [CrossRef]
38. Rotter, I.; Kosik-Bogacka, D.; Dołęgowska, B.; Safranow, K.; Lubkowska, A.; Laszczyńska, M. Relationship between the concentrations of heavy metals and bioelements in aging men with metabolic syndrome. *Int. J. Environ. Res. Public Health* **2015**, *12*, 3944–3961. [CrossRef] [PubMed]
39. Van Wyk, E.; Van der Bank, F.H.; Verdoorn, G.H.; Hofmann, D. Selected mineral and heavy metal concentrations in blood and tissues of vultures in different regions of South Africa. *S. Afr. J. Anim. Sci.* **2001**, *31*, 57–64.
40. Jan, A.T.; Azam, M.; Siddiqui, K.; Ali, A.; Choi, I.; Haq, Q.M. Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants. *Int. J. Mol. Sci.* **2015**, *16*, 29592–29630. [CrossRef]
41. Wang, H.; Shan, X.Q.; Wen, B.; Zhang, S.; Wang, Z.J. Responses of antioxidative enzymes to accumulation of copper in a copper hyperaccumulator of *Commoelina communis*. *Arch. Environ. Contam. Toxicol.* **2004**, *47*, 185–192. [CrossRef]
42. Dudek-Adamska, D.; Lech, T.; Konopka, T.; Kościelniak, P. Chromium in Postmortem Material. *Biol. Trace Elem. Res.* **2018**, *186*, 370–378. [CrossRef]
43. Sall, M.L.; Diaw, A.K.D.; Gningue-Sall, D.; Efremova Aaron, S.; Aaron, J.J. Toxic heavy metals: Impact on the environment and human health, and treatment with conducting organic polymers, a review. *Environ. Sci. Pollut. Res. Int.* **2020**, *27*, 29927–29942. [CrossRef]
44. Mitra, S.; Chakraborty, A.J.; Tareq, A.M.; Emran, T.B.; Nainu, F.; Khusro, A.; Idris, A.M.; Khandaker, M.U.; Osman, H.; Alhumaydhi, F.A.; et al. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. *J. King Saud Univ.-Sci.* **2022**, *34*, 101865. [CrossRef]
45. Järup, L. Hazards of heavy metal contamination. *Br. Med. Bull.* **2003**, *68*, 167–182. [CrossRef]
46. Ociepa-Kubicka, A.; Ociepa, E. Toxic effects of heavy metals on plants, animals and humans. In *Inżynieria i Ochrona Środowiska*; Wydawnictwo Politechniki Częstochowskiej: Częstochowa, Poland, 2012; Volume 15, pp. 169–180.
47. Kot, K.; Kosik-Bogacka, D.; Łanocha-Arendarczyk, N.; Ciosek, Ż. Wpływ związków rtęci na organizm człowieka. *Farm. Współczesna* **2016**, *9*, 210–216.
48. Compston, J.E. Skeletal actions of intermittent parathyroid hormone: Effects on bone remodelling and structure. *Bone* **2007**, *40*, 1447–1452. [CrossRef]
49. Wilson, L.R.; Tripkovic, L.; Hart, K.H.; Lanham-New, S.A. Vitamin D deficiency as a public health issue: Using Vitamin D2 or Vitamin D3 in future fortification strategies. *Proc. Nutr. Soc.* **2017**, *76*, 392–399. [CrossRef]
50. Botelho, J.; Machado, V.; Proença, L.; Delgado, A.S.; Mendes, J.J. Vitamin D Deficiency and Oral Health: A Comprehensive Review. *Nutrients* **2020**, *12*, 1471. [CrossRef]
51. Krawiec, M.; Dominiak, M. Prospective evaluation of vitamin D levels in dental treated patients: A screening study. *Dent. Med. Probl.* **2021**, *58*, 321–326. [CrossRef] [PubMed]
52. Albalawi, O.; Almubark, R.; Almarshad, A.; Alqahtani, A.S. The Prevalence of Vitamin and Mineral Deficiencies and High Levels of Non-Essential Heavy Metals in Saudi Arabian Adults. *Healthcare* **2022**, *10*, 2415. [CrossRef] [PubMed]
53. Cho, G.J.; Park, H.T.; Shin, J.H.; Hur, J.Y.; Kim, S.H.; Lee, K.W.; Kim, T. The relationship between blood mercury level and osteoporosis in postmenopausal women. *Menopause J. N. Am. Menopause Soc.* **2012**, *19*, 576–581. [CrossRef] [PubMed]
54. *PN-EN 13805:2003*; Food Products-Determination of Trace Elements-Pressure Mineralization. European Commission: Brussels, Belgium, 2003.
55. Całkosiński, I.; Rosińczuk-Tonderys, J.; Bronowicka-Szydełko, A.; Dzierzba, K.; Bazan, J.; Dobrzyński, M.; Majda, J.; Gamian, A. Effect of tocopherol on biochemicalbloodparameters in pleuritis-inducedratstreated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol. Ind. Health* **2015**, *31*, 510–522. [CrossRef]
56. Bryła, E.; Dobrzyński, M.; Konkol, D.; Kuropka, P.; Styczyńska, M.; Korczyński, M. Toxic Metals Content in Impacted Third Molars and Adjacent Bone Tissue in Different Groups of Patients. *Materials* **2021**, *14*, 793. [CrossRef] [PubMed]
57. Calkosinski, I.; Rosinczuk-Tonderys, J.; Dobrzynski, M.; Palka, L.; Bazan, J. Occurrence of disseminated intravascular coagulation in 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced pneumonia in the rat. *Adv. Exp. Med. Biol.* **2013**, *788*, 283–292. [CrossRef]
58. Liu, Y.; Liu, J.; Habeebu, S.M.; Waalkes, M.P.; Klaassen, C.D. Metallothionein-I/II null mice are sensitive to chronic oral cadmium-induced nephrotoxicity. *Toxicol. Sci.* **2000**, *57*, 167–176. [CrossRef] [PubMed]

59. Sitarz-Palczak, E.; Kalembkiewicz, J. Study of Remediation of Soil Contamined with Heavy Metals by Coal Fly Ash. *J. Environ. Prot.* **2012**, *3*, 1373–1383. [CrossRef]
60. Kabata-Pendias, A.; Pendias, H. *Biogeochemistry of Trace Elements*; Polish Scientific Publishing Company: Varsov, Poland, 1999.
61. Niemi, A.; Venäläinen, E.R.; Hirvi, T.; Hirn, J.; Karppanen, E. The lead, cadmium and mercury concentrations in muscle, liver and kidney from Finnish pigs and cattle during 1987-1988. *Z. Lebensm.-Unters. Forsch.* **1991**, *192*, 427–429. [CrossRef]
62. Wong, A.C.; Ryan, A.F. Mechanisms of sensorineural cell damage, death and survival in the cochlea. *Front. Aging. Neurosci.* **2015**, *7*, 58. [CrossRef] [PubMed]
63. Dusek, P.; Litwin, T.; Czlonkowska, A. Wilson disease and other neurodegenerations with metal accumulations. *Neurol. Clin.* **2015**, *33*, 175–204. [CrossRef]
64. Mikulewicz, M.; Chojnacka, K.; Gedrange, T.; Górecki, H. Reference values of elements in human hair: A systematic review. *Environ. Toxicol. Pharmacol.* **2013**, *36*, 1077–1086. [CrossRef]
65. Mikulewicz, M.; Chojnacka, K.; Zielińska, A.; Michalak, I. Exposure to metals from orthodontic appliances by hair mineral analysis. *Environ. Toxicol. Pharmacol.* **2011**, *32*, 10–16. [CrossRef] [PubMed]
66. Li, Y.; Yang, L.; Wang, W.; Li, H.; Lv, J.; Zou, X. Trace element concentrations in hair of healthy Chinese centenarians. *Sci. Total Environ.* **2011**, *409*, 1385–1390. [CrossRef] [PubMed]
67. Mikulewicz, M.; Wołowiec, P.; Michalak, I.; Chojnacka, K.; Czopor, W.; Berniczei-Royko, A.; Vegh, A.; Gedrange, T. Mapping chemical elements on the surface of orthodontic appliance by SEM-EDX. *Med. Sci. Monit.* **2014**, *20*, 860–865. [CrossRef] [PubMed]
68. Mikulewicz, M.; Wołowiec, P.; Janeczek, M.; Gedrange, T.; Chojnacka, K. The release of metal ions from orthodontic appliances animal tests. *Angle Orthod.* **2014**, *84*, 673–679. [CrossRef] [PubMed]
69. Shishniashvili, T.; Suladze, N.; Margvelashvili, V. Primary Teeth and Hair as Indicators of Environmental Pollution. *J. Clin. Pediatr. Dent.* **2016**, *40*, 152–155. [CrossRef] [PubMed]
70. Alomary, A.; Al-Momani, I.F.; Massadeh, A.M. Lead and cadmium in human teeth from Jordan by atomic absorption spectrometry: Some factors influencing their concentrations. *Sci. Total Environ.* **2006**, *369*, 69–75. [CrossRef]
71. Rayad, S.; Dobrzyński, M.; Kuźniarski, A.; Styczyńska, M.; Diakowska, D.; Gedrange, T.; Klimas, S.; Gębarowski, T.; Dominiak, M. An In-Vitro Evaluation of Toxic Metals Concentration in the Third Molars from Residents of the Legnica-Głogów Copper Area and Risk Factors Determining the Accumulation of Those Metals: A Pilot Study. *Appl. Sci.* **2023**, *13*, 2904. [CrossRef]
72. Strugała-Stawik, H.; Rudkowski, Z.; Pastuszek, B.; Morawiec, K. Biomonitoring of lead in blood of children—Short assessment of results 1991–2009. *Med. Srod.* **2010**, *13*, 11–14.
73. Collaborative on Health and the Environment's Learning and Developmental Disabilities. Scientific Consensus Statement on Environmental Agents Associated with Neurodevelopmental Disorders. 2008. Available online: https://www.healthandenvironment.org/docs/xaruploads/LDDIStatement.pdf (accessed on 17 August 2023).
74. Wilhelm, M.; Heinzow, B.; Angerer, J.; Schulz, C. Reassessment of critical lead effects by the German Human Biomonitoring Commission results in suspension of the human biomonitoring values (HBM I and HBM II) for lead in blood in children adults. *Int. J. Hyg. Environ. Health* **2010**, *4*, 233–320. [CrossRef] [PubMed]
75. Cyran, M. Wpływ środowiskowego narażenia na rtęć na funkcjonowanie organizmu człowieka. *Med. Srod.* **2013**, *16*, 55–58.
76. Rzymski, P.; Tomczyk, K.; Rzymski, P.; Poniedziałek, B.; Opala, T.; Wilczak, M. Impact of heavy metals on the female reproductive system. *Ann. Agric. Environ. Med.* **2015**, *22*, 259–264. [CrossRef]
77. Eide, R.; Wesenberg, G.B.; Fosse, G. Mercury in primary teeth in preindustrial Norway. *Scand. J. Dent. Res.* **1993**, *101*, 1–4. [CrossRef]
78. Tvinnereim, H.; Eide, R.; Riise, T. Heavy metals in human primary teeth: Some factors influencing the metal concentrations. *Sci. Total Environ.* **2000**, *255*, 21–27. [CrossRef]
79. Eide, R.; Schiønning, J.D.; Ernst, E.; Hansen, I.M.; Wesenberg, G.R. Mercury content in rat teeth after administration of organic and inorganic mercury. The effects of interrupted exposure and of selenite. *Acta Odontol. Scand.* **1995**, *53*, 12–16. [CrossRef] [PubMed]
80. Giżejewska, A.; Spodniewska, A.; Barski, D. Concentration of lead, cadmium, and mercury in tissues of European beaver (Castor fiber) from the north-eastern Poland. *J. Vet. Res.* **2014**, *58*, 77–80. [CrossRef]
81. Brodziak-Dopierała, B.; Fischer, A.; Chrzanowska, M.; Ahnert, B. Mercury Exposure from the Consumption of Dietary Supplements Containing Vegetable, Cod Liver, and Shark Liver Oils. *Int. J. Environ. Res. Public Health* **2023**, *20*, 2129. [CrossRef]
82. Stanek, M.; Dąbrowski, J.; Różański, S.; Janicki, B.; Długosz, J. Heavy Metals Bioaccumulation in Tissues of Spiny-Cheek Crayfish (*Orconectes limosus*) from Lake Gopło: Effect of Age and Sex. *Bull. Environ. Contam. Toxicol.* **2017**, *98*, 740–746. [CrossRef] [PubMed]

Journal of
Clinical Medicine

MDPI

Article

Comparative Analysis of Heavy Metal Content in Impacted Third Molars from Industrial and Non-Industrial Areas and Its Effect on the Isolation, Culture, and Proliferation of Dental Stem Cells (DSCs)

Benita Wiatrak [1], Sadri Rayad [2], Tomasz Gębarowski [3,*], Jakub Hadzik [4], Marzena Styczyńska [5], Tomasz Gedrange [4], Maciej Dobrzyński [6], Ewa Barg [7] and Marzena Dominiak [4]

[1] Department of Pharmacology, Faculty of Medicine, Wroclaw Medical University, Mikulicza-Radeckiego 2, 50-345 Wroclaw, Poland; benita.wiatrak@umw.edu.pl
[2] Academic Dental Polyclinic of Dental Center, Technology Transfer Ltd., Krakowska 26, 50-425 Wroclaw, Poland; sadri.rayad@gmail.com
[3] Department of Biostructure and Animal Physiology, The Wroclaw University of Environmental and Life Sciences, Kożuchowska 1/3, 51-631 Wroclaw, Poland
[4] Department of Dental Surgery, Wroclaw Medical University, Krakowska 26, 50-425 Wroclaw, Poland; jakub.hadzik@umw.edu.pl (J.H.); tomasz.gedrange@umw.edu.pl (T.G.); marzena.dominiak@umw.edu.pl (M.D.)
[5] Department of Human Nutrition, Wroclaw University of Environmental and Life Sciences, Chelmonskiego 37/41, 51-630 Wroclaw, Poland; marzena.styczynska@upwr.edu.pl
[6] Department of Pediatric Dentistry and Preclinical Dentistry, Wroclaw Medical University, Krakowska 26, 50-425 Wroclaw, Poland; maciej.dobrzynski@umw.edu.pl
[7] Department of Basic Medical Sciences, Wroclaw Medical University, Borowska 211A, 50-556 Wroclaw, Poland; ewa.barg@umw.edu.pl
* Correspondence: tomasz.gebarowski@upwr.edu.pl

check for updates

Citation: Wiatrak, B.; Rayad, S.; Gębarowski, T.; Hadzik, J.; Styczyńska, M.; Gedrange, T.; Dobrzyński, M.; Barg, E.; Dominiak, M. Comparative Analysis of Heavy Metal Content in Impacted Third Molars from Industrial and Non-Industrial Areas and Its Effect on the Isolation, Culture, and Proliferation of Dental Stem Cells (DSCs). *J. Clin. Med.* **2024**, *13*, 5465. https://doi.org/10.3390/jcm13185465

Academic Editor: Lei Liu

Received: 3 August 2024
Revised: 7 September 2024
Accepted: 10 September 2024
Published: 14 September 2024

Abstract: Background: This study investigates the impact of environmental pollution on the quality and viability of dental stem cells (DSCs) from impacted third molars. By comparing DSCs from patients in industrial areas with high air pollution and those from non-industrial regions, the research assesses the adverse effects of heavy metals on stem cell proliferation. **Methods**: Impacted lower third molars were collected from 28 patients—10 from industrial and 18 from non-industrial areas. Patients were divided into two age groups: 18–27 years and 28–38 years old. Dental pulp was extracted under sterile conditions, and DSCs were isolated and cultured. Heavy metal concentrations in dental tissues were measured using atomic absorption/emission spectrometry. **Results**: The study found significantly higher concentrations of copper and lead in the dental tissues of patients in industrial areas. Cell viability was lower in samples from these areas, with a statistically significant difference in average doubling time and the number of cells obtained after the first passage. There was no significant impact of gender on heavy metal content, except for higher iron levels in men. **Conclusions**: Exposure to industrial pollutants negatively affects the viability and proliferation of DSCs, but there are no differences in differentiation in the osteogenic medium regarding cell mineralization. These studies highlight the importance of environmental factors for oral health, suggesting that residents of polluted areas may face greater difficulties in dental and regenerative treatments. Further research is needed to develop strategies to mitigate the effects and improve clinical outcomes for affected populations.

Keywords: regenerative medicine; DPSCs; tissue regeneration; oral medicine

1. Introduction

Medicine and modern dentistry increasingly utilize the potential of transplantation. Unfortunately, not every case yields a satisfactory clinical result [1–3]. One common approach is bone transplantation, using material from the patient (autogenous), another donor (allogeneic), or a synthetic product (xenogeneic). While autologous bone grafting remains the "gold standard", harvesting from sites like the hip or rib is invasive and painful, leading to the use of allogeneic or xenogeneic methods, which carry different risks [1,2].

Allogeneic materials are purified using various protocols, such as heat treatment or gamma radiation, removing over 95% of leukocytes and plasma components. Though traditionally considered low risk for alloimmunization, bone grafts can trigger immune responses, which may impact treatment outcomes [4–7].

Bone grafts are often necessary in maxillofacial surgery and orthodontic treatments, where bone loss is common, even in younger patients. The goal of using stem cells is rapid bone regeneration, restoring its shape, function, and blood supply, making it an excellent option for craniofacial defects or bone atrophy caused by trauma, disease, or cancer [8–10].

Stem cells from bone marrow (BMSCs) were first used in medicine, but obtaining them is invasive and yields fewer cells than from dental pulp. Adipose tissue is another source, but isolating stem cells requires large volumes of fat and poses risks like brain or heart embolism [11–13]. In contrast, dental pulp stem cells (DSCs) offer a safer and more efficient option for obtaining mesenchymal stem cells (MSCs) [14].

The dental pulp contains odontoblasts, fibroblasts, and DSCs, including dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHEDs), and periodontal ligament stem cells (PDLSCs). These cells exhibit multipotent characteristics, making them valuable for regenerative treatments. Extracted teeth, often discarded, can be a rich source of DPSCs [15].

Environmental factors, particularly heavy metals, can significantly affect the quality of DSCs. Studies show that metals like cadmium, lead, and mercury accumulate in dental tissues, posing health risks. Teeth can serve as biomarkers for environmental and occupational exposure to these metals [16].

This study aimed to conduct a detailed assessment of the quality of cells obtained from patients who required the extraction of third molars. The study aimed to compare the quality of cells from patients living in industrial areas, characterized by high levels of air pollution, with the quality of cells from patients in areas with lower pollution levels. The analysis aimed to identify potential differences in cell quality that may be associated with exposure to environmental pollutants and to assess the impact of these pollutants on the cellular health of patients. The results of the study may provide valuable information on the impact of the environment on oral health and the overall health condition of patients.

2. Materials and Methods

Before conducting the study, approval was obtained from the Bioethics Committee of Wroclaw Medical University, Wrocław, Poland (approval number: KB-246/2019 and KB-34/2020).

2.1. Materials

The material consisted of impacted lower third molars collected from 28 patients: 10 residents of the Glogow-Legnica district (industrial) and 18 residents of Wroclaw (non-industrial city), who underwent third molars extraction surgery [17]. Patients of both sexes were divided into two groups: young (18–27 years old) and mature (28–38 years old). During the study, data were collected regarding gender, age, place of permanent residence, lack of occupational exposure to toxic metals, lack of smoking habits, and the use of dietary supplements. The assignment of patients to industrial or non-industrial area groups was based on self-reported place of residence, without specific inquiries into dietary habits, smoking, or occupational exposure to heavy metals. This lack of control over additional influencing factors presents a limitation of the study, as these factors could potentially affect heavy metal accumulation and DSC characteristics. The sample selection was not randomized but aimed to include a balanced number of patients from industrial and non-industrial areas, representing typical environmental exposure scenarios in Lower

Silesia. Randomization was not possible due to logistical limitations, but we sought to minimize biases by excluding patients with occupational exposure to toxic metals and ensuring a similar age distribution between the groups.

2.2. Pulp Extraction

Before the entire procedure, the patient is informed of the possible risks and complications associated with the procedure, and then the performing doctor obtains the patient's written and informed consent to perform the extraction. The tooth extraction procedure was carried out under local anesthesia with the patient fully conscious. The procedure was carried out in accordance with the principles of current medical knowledge and good medical practice, and the technique of the procedure was adapted each time to the clinical situation. The most common procedure involves the creation of a mucosal and bone flap; after flap dehiscence, the tooth is chiseled. The extracted tooth is then secured for the next stage to obtain material.

The cutting of the tooth to obtain the pulp tissue had to take place outside the mouth under sterile conditions so as not to cause contamination with oral bacterial flora. After removal, the teeth were stored in a cool antibiotic solution (0.5 mg/mL gentamicin—Biological Industries, Beit Haemek, Israel) in a refrigerator (temperature: 6 °C). The wisdom teeth were then cut with a sterile diamond disc with water cooling with sterile physiological saline (Biological Industries, Beit Haemek, Israel). The most effective was a combination of longitudinal and transverse cuts depending on the morphology of the tooth in question, leading to a weakening of the enamel and dentine structures, while not leading to a violation of the tooth chamber. With the use of hand tools (dental levers), access to the chamber was gained, and the intact tooth pulp was immediately transferred to the antibiotic solution.

2.3. Isolation of DCSCs

The obtained dental pulp was placed in the transport medium at 4 °C for up to 24 h (usually 18 h). The pulp was usually collected in the afternoon, and isolation was performed the next morning. After transport to the laboratory, the pulp was rinsed using a PBS/gentamicin solution (Biological Industries, Beit Haemek, Israel). Then the isolation process was carried out. Isolation was performed by cutting with scissors in 5 mL tubes to obtain a homogeneous suspension. The pulp was then digested using collagenase II at a concentration of 4 mg/mL in Hanks' Balanced Salt Solution (HBSS). Before digestion, the collagenase solution (Sigma-Aldrich, Darmstadt, Germany) was filtered through 0.22 µm filters (Sigma-Aldrich, Darmstadt, Germany). Digestion time was 1 h at 37 °C. After this time, the suspension was neutralized with a complete medium and centrifuged at 200 G for 5 min at 4 °C. After centrifugation, the obtained pellet was suspended in the medium, and the viability and number of obtained cells were assessed. The obtained suspension was placed in culture flasks with a surface area of 25 cm^2 (TPP Techno Plastic Products AG, Trasadingen, Switzerland). The first culture assessment was performed after 72 h, after which the culture medium was changed. The culture was then assessed every 48–72 h, and if confluence above 80% was achieved, the passage procedure was performed by detaching the cells using the TrypLE solution (Thermo Fisher Scientific, Waltham, MA, USA). After the first passage, the cells were further cultured in flasks with a surface area of 74 cm^2, and their further assessment was performed—growth, surface antigen expression, and differentiation into other cell types were assessed.

2.4. Cell Viability

The assessment of cell viability and counting was performed using a ready-made AO PI dual fluorescence kit (Sigma-Aldrich, Darmstadt, Germany) for analyzing concentration and viability according to the manufacturer's instructions. The measurement was carried out using the Countstar reader (Alit Biotech Co., Ltd., Shanghai, China). The evaluation of the cell growth rate was conducted using the Lionheart FX (Agilent Technologies, Inc. Santa

Clara, CA, USA) automated microscope (Figure 1). This microscope maintains humidity and temperature as in standard culture conditions in a CO_2 incubator. This allowed for real-time monitoring of cell proliferation growth over 48–72 h.

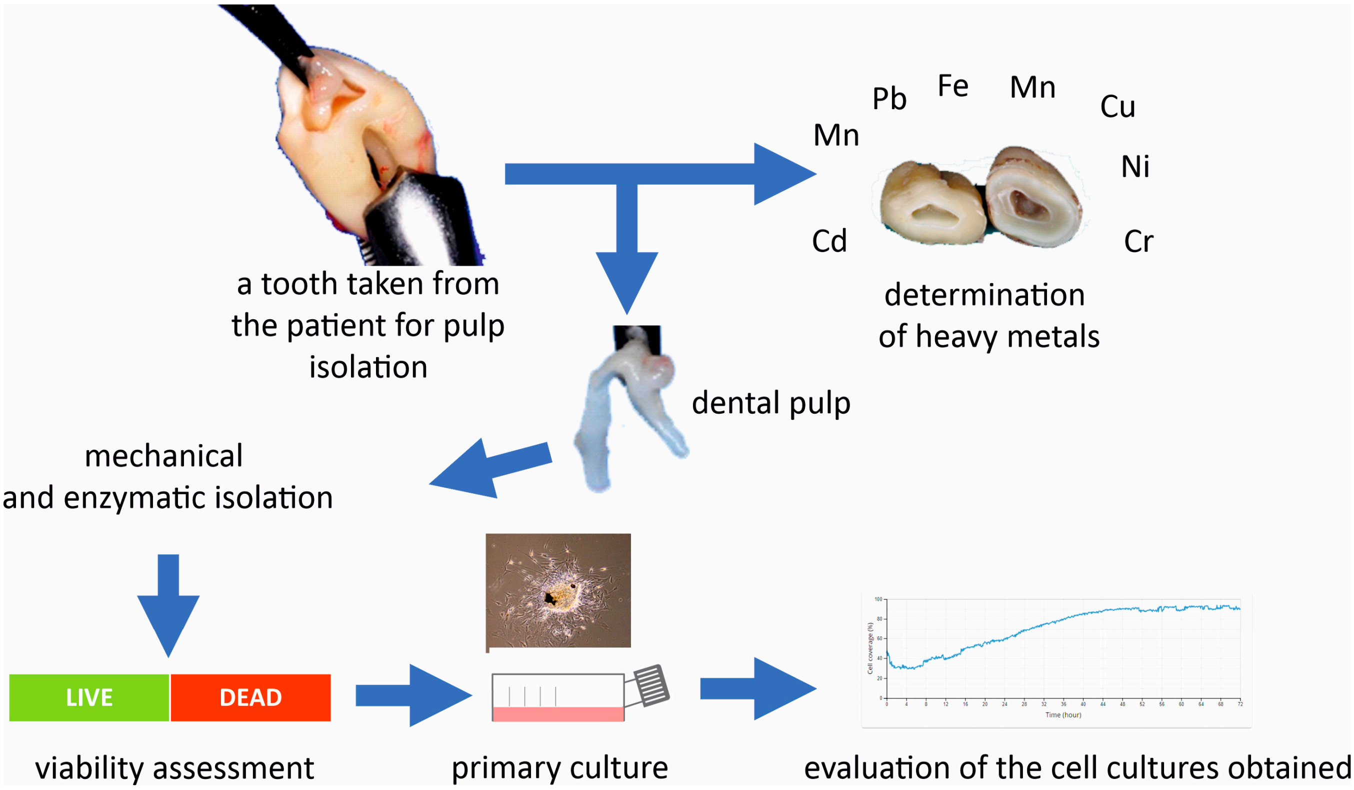

Figure 1. Scheme of in vitro tests performed.

2.5. Induction of Differentiation DSCs

When the cells reached approximately 80% confluence, the medium was replaced with osteogenic differentiation medium (0.5 mL/well; 24-well plate format). The cells were incubated with MSCgo Osteogenic XF (cat. no. 05-440-1, Biological Industries, Beit Haemek, Israel) or MSCgo Rapid Osteogenic XF (cat. no. 05-442-1, Biological Industries, Beit Haemek, Israel) for a period ranging from 10 to 21 days at 37 °C in a 5% CO_2 atmosphere. The differentiation medium was replaced every 2 to 3 days.

The differentiation and mineralization were assessed using a 2% Alizarin Red S (ARS—Sigma-Aldrich, Darmstadt, Germany) staining solution. ARS staining was performed to qualitatively and quantitatively evaluate the extent of calcium deposition, which is indicative of osteogenic differentiation.

2.6. Mineralization of Research Material

The samples were "wet" mineralized in a closed microwave mineralization system MARS 6 (CEM, Matthews, NC, USA). Next, 5 cm^3 of concentrated nitric acid (V) p.a was added to homogeneous sample (from 0.1 g to 0.5 g) in preparation vessels. Then the samples were mineralized in the microwave sample preparation system. The minerals were quantitatively transferred to 10 cm^3 measuring vessels with redistilled water. The mineralization was carried out in accordance with the Polish Standard PN-EN 13805:2003 "Foodstuffs. Determination of trace elements. Pressure mineralization" [18].

2.7. Determination of Elements in the Research Material

Determination of toxic heavy metals (Mn, Cr, Ni, Cu, Fe, Pb, Cd, Zn) content in materials by atomic absorption/emission spectrometry an acetylene/air flame was carried out by atomic absorption or emission spectrometry using a SpectrAA atomic absorption spectrometer with a Varian AA240FS flame attachment (Varian Inc., Palo Alto, CA, USA).

The analyses were performed in a food testing laboratory at the Department of Food Nutrition, University of Environmental and Life Sciences in Wroclaw.

The accuracy of the method was confirmed on the basis of the Standard Reference Material, Bone Meal, NIST-1486 (National Institute of Standards and Technology, Gaithersburg, MD, USA), and the measurement uncertainty was estimated at 5%.

The elements were determined according to the following standards [18]:

- Cu, Cr, Zn, Fe, Cd, Zn—PN-EN 14082: 2004 Food products—Determination of trace elements—Determination of lead, cadmium, zinc, copper, iron, and chromium by atomic absorption spectrometry (AAS) after dry mineralization [19].
- Ni—PN-A-86939-6:1998 Vegetable and animal oils and fats—Determination of heavy metal content by atomic emission spectrometry—Determination of nickel content [20].
- Mn—PN-EN ISO 6869:2002 Feed—Determination of calcium, copper, iron, magnesium, manganese, potassium, sodium, and zinc content—Atomic absorption spectrometry method [21].

2.8. Statistical Analysis

Data are presented as mean value and standard deviation (SD). The compliance of the distributions of quantitative variables with the theoretical normal distribution was checked using the Shapiro–Wilk test. The equality of variances was assessed using the Levene test. Depending on the results of the Shapiro–Wilk and Levene tests, the significance of differences between the mean values of quantitative parameters (place of residence, gender, age group) was assessed. The Student's *t*-test for independent variables or the non-parametric Whitney U test was used. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using Statistica 12 (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results

3.1. Characteristics of Patients of Both Groups

The study was conducted from 2020 to 2021. The inclusion criteria were the clinical necessity of third molar extractions; a total of 28 patients were recruited from two regions in Lower Silesia, Poland (the industrial area of Glogow-Legnica) and a non-industrial area (Wroclaw). Specifically, 10 patients were from the industrial-mining region of Glogow-Legnica, and 18 were from the non-industrial area.

Despite the disparity in group sizes, no statistically significant age differences were observed between the groups (Table 1). The demographic characteristic that distinguished the populations was gender; in the non-industrial group, the majority of patients were women (38.3% vs. 16.7%, p = NS), but this difference was not statistically significant. Group classification was based on patients' self-reported place of residence during interviews. The Legnica mining area group comprised patients who reported residing in this area for at least 18 years.

Table 1. Characteristics of patients of both groups.

Feature	Industrial District (n = 10)		Non-Industrial District (n = 18)		All		*p*-Value
	n	%	n	%	n	%	
Sex							
Women	6	60.0	15	83.3	21	75.0	0.327
Men	4	40.0	3	16.7	7	25.0	
Age group							
18–27 years	7	70.0	14	77.8	21	75.0	0.757
28–38 years	3	30.0	4	22.2	7	25.0	

Table 1. *Cont.*

Feature	Industrial District (n = 10)		Non-Industrial District (n = 18)		All		*p*-Value
	n	%	n	%	n	%	
Age (years)							
Mean ± SD	24.0 ± 5.9		26.0 ± 4.4		25.0 ± 5.0		0.300

3.2. Content of Heavy Metals

Basic statistics of the content of toxic metals in the tested samples of patients from both groups are presented in Tables 2–4. Table 2 shows a comparison of the metal content based on the area inhabited by the patients. Table 3 presents a comparison based on gender, and Table 4 illustrates the level of heavy metals according to age.

Table 2. Content of heavy metals in teeth from people from industrial and non-industrial areas.

Heavy Metals [µg/g]	Industrial District (Mean ± SD)	Non-Industrial District (Mean ± SD)	*p*-Value
Mn	0.164 ± 0.090	0.239 ± 0.229	0.331
Cr	0.000 ± 0.000	0.088 ± 0.372	0.446
Ni	0.000 ± 0.000	0.000 ± 0.000	1.000
Cu	0.310 ± 0.266	0.122 ± 0.125	0.017
Fe	4.899 ± 2.775	3.925 ± 4.924	0.571
Cd	0.064 ± 0.033	0.062 ± 0.021	0.814
Pb	0.551 ± 0.379	0.324 ± 0.203	0.048
Zn	86.300 ± 29.104	103.243 ± 30.305	0.163

Table 3. Content of heavy metals in teeth taken from patients of different sexes.

Heavy Metals [µg/g]	Women	Men	*p*-Value
Mn	0.221 ± 0.216	0.185 ± 0.099	0.676
Cr	0.000 ± 0.000	0.225 ± 0.596	0.083
Ni	0.000 ± 0.000	0.000 ± 0.000	1.000
Cu	0.183 ± 0.168	0.210 ± 0.307	0.765
Fe	3.116 ± 2.238	7.742 ± 6.770	0.0097
Cd	0.064 ± 0.026	0.058 ± 0.026	0.572
Pb	0.405 ± 0.314	0.406 ± 0.242	0.992
Zn	100.576 ± 30.177	87.041 ± 31.377	0.318

The total heavy metal content, measured in µg/g, revealed significantly higher concentrations of copper and lead in patients from industrial areas compared to those from non-industrial regions ($p = 0.017$ and $p = 0.048$, respectively). Specifically, the copper levels in individuals residing in industrial areas were 0.310 ± 0.266, whereas in non-industrial areas, they were 0.122 ± 0.123. This indicates that residents of non-industrial areas had a 59.6% lower total copper content (Table 2). Similarly, the lead levels in patients from industrial areas were 0.552 ± 0.379, compared to 0.324 ± 0.203 from non-industrial areas, showing that residents of non-industrial areas had a 41.3% lower total lead content.

There was no statistically significant effect of patient gender on the content of manganese, chromium, nickel, copper, cadmium, lead, and zinc in teeth ($p > 0.05$). The only statistically significant difference was observed in the iron content ($p = 0.0097$), which was higher in men. The iron content values were 7.742 ± 6.770 for men and 3.116 ± 2.238 for women. This indicates that women had a 59.8% lower total iron content compared to men (Table 3).

Table 4. Content of heavy metals in teeth taken from patients of different ages.

Heavy Metals [μg/g]	18–27	28–38	*p*-Value
Mn	0.176 ± 0.091	0.302 ± 0.328	0.121
Cr	0.079 ± 0.353	0.000 ± 0.000	0.537
Ni	0.000 ± 0.000	0.000 ± 0.000	1.000
Cu	0.141 ± 0.150	0.310 ± 0.278	0.047
Fe	4.783 ± 4.728	2.997 ± 2.523	0.324
Cd	0.063 ± 0.024	0.061 ± 0.032	0.870
Pb	0.372 ± 0.306	0.487 ± 0.260	0.362
Zn	93.534 ± 26.609	106.337 ± 39.084	0.325

There was no statistically significant effect of patients' age on the content of manganese, chromium, nickel, iron, cadmium, lead, and zinc in teeth ($p > 0.05$). The only statistically significant difference was observed in the copper content ($p = 0.047$), which was higher in the 28–38 age group compared to the 18–27 age group. The copper content values were 0.310 ± 0.276 for the older patients and 0.141 ± 0.150 for the younger patients. This indicates that younger patients had a 54.5% lower total copper content in the 18–27 age group compared to the 28–38 age group (Table 4).

3.3. The Influence of the Living Environment on Isolated DSCs

The basic statistics for assessing the viability of cells isolated from the dental pulp in the examined samples of patients from both groups are presented in Tables 5–7. Table 5 shows a comparison of DSC (dental stem cell) cultures based on the patients' places of residence. Table 6 shows the comparison by gender, and Table 7 shows the evaluation of cell cultures depending on the age of the patients.

Table 5. Evaluation of DSC cultures from human teeth from industrial and non-industrial areas.

Parameters	Industrial District (Mean ± SD)	Non-Industrial District (Mean ± SD)	*p*-Value
Viability cells	84.410 ± 8.832	93.944 ± 5.208	0.001
Number of cells obtained (million) in the first passage	1.479 ± 0.250	1.746 ± 0.150	0.002
24 h after isolation and the first passage	9.3 ± 2.6	5.7 ± 0.8	<0.001
Average doubling time (h)	41.2 ± 22.0	17.1 ± 4.8	<0.001

Table 6. Evaluation of DSC cultures from human teeth from patients of different sexes.

Parameters	Women	Men	*p*-Value
Viability cells	91.033 ± 7.424	89.057 ± 10.204	0.583
Number of cells obtained (million) in the first passage	1.657 ± 0.202	1.632 ± 0.312	0.802
24 h after isolation and the first passage	6.7 ± 2.1	7.9 ± 3.2	0.261
Average doubling time (h)	23.5 ± 14.5	32.3 ± 25.3	0.307

Table 7. Evaluation of DSC cultures from human teeth from patients of different ages.

Parameters	18–27 Years	28–38 Years	*p*-Value
Viability cells	92.455 ± 7.254	85.750 ± 8.374	0.044
Number of cells obtained (million) in the first passage	1.709 ± 0.174	1.506 ± 0.291	0.031
24 h after isolation and the first passage	6.5 ± 1.9	8.1 ± 3.2	0.108
Average doubling time (h)	21.6 ± 11.5	36.1 ± 26.0	0.048

Cell culture viability was statistically significantly lower in dental pulp samples from industrial areas. Specifically, the viability of cells from individuals residing in industrial areas was 84.410 ± 8.832, compared to 93.944 ± 5.208 in non-industrial areas (Table 5). Figure 2 shows an example culture of isolated dental stem cells (DSCs). In the case of cells isolated from pulp from industrial areas, we observe a few single cells after 48 h of incubation after isolation and many more from pulp from non-industrial areas. Similarly, the average culture doubling time was statistically significantly shorter in cultures of DSCs isolated from pulp from non-industrial areas, being more than twice as short: 41.2 ± 22.0 h in industrial areas versus 17.1 ± 4.8 h in non-industrial areas. The time to the first passage 24 h post-isolation was also statistically significantly shorter in the group of cell cultures isolated from non-industrial areas. Additionally, the number of cells after the first passage was statistically significantly higher in the group from non-industrial areas. The average number of cells after the first passage was 1.479 ± 0.250 million for tooth pulps from industrial areas (Figure 2B) and 1.746 ± 0.150 million for material from non-industrial areas (Figure 2D). This indicates that the efficiency of cell isolation from industrial areas is 15.3% lower compared to non-industrial areas (Table 5).

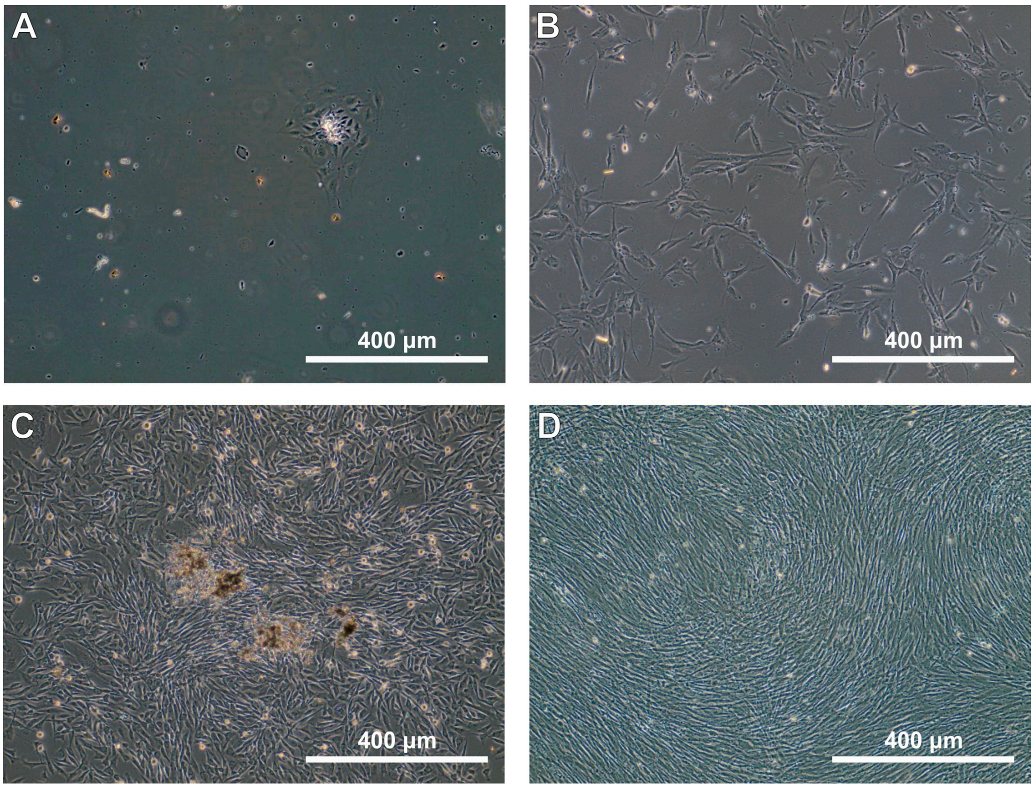

Figure 2. DSC cultures 48 h after isolation (**A**,**B**) and before the first passage (**C**,**D**). Cells isolated from an industrial district (**A**,**C**) from a non-industrial district (**B**,**D**).

There was no statistically significant effect of patient age on any of the assessed culture parameters of cells isolated from dental pulp ($p > 0.05$) (Table 6).

Cell culture viability was statistically significantly lower in dental pulp samples from the 28–38 age group. Specifically, cell viability in individuals over 27 years of age was 85.750 ± 8.374, compared to 92.455 ± 7.254 in younger donors (Table 7). Similarly, the mean culture doubling time was statistically significantly shorter in DSC cultures isolated from the pulp of younger individuals, being 21.6 ± 11.5 h in donors under 27 years of age compared to 36.1 ± 26.0 h in older individuals. Additionally, the number of cells after the first passage was statistically significantly higher in the 18–27 age group. The average number of cells after the first passage was 1.709 ± 0.174 million for material from people under 27 years of age, compared to 1.506 ± 0.291 million for the pulp of teeth from people over 27 years of age. This indicates that the efficiency of cell isolation from older individuals is 11.9% lower than in younger donors (Table 7).

3.4. The Influence of DSCs Differentiation

The initial seeding density allowed for optimal cell growth and confluence. The use of pre-coated plates with MSC Attachment Solution facilitated cell adhesion and uniform growth. Transitioning to an osteogenic differentiation medium at approximately 80% confluence ensured a robust initiation of the differentiation process. Extended incubation times were correlated with increased mineralization, as evidenced by more intense Alizarin Red S (ARS) staining.

Cells incubated for longer periods (up to 21 days) in the osteogenic medium demonstrated higher levels of mineralization. The periodic replacement of the differentiation medium every 2 to 3 days was crucial for maintaining the differentiation process and providing necessary nutrients. The ARS staining provided clear evidence of mineral deposition. The intensity of the ARS staining was directly proportional to the incubation time, confirming successful osteogenic differentiation. Quantitative analysis of the ARS staining can further elucidate the extent of mineralization. Importantly, there were no statistically significant differences in the level of differentiation and mineralization (Figure 3) regardless of the origin of the explant—whether the tooth pulp came from a person living in an industrial area or not and whether it came from a woman or a man.

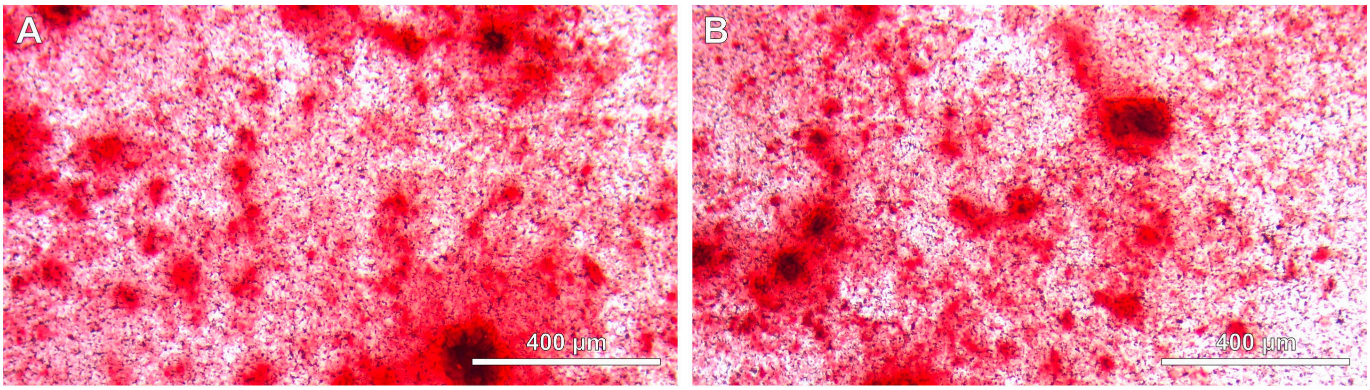

Figure 3. DSC cultures after differentiation in osteogenic medium: (**A**) patient from the industrial district, (**B**) patient from the non-industrial district.

4. Discussion

Stem cells isolated from dental pulp (DPSCs) show significant potential in regenerative medicine. Currently, research is being conducted on their application in treating conditions such as neurodegenerative diseases, spinal cord injuries, and heart diseases [22]. DPSCs have a high capacity for differentiating into various cell types, making them a promising tool for regenerating damaged tissues. The application of DPSCs in the regeneration of dental pulp and dentin tissue has direct significance for aesthetic dentistry, especially in the context of preserving and restoring teeth and periodontal tissue, which is important for the aesthetics of the smile and oral health [23]. The potential clinical applications of DPSCs, including their ability to regenerate bone, are significant in the context of reconstructing bone structures in the craniofacial area, and thus for facial aesthetics [24]. Research is being conducted into using lasers to help grow new sets of teeth, e.g., by using lasers to activate stem cells, researchers were able to stimulate tooth growth in rats [25]. Future research focuses on improving the efficiency of differentiating these cells and developing new methods for delivering them to injury sites. Understanding the tissue microenvironment and immune mechanisms is crucial for enhancing the effectiveness of therapies using DPSCs [22].

The isolation of DPSCs is quite efficient; after a few passages, several million cells can be obtained from a small amount of pulp. The undeniable advantage of this method of obtaining stem cells is the minimally invasive nature of the material collection, which is medically indicated, in contrast to cells obtained from bone marrow or adipose tissue [15].

As indicated by the team led by Juli Baronova, the problem of reconstructing teeth from isolated stem cells from dental pulp is quite complex and costly. There is still a lack of developed materials for guiding regeneration in the tissue engineering process and for releasing drugs to prevent, among other things, inflammatory conditions [15].

In recent studies, the utilization of autogenous dentin has been proposed as an innovative alternative for preserving alveolar bone post-extraction. This method has shown promising results in maintaining bone volume and quality, offering a viable option for bone regeneration [26,27].

We decided to investigate whether, in today's times, with such a developed economy but significant environmental pollution, this has an impact on the quality and quantity of cells obtained from dental pulp. The material collected came from industrial areas of Lower Silesia in Poland and the Wroclaw agglomeration.

Wychowanski and Malkiewicz analyzed the occurrence of trace elements in the enamel and dentin of impacted third molars in patients from urban and agricultural areas of Masovia, Poland [28]. They collected dental material from 30 patients aged 26–37. Their research indicates that the enamel and dentin of individuals living in cities contain significantly higher levels of lead and cadmium compared to those living in agricultural areas, as well as elevated levels of manganese and chromium [28]. Similarly, Bryła and co-authors noted an increased cadmium content in the dental tissues of residents of the Wroclaw agglomeration, which increased with age but was independent of gender [29]. In our research, we observed statistically significantly higher levels of copper and lead deposited in teeth from industrial areas. Additionally, similar to Wychowanski's findings, higher but not statistically significant levels of manganese, chromium, and zinc were observed in the urban agglomeration compared to the Glogow-Legnica district. Consequently, this affected the viability of cell cultures immediately after isolation. A statistically significant difference was observed: in non-industrial areas, the viability level was above 90%, while in industrial areas, it was around 84%. Cells of patients from non-industrial areas proliferated significantly faster, and in the first passage, about 300,000 more cells were obtained. At the same time, the yield of cells was sufficient in both cases for potential transfer to scaffolds. An important aspect is the demonstration that in clinical practice, where tooth extractions are often performed after the working hours of cell culture laboratories, especially in emergency cases, storing the pulp for 24–48 hours does not affect the viability of isolated cells. This is of great importance from the perspective of the patient and the dentist in the potential future use of tissue engineering. Most importantly, this study showed that regardless of the source of tooth pulp for isolating DPSCs, there was no effect on osteogenic differentiation.

In this study, no significant differences were observed in the content of heavy metal elements between genders, except of iron levels. Our findings diverge from those reported by Bryła et al. [29]. In our group, we identified a statistically significant higher iron content in samples obtained from men. This phenomenon can be attributed to the fact that iron is a microelement present in bones that accumulates with age, and its concentration is primarily dependent on serum levels. In conditions of iron excess, the hepatic protein hepcidin inhibits the absorption of iron from erythrocytes into the bloodstream and reduces the release of iron from macrophages. Maciejewska reported similar findings, highlighting the impact of other microelements on bone development [30]. Furthermore, WHO guidelines on iron supplementation recommend that women of reproductive age, particularly those who menstruate, should take iron prophylactically to prevent deficiencies. Research indicates that iron supplementation in menstruating women enhances hemoglobin levels and mitigates the risk of anemia [31]. Consequently, despite supplementation, physiological processes such as menstruation, pregnancy, childbirth, and lactation result in lower iron deposition in bones in women compared to men. However, this difference did not affect the effectiveness of isolated DPSCs.

In our study, we observed a statistically significant increase in copper content in the bones of teeth in the 28–38 years age group compared to the 18–27 years group. These results are consistent with the findings of Fischer and colleagues, who noted an increase in copper and other elements in the permanent teeth of children aged 5–14 years [32]. On the other hand, Bryła and colleagues did not observe differences in copper content regardless of age [29]. This discrepancy may be because Fischer's study included children up to 14 years old, who are in a phase of continuous development and have been exposed to environmental factors for a shorter period. After the complete saturation of the hard tissues of the tooth with minerals, which can take several years after the tooth has rooted, this level remains constant throughout life. In our study, we analyzed only third molars, which erupt late, usually between the ages of 17 and 24, although this time can vary individually [33]. Therefore, the saturation of elements in these teeth may be prolonged, explaining the differences. However, copper content does not seem to affect the quality and quantity of isolated DSCs. Differences in the yield of isolated DSCs depending on the age of the patient may be because stem cells in older individuals tend to divide more slowly and have a reduced capacity for proliferation. This may be caused by changes in signaling pathways, such as the mTOR pathway, which regulates cell growth and the cell cycle. Stem cells in older individuals may have altered metabolism, affecting their ability to divide and differentiate, leading to a reduced number of stem cells available for isolation [34,35].

Limitations: While this study offers important insights into the effects of environmental pollution on DSC viability and proliferation, several limitations should be acknowledged. First, the relatively small sample size (28 patients) limits the generalizability of the findings. The statistical power of the study was estimated at 49.7%, which is moderate but reflects a typical range for preliminary investigations. This level of power still enabled the detection of statistically significant differences between the groups, but a larger sample size would enhance the robustness and reliability of the conclusions.

Moreover, the classification of patients based solely on self-reported place of residence without controlling for dietary habits, smoking, or occupational exposure could introduce bias. These factors are known to affect heavy metal accumulation and may have contributed to variability in DSC viability. Future studies with larger cohorts and more controlled variables are necessary to confirm these findings and further explore the environmental impact on DSC proliferation. Additionally, this study's cross-sectional design limits our ability to observe changes in heavy metal content and DSC viability over time. Future longitudinal studies are planned to track these changes and offer a more comprehensive understanding of how prolonged exposure to environmental pollutants impacts DSC characteristics.

5. Conclusions

This study highlights the significant impact of environmental pollution on the quality and viability of dental stem cells (DSCs) extracted from impacted third molars. By comparing DSCs from patients from industrial and non-industrial areas, the research reveals the adverse effects of heavy metals on oral health and stem cell proliferation. Findings show higher copper and lead levels in dental tissues from patients in industrial areas, correlating with lower cell culture viability and slower proliferation. These results suggest that pollutants compromise the regenerative potential of DSCs. While these findings provide valuable insights into the impact of environmental pollution on the viability and proliferation of DSCs, it is important to note that this study represents preliminary research.

Our primary goal is to develop a reproducible and scalable methodology for isolating and utilizing DSCs in clinical practice. The ultimate objective is to register a DSC-based product with the European Medicines Agency (EMA) as an Advanced Therapy Medicinal Product (ATMP) and establish a reliable production process. This would enable the use of DSCs in regenerative treatments, particularly for patients exposed to environmental pollutants, offering a personalized approach to regenerative medicine.

The concentration of essential elements and activity of dental pulp stem cells are crucial for effective bone regeneration in the maxillofacial region. Sufficient levels of calcium and phosphate support mineralization, while stem cell activity enhances tissue formation, improving graft stability and durability.

The study also emphasizes the need to consider environmental factors in dental medicine. While no significant gender differences in heavy metal content were found, except elevated iron levels in men, age-related variations in copper were observed. These findings are key for developing targeted interventions for populations exposed to pollution. Overall, this research emphasizes the need for stricter environmental regulations and public health policies to reduce exposure to harmful pollutants. Further studies are needed to explore mitigation strategies and enhance clinical outcomes for individuals affected by environmental pollution.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm13185465/s1.

Author Contributions: Conceptualization, T.G. (Tomasz Gedrange) and M.D. (Marzena Dominiak); methodology, T.G. (Tomasz Gębarowski), J.H. and M.S.; formal analysis, B.W. and S.R.; investigation, S.R., T.G. (Tomasz Gębarowski), J.H. and M.S.; resources, E.B., M.D. (Marzena Dominiak) and T.G. (Tomasz Gedrange); data curation, B.W., S.R. and T.G. (Tomasz Gębarowski); writing—original draft preparation, B.W. and S.R.; writing—review and editing, B.W., T.G. (Tomasz Gębarowski), E.B., T.G. (Tomasz Gedrange), M.D. (Maciej Dobrzyński) and M.D. (Marzena Dominiak); visualization, B.W. and T.G. (Tomasz Gębarowski); supervision, M.D. (Marzena Dominiak) and M.D. (Maciej Dobrzyński); project administration, T.G. (Tomasz Gębarowski); funding acquisition, M.D. (Marzena Dominiak). All authors have read and agreed to the published version of the manuscript.

Funding: The research was carried out as part of the project: "Development of a method for obtaining and isolating mesenchymal stromal cells (MSCs) from teeth for the regeneration of bone defects in dentistry" co-financed by the European Union from the European Regional Development Fund under the Smart Growth Operational Program 2014–2020. The project was carried out as part of the National Center for Research and Development competition "Strategic research programs for the economy". Beneficiary: Medical Center of Innovation Wrocław (MCIW) sp. z o.o. and Wrocław Medical University. Project number POIR.04.01.01-00-0006/19-00.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. Approval was obtained from the Bioethics Committee of Wroclaw Medical University: KB-246/2019 (18 March 2019) and KB-34/2020 (16 January 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in the study are included in the Supplementary Material; further inquiries can be directed to the corresponding author.

Conflicts of Interest: Author Sadri Rayad was employed by the company Academic Dental Polyclinic of Dental Center, Technology Transfer Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Ciszyński, M.; Dominiak, S.; Dominiak, M.; Gedrange, T.; Hadzik, J. Allogenic Bone Graft in Dentistry: A Review of Current Trends and Developments. *Int. J. Mol. Sci.* **2023**, *24*, 16598. [CrossRef] [PubMed]
2. Hadzik, J.; Błaszczyszyn, A.; Gedrange, T.; Dominiak, M. Soft-Tissue Augmentation around Dental Implants with a Connective Tissue Graft (CTG) and Xenogeneic Collagen Matrix (CMX)—5-Year Follow-Up. *J. Clin. Med.* **2023**, *12*, 924. [CrossRef] [PubMed]
3. Glowacka-Sobotta, A.; Ziental, D.; Czarczynska-Goslinska, B.; Michalak, M.; Wysocki, M.; Güzel, E.; Sobotta, L. Nanotechnology for Dentistry: Prospects and Applications. *Nanomaterials* **2023**, *13*, 2130. [CrossRef] [PubMed]
4. Ward, W.G.; Gautreaux, M.D.; Lippert, D.C.; Boles, C. HLA Sensitization and Allograft Bone Graft Incorporation. *Clin. Orthop. Relat. Res.* **2008**, *466*, 1837–1848. [CrossRef]
5. O'Sullivan, E.D.; Battle, R.K.; Zahra, S.; Keating, J.F.; Marson, L.P.; Turner, D.M. Allosensitization Following Bone Graft. *Am. J. Transplant.* **2017**, *17*, 2207–2211. [CrossRef]
6. Lorenz, J.; Schlee, M.; Al-Maawi, S.; Chia, P.; Sader, R.; Ghanaati, S. Variant Purification of an Allogeneic Bone Block. *Acta Stomatol. Croat.* **2017**, *51*, 141–147. [CrossRef]
7. Ghanaati, S.; Barbeck, M.; Booms, P.; Lorenz, J.; Kirkpatrick, C.J.; Sader, R.A. Potential Lack of "Standardized" Processing Techniques for Production of Allogeneic and Xenogeneic Bone Blocks for Application in Humans. *Acta Biomater.* **2014**, *10*, 3557–3562. [CrossRef]
8. Charavet, C.; Lecloux, G.; Vandenberghe, B.; Lambert, F. Buccal Bone Regeneration Combined with Piezocision in Adult Orthodontic Patients: Clinical, 3D Radiographic, and Patient-Reported Outcomes. *J. Stomatol. Oral Maxillofac. Surg.* **2021**, *122*, 549–556. [CrossRef]
9. Dominiak, M.; Dominiak, S.; Targonska, S.; Gedrange, T. Three-Dimensional Bone Block Planning for Mandibular Sagittal Bone Defect Reconstruction. *J. Healthc. Eng.* **2020**, *2020*, 8829288. [CrossRef]
10. Dominiak, M.; Hnitecka, S.; Olchowy, C.; Dominiak, S.; Gedrange, T. Possible Treatment of Severe Bone Dehiscences Based on 3D Bone Reconstruction—A Description of Treatment Methodology. *Appl. Sci.* **2021**, *11*, 10299. [CrossRef]
11. Fraser, J.K.; Schreiber, R.; Strem, B.; Zhu, M.; Alfonso, Z.; Wulur, I.; Hedrick, M.H. Plasticity of Human Adipose Stem Cells toward Endothelial Cells and Cardiomyocytes. *Nat. Clin. Pract. Cardiovasc. Med.* **2006**, *3*, S33–S37. [CrossRef] [PubMed]
12. Wang, S.; Qu, X.; Zhao, R.C. Clinical Applications of Mesenchymal Stem Cells. *J. Hematol. Oncol.* **2012**, *5*, 19. [CrossRef] [PubMed]
13. Mizuno, H. Adipose-Derived Stem Cells for Tissue Repair and Regeneration: Ten Years of Research and a Literature Review. *J. Nippon. Med. Sch.* **2009**, *76*, 56–66. [CrossRef] [PubMed]
14. Shi, S.; Robey, P.G.; Gronthos, S. Comparison of Human Dental Pulp and Bone Marrow Stromal Stem Cells by CDNA Microarray Analysis. *Bone* **2001**, *29*, 532–539. [CrossRef]
15. Baranova, J.; Büchner, D.; Götz, W.; Schulze, M.; Tobiasch, E. Tooth Formation: Are the Hardest Tissues of Human Body Hard to Regenerate? *Int. J. Mol. Sci.* **2020**, *21*, 4031. [CrossRef]
16. Malara, P.; Fischer, A.; Malara, B. Selected Toxic and Essential Heavy Metals in Impacted Teeth and the Surrounding Mandibular Bones of People Exposed to Heavy Metals in the Environment. *J. Occup. Med. Toxicol.* **2016**, *11*, 56. [CrossRef]
17. Castagna, V.; Pardo, A.; Lanaro, L.; Signoriello, A.; Albanese, M. Periodontal Healing after Lower Third Molars Extraction: A Clinical Evaluation of Different Flap Designs. *Healthcare* **2022**, *10*, 1587. [CrossRef]
18. *PN-EN 13805:2003*; Foodstuffs. Determination of trace elements. Pressure mineralization. Polish Committee for Standardization: Warsaw, Poland, 2004.
19. *PN-EN 14082:2004*; Food Products—Determination of trace Elements—Determination of Lead, Cadmium, Zinc, Copper, Iron, and Chromium by Atomic Absorption Spectrometry (AAS) after Dry Mineralization. Polish Committee for Standardization: Warsaw, Poland, 2004.
20. *PN-A-86939-6:1998*; Vegetable and Animal Oils and Fats—Determination of Heavy Metal Content by Atomic Emission Spectrometry—Determination of Nickel Content. Polish Committee for Standardization: Warsaw, Poland, 1998.
21. *PN-EN ISO 6869:2002*; Feed—Determination of Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Sodium, and Zinc Content—Atomic Absorption Spectrometry Method. Polish Committee for Standardization: Warsaw, Poland, 2002.
22. Li, B.; Ouchi, T.; Cao, Y.; Zhao, Z.; Men, Y. Dental-Derived Mesenchymal Stem Cells: State of the Art. *Front. Cell Dev. Biol.* **2021**, *9*, 654559. [CrossRef]
23. Nakashima, M.; Iohara, K. Regeneration of Dental Pulp by Stem Cells. *Adv. Dent. Res.* **2011**, *23*, 313–319. [CrossRef]
24. d'Aquino, R.; De Rosa, A.; Laino, G.; Caruso, F.; Guida, L.; Rullo, R.; Checchi, V.; Laino, L.; Tirino, V.; Papaccio, G. Human Dental Pulp Stem Cells: From Biology to Clinical Applications. *J. Exp. Zool. B Mol. Dev. Evol.* **2009**, *312B*, 408–415. [CrossRef]
25. Zhai, Q.; Dong, Z.; Wang, W.; Li, B.; Jin, Y. Dental Stem Cell and Dental Tissue Regeneration. *Front. Med.* **2019**, *13*, 152–159. [CrossRef] [PubMed]
26. Artzi, Z.; Netanely, E.; Renert, U. Autogenous Particulate Dentin in Socket Site Preservation Procedures: Histologic and Histomorphometric Observations. *Int. J. Oral Maxillofac. Implant.* **2022**, *37*, 373–380. [CrossRef] [PubMed]
27. Dłucik, R.; Orzechowska-Wylęgała, B.; Dłucik, D.; Bogus, K. Histological Examination of Tooth-Derived Biomaterials Obtained from Different Devices. *Expert. Rev. Med. Devices* **2023**, *20*, 979–988. [CrossRef] [PubMed]
28. Wychowanski, P.; Malkiewicz, K. Evaluation of Metal Ion Concentration in Hard Tissues of Teeth in Residents of Central Poland. *Biomed. Res. Int.* **2017**, *2017*, 6419709. [CrossRef]

29. Bryła, E.; Dobrzyński, M.; Konkol, D.; Kuropka, P.; Styczyńska, M.; Korczyński, M. Toxic Metals Content in Impacted Third Molars and Adjacent Bone Tissue in Different Groups of Patients. *Materials* **2021**, *14*, 793. [CrossRef]
30. Maciejewska, K.; Drzazga, Z.; Kaszuba, M. Role of Trace Elements (Zn, Sr, Fe) in Bone Development: Energy Dispersive X-Ray Fluorescence Study of Rat Bone and Tooth Tissue. *BioFactors* **2014**, *40*, 425–435. [CrossRef]
31. Dudek, M.; Kocyłowski, R.; Kokocińska, K.; Kuźniacka, I.; Lewicka, I.; Suliburska, J. Ocena Podaży Żelaza i Kwasu Foliowego u Kobiet w Wieku Rozrodczym. *Forum Zaburzeń Metab.* **2017**, *8*, 88–95.
32. Fischer, A.; Wiechuła, D.; Przybyła-Misztela, C. Changes of Concentrations of Elements in Deciduous Teeth with Age. *Biol. Trace Elem. Res.* **2013**, *154*, 427–432. [CrossRef]
33. Gambier, A.; Rérolle, C.; Faisant, M.; Lemarchand, J.; Paré, A.; Saint-Martin, P. Contribution of Third Molar Eruption to the Estimation of the Forensic Age of Living Individuals. *Int. J. Leg. Med.* **2019**, *133*, 625–632. [CrossRef]
34. Lengefeld, J.; Cheng, C.W.; Maretich, P.; Blair, M.; Hagen, H.; McReynolds, M.R.; Sullivan, E.; Majors, K.; Roberts, C.; Kang, J.H.; et al. Cell Size Is a Determinant of Stem Cell Potential during Aging. *Sci. Adv.* **2021**, *7*, 271. [CrossRef]
35. Chong, P.P.; Selvaratnam, L.; Abbas, A.A.; Kamarul, T. Factors Influencing the Successful Isolation and Expansion of Aging Human Mesenchymal Stem Cells. *Open Life Sci.* **2018**, *13*, 279–284. [CrossRef]

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Sylwia Klimas [2], Maciej Janeczek [3], Agata Małyszek [3], Marta Bort [4], Andrzej Małysa [4], Marzena Dominiak [5], Maciej Dobrzyński [2]: *Studies on the content of toxic metals in teeth: A narrative review of literature. Dent. Med. Probl.* doi:10.17219/dmp/193406.

[1] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław
[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław
[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław
[4] Katedra i Zakład Stomatologii Doświadczalnej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław
[5] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

Sadri Rayad – A,B,C,D,E
Sylwia Klimas – B,C,D
Maciej Janeczek - B,C,D
Agata Małyszek - B,C,D
Marta Bort - D,E
Andrzej Małysa - D,E
Marzena Dominiak - E,F
Maciej Dobrzyński - A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

S. Sylwia Klimas

Sylwia Klimas
LEKARZ DENTYSTA
3647285

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Sylwia Klimas [2], Maciej Janeczek [3], Agata Małyszek [3], Marta Bort [4], Andrzej Małysa [4], Marzena Dominiak [5], Maciej Dobrzyński [2]: *Studies on the content of toxic metals in teeth: A narrative review of literature. Dent. Med. Probl.* doi:10.17219/dmp/193406.

[1] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

[4] Katedra i Zakład Stomatologii Doświadczalnej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

[5] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

Sadri Rayad – A,B,C,D,E
Sylwia Klimas – B,C,D
Maciej Janeczek - B,C,D
Agata Małyszek - B,C,D

Marta Bort - D,E
Andrzej Małysa - D,E
Marzena Dominiak - E,F
Maciej Dobrzyński - A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Kierownik Katedry
Biostruktury i Fizjologii Zwierząt
prof. dr hab. n. wet. Maciej Janeczek
specjalista chirurg

Maciej Janeczek

....
czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Sylwia Klimas [2], Maciej Janeczek [3], Agata Małyszek [3], Marta Bort [4], Andrzej Małysa [4], Marzena Dominiak [5], Maciej Dobrzyński [2]: *Studies on the content of toxic metals in teeth: A narrative review of literature. Dent. Med. Probl.* doi:10.17219/dmp/193406.

[1] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

[4] Katedra i Zakład Stomatologii Doświadczalnej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

[5] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

Sadri Rayad – A,B,C,D,E
Sylwia Klimas – B,C,D
Maciej Janeczek - B,C,D
Agata Małyszek - B,C,D
Marta Bort - D,E
Andrzej Małysa - D,E
Marzena Dominiak - E,F
Maciej Dobrzyński - A,E,F

A – koncepcja i projekt badawczy;

B – gromadzenie danych;

C – analiza i interpretacja danych;

D – napisanie artykułu;

E – krytyczna ocena artykułu;

F – ostateczna akceptacja artykułu

Agata Małyszek

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Sylwia Klimas [2], Maciej Janeczek [3], Agata Małyszek [3], Marta Bort [4], Andrzej Małysa [4], Marzena Dominiak [5], Maciej Dobrzyński [2]: *Studies on the content of toxic metals in teeth: A narrative review of literature. Dent. Med. Probl.* doi:10.17219/dmp/193406.

[1] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław
[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław
[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław
[4] Katedra i Zakład Stomatologii Doświadczalnej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław
[5] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

Sadri Rayad – A,B,C,D,E
Sylwia Klimas – B,C,D
Maciej Janeczek - B,C,D
Agata Małyszek - B,C,D
Marta Bort - D,E
Andrzej Małysa - D,E
Marzena Dominiak - E,F
Maciej Dobrzyński - A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Marta Bort

LEKARZ STOMATOLOG
3555860

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Sylwia Klimas [2], Maciej Janeczek [3], Agata Małyszek [3], Marta Bort [4], Andrzej Małysa [4], Marzena Dominiak [5], Maciej Dobrzyński [2]: *Studies on the content of toxic metals in teeth: A narrative review of literature. Dent. Med. Probl.* doi:10.17219/dmp/193406.

[1] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław
[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław
[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław
[4] Katedra i Zakład Stomatologii Doświadczalnej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław
[5] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

Sadri Rayad – A,B,C,D,E
Sylwia Klimas – B,C,D
Maciej Janeczek - B,C,D
Agata Małyszek - B,C,D
Marta Bort - D,E
Andrzej Małysa - D,E
Marzena Dominiak - E,F
Maciej Dobrzyński - A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

dr n. med. Andrzej Małysa
lekarz dentysta
3248791

Andrzej 1.10.24

....
czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Sylwia Klimas [2], Maciej Janeczek [3], Agata Małyszek [3], Marta Bort [4], Andrzej Małysa [4], Marzena Dominiak [5], Maciej Dobrzyński [2]: *Studies on the content of toxic metals in teeth: A narrative review of literature. Dent. Med. Probl.* doi:10.17219/dmp/193406.

[1] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

[4] Katedra i Zakład Stomatologii Doświadczalnej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

[5] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

Sadri Rayad – A,B,C,D,E
Sylwia Klimas – B,C,D
Maciej Janeczek - B,C,D
Agata Małyszek - B,C,D

Marta Bort - D,E
Andrzej Małysa - D,E
Marzena Dominiak - E,F
Maciej Dobrzyński - A,E,F

A – koncepcja i projekt badawczy;

B – gromadzenie danych;

C – analiza i interpretacja danych;

D – napisanie artykułu;

E – krytyczna ocena artykułu;

F – ostateczna akceptacja artykułu

Uniwersytet Medyczny we Wrocławiu
KATEDRA I ZAKŁAD CHIRURGII
STOMATOLOGICZNEJ
kierownik
prof. dr hab. Marzena Dominiak

....
czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Sylwia Klimas [2], Maciej Janeczek [3], Agata Małyszek [3], Marta Bort [4], Andrzej Małysa [4], Marzena Dominiak [5], Maciej Dobrzyński [2]: *Studies on the content of toxic metals in teeth: A narrative review of literature. Dent. Med. Probl.* doi:10.17219/dmp/193406.

[1] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

[4] Katedra i Zakład Stomatologii Doświadczalnej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

[5] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

Sadri Rayad – A,B,C,D,E
Sylwia Klimas – B,C,D
Maciej Janeczek - B,C,D
Agata Małyszek - B,C,D
Marta Bort - D,E
Andrzej Małysa - D,E
Marzena Dominiak - E,F
Maciej Dobrzyński - A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Uniwersytet Medyczny we Wrocławiu
Wydział Lekarsko-Stomatologiczny
KATEDRA I ZAKŁAD
STOMATOLOGII DZIECIĘCEJ
I STOMATOLOGII PRZEDKLINICZNEJ
kierownik
dr hab. n. med. Maciej Dobrzyński, profesor uczelni

....
czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2], Amadeusz Kuźniarski [3], Marzena Styczyńska[4], Dorota Diakowska[5], Tomasz Gedrange[6], Sylwia Klimas[2], Tomasz Gębarowski[7] and Marzena Dominiak[6]: *An In-Vitro Evaluation of Toxic Metals Concentration in the Third Molars from Residents of the Legnica-Głogów Copper Area and Risk Factors Determining the Accumulation of Those Metals: A Pilot Study. Appl. Sci.* 2023, 13, 2904. https://doi.org/10.3390/app13052904

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław), ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra I Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D
Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Uniwersytet Medyczny we Wrocławiu
Wydział Lekarsko-Stomatologiczny
KATEDRA I ZAKŁAD
STOMATOLOGII DZIECIĘCEJ
I STOMATOLOGII PRZEDKLINICZNEJ
Kierownik
dr hab. n. med. Maciej Dobrzyński, profesor uczelni

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2] , Amadeusz Kuźniarski [3] , Marzena Styczyńska[4] , Dorota Diakowska[5] , Tomasz Gedrange[6], Sylwia Klimas[2], Tomasz Gębarowski[7] and Marzena Dominiak[6]: *An In-Vitro Evaluation of Toxic Metals Concentration in the Third Molars from Residents of the Legnica-Głogów Copper Area and Risk Factors Determining the Accumulation of Those Metals: A Pilot Study. Appl. Sci.* 2023, 13, 2904. https://doi.org/10.3390/app13052904

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław), ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra I Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D
Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;

B – gromadzenie danych;

C – analiza i interpretacja danych;

D – napisanie artykułu;

E – krytyczna ocena artykułu;

F – ostateczna akceptacja artykułu

Amadeusz Kuźniarski
LEKARZ DENTYSTA
3375751

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2] , Amadeusz Kuźniarski [3] , Marzena Styczyńska[4] , Dorota Diakowska[5] , Tomasz Gedrange[6], Sylwia Klimas[2], Tomasz Gębarowski[7] and Marzena Dominiak[6]: *An In-Vitro Evaluation of Toxic Metals Concentration in the Third Molars from Residents of the Legnica-Głogów Copper Area and Risk Factors Determining the Accumulation of Those Metals: A Pilot Study. Appl. Sci.* 2023, 13, 2904. https://doi.org/10.3390/app13052904

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław), ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra I Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D
Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;

B – gromadzenie danych;

C – analiza i interpretacja danych;

D – napisanie artykułu;

E – krytyczna ocena artykułu;

F – ostateczna akceptacja artykułu

Marzena Styczyńska
czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2], Amadeusz Kuźniarski [3], Marzena Styczyńska[4], Dorota Diakowska[5], Tomasz Gedrange[6], Sylwia Klimas[2], Tomasz Gębarowski[7] and Marzena Dominiak[6]: *An In-Vitro Evaluation of Toxic Metals Concentration in the Third Molars from Residents of the Legnica-Głogów Copper Area and Risk Factors Determining the Accumulation of Those Metals: A Pilot Study. Appl. Sci.* 2023, 13, 2904. https://doi.org/10.3390/app13052904

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław), ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra I Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D

Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;

B – gromadzenie danych;

C – analiza i interpretacja danych;

D – napisanie artykułu;

E – krytyczna ocena artykułu;

F – ostateczna akceptacja artykułu

Dorota Diakowska
....
czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2], Amadeusz Kuźniarski [3], Marzena Styczyńska [4], Dorota Diakowska [5], Tomasz Gedrange [6], Sylwia Klimas [2], Tomasz Gębarowski [7] and Marzena Dominiak [6]: *An In-Vitro Evaluation of Toxic Metals Concentration in the Third Molars from Residents of the Legnica-Głogów Copper Area and Risk Factors Determining the Accumulation of Those Metals: A Pilot Study. Appl. Sci.* 2023, 13, 2904. https://doi.org/10.3390/app13052904

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław), ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra I Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D
Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Dental Surgery Department
of Silesian Piast Medical Uniwersity
of Wrocław, Poland

prof. dr hab. Tomasz Gedrange

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2], Amadeusz Kuźniarski [3], Marzena Styczyńska[4], Dorota Diakowska[5], Tomasz Gedrange[6], Sylwia Klimas[2], Tomasz Gębarowski[7] and Marzena Dominiak[6]: *An In-Vitro Evaluation of Toxic Metals Concentration in the Third Molars from Residents of the Legnica-Głogów Copper Area and Risk Factors Determining the Accumulation of Those Metals: A Pilot Study. Appl. Sci.* 2023, 13, 2904. https://doi.org/10.3390/app13052904

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław), ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra I Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D
Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Sylwia Klimas

Sylwia Klimas
LEKARZ DENTYSTA
3647285

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2] , Amadeusz Kuźniarski [3] , Marzena Styczyńska[4] , Dorota Diakowska[5] , Tomasz Gedrange[6], Sylwia Klimas[2], Tomasz Gębarowski[7] and Marzena Dominiak[6]: *An In-Vitro Evaluation of Toxic Metals Concentration in the Third Molars from Residents of the Legnica-Głogów Copper Area and Risk Factors Determining the Accumulation of Those Metals: A Pilot Study. Appl. Sci.* 2023, 13, 2904. https://doi.org/10.3390/app13052904

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław), ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra I Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D
Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;

B – gromadzenie danych;

C – analiza i interpretacja danych;

D – napisanie artykułu;

E – krytyczna ocena artykułu;

F – ostateczna akceptacja artykułu

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2], Amadeusz Kuźniarski [3], Marzena Styczyńska [4], Dorota Diakowska [5], Tomasz Gedrange [6], Sylwia Klimas [2], Tomasz Gębarowski [7] and Marzena Dominiak [6]: *An In-Vitro Evaluation of Toxic Metals Concentration in the Third Molars from Residents of the Legnica-Głogów Copper Area and Risk Factors Determining the Accumulation of Those Metals: A Pilot Study. Appl. Sci.* 2023, 13, 2904. https://doi.org/10.3390/app13052904

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław), ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra I Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50 – 425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D
Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Uniwersytet Medyczny we Wrocławiu
KATEDRA I ZAKŁAD CHIRURGII
STOMATOLOGICZNEJ
Kierownik
prof. dr hab. Marzena Dominiak

....
czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2], Amadeusz Kuźniarski [3], Marzena Styczyńska [4], Dorota Diakowska [5], Tomasz Gedrange [6], Sylwia Klimas[2], Tomasz Gębarowski [7], Marzena Dominiak [6]: *Mercury Content in Impacted Wisdom Teeth from Patients of the Legnica–Głogów Copper Area — An In Vitro Pilot Study. J. Xenobiot.* 2023, 13, 463–478. https://doi.org/10.3390/jox13030029

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra i Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D
Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Uniwersytet Medyczny we Wrocławiu
Wydział Lekarsko-Stomatologiczny
KATEDRA I ZAKŁAD
STOMATOLOGII DZIECIĘCEJ
I STOMATOLOGII PRZEDKLINICZNEJ
Kierownik
dr hab. n. med. Maciej Dobrzyński, profesor uczelni

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2], Amadeusz Kuźniarski [3], Marzena Styczyńska [4], Dorota Diakowska [5], Tomasz Gedrange [6], Sylwia Klimas[2], Tomasz Gębarowski [7], Marzena Dominiak [6]: *Mercury Content in Impacted Wisdom Teeth from Patients of the Legnica–Głogów Copper Area — An In Vitro Pilot Study. J. Xenobiot.* 2023, 13, 463–478. https://doi.org/10.3390/jox13030029

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra i Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D
Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;

B – gromadzenie danych;

C – analiza i interpretacja danych;

D – napisanie artykułu;

E – krytyczna ocena artykułu;

F – ostateczna akceptacja artykułu

Amadeusz Kuźniarski
LEKARZ DENTYSTA
3375751

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2], Amadeusz Kuźniarski [3], Marzena Styczyńska [4], Dorota Diakowska [5], Tomasz Gedrange [6], Sylwia Klimas[2], Tomasz Gębarowski [7], Marzena Dominiak [6]: *Mercury Content in Impacted Wisdom Teeth from Patients of the Legnica–Głogów Copper Area — An In Vitro Pilot Study. J. Xenobiot.* 2023, 13, 463–478. https://doi.org/10.3390/jox13030029

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra i Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D
Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;

B – gromadzenie danych;

C – analiza i interpretacja danych;

D – napisanie artykułu;

E – krytyczna ocena artykułu;

F – ostateczna akceptacja artykułu

Marzena Styczyńska
....
czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2], Amadeusz Kuźniarski [3], Marzena Styczyńska [4], Dorota Diakowska [5], Tomasz Gedrange [6], Sylwia Klimas[2], Tomasz Gębarowski [7], Marzena Dominiak [6]: *Mercury Content in Impacted Wisdom Teeth from Patients of the Legnica–Głogów Copper Area — An In Vitro Pilot Study. J. Xenobiot.* 2023, 13, 463–478. https://doi.org/10.3390/jox13030029

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra i Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D
Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;

B – gromadzenie danych;

C – analiza i interpretacja danych;

D – napisanie artykułu;

E – krytyczna ocena artykułu;

F – ostateczna akceptacja artykułu

Dorota Diakowska
....
czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2], Amadeusz Kuźniarski [3], Marzena Styczyńska [4], Dorota Diakowska [5], Tomasz Gedrange [6], Sylwia Klimas[2], Tomasz Gębarowski [7], Marzena Dominiak [6]: *Mercury Content in Impacted Wisdom Teeth from Patients of the Legnica–Głogów Copper Area — An In Vitro Pilot Study. J. Xenobiot.* 2023, 13, 463–478. https://doi.org/10.3390/jox13030029

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra i Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D

Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Dental Surgery Department
of Silesian Piast Medical Uniwersity
of Wrocław, Poland

prof. dr hab. Tomasz Gedrange

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2], Amadeusz Kuźniarski [3], Marzena Styczyńska [4], Dorota Diakowska [5], Tomasz Gedrange [6], Sylwia Klimas[2], Tomasz Gębarowski [7], Marzena Dominiak [6]: *Mercury Content in Impacted Wisdom Teeth from Patients of the Legnica–Głogów Copper Area — An In Vitro Pilot Study. J. Xenobiot.* 2023, 13, 463–478. https://doi.org/10.3390/jox13030029

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra i Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D
Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Sylwia Klimas

Sylwia Klimas
LEKARZ DENTYSTA
3647285

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2], Amadeusz Kuźniarski [3], Marzena Styczyńska [4], Dorota Diakowska [5], Tomasz Gedrange [6], Sylwia Klimas[2], Tomasz Gębarowski [7], Marzena Dominiak [6]: *Mercury Content in Impacted Wisdom Teeth from Patients of the Legnica–Głogów Copper Area — An In Vitro Pilot Study. J. Xenobiot.* 2023, 13, 463–478. https://doi.org/10.3390/jox13030029

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra i Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D
Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;

B – gromadzenie danych;

C – analiza i interpretacja danych;

D – napisanie artykułu;

E – krytyczna ocena artykułu;

F – ostateczna akceptacja artykułu

Tomasz Gedrange

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Sadri Rayad [1], Maciej Dobrzyński [2], Amadeusz Kuźniarski [3], Marzena Styczyńska [4], Dorota Diakowska [5], Tomasz Gedrange [6], Sylwia Klimas[2], Tomasz Gębarowski [7], Marzena Dominiak [6]: *Mercury Content in Impacted Wisdom Teeth from Patients of the Legnica–Głogów Copper Area — An In Vitro Pilot Study. J. Xenobiot.* 2023, 13, 463–478. https://doi.org/10.3390/jox13030029

[1] Medyczne Centrum Innowacji Wrocław Sp. z o. o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[2] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[3] Katedra i Zakład Protetyki Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[4] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[5] Zakład Nauk Podstawowych, Uniwersytet Medyczny we Wrocławiu, ul. Chałubińskiego 4, 50-368 Wrocław

[6] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław

[7] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

Sadri Rayad – A,B,C,D,F
Maciej Dobrzyński – A,E,F
Amadeusz Kuźniarski – B,D
Marzena Styczyńska – B,C
Dorota Diakowska – B,C,D
Tomasz Gedrange – E,F
Sylwia Klimas – D
Tomasz Gębarowski – D,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Uniwersytet Medyczny we Wrocławiu
KATEDRA I ZAKŁAD CHIRURGII
STOMATOLOGICZNEJ
kierownik
prof. dr hab. Marzena Dominiak

....
czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Benita Wiatrak [1], Sadri Rayad [2], Tomasz Gębarowski [3], Jakub Hadzik [4], Marzena Styczyńska [5], Tomasz Gedrange [4], Maciej Dobrzyński [6], Ewa Barg [7], Marzena Dominiak [4]: *Comparative Analysis of Heavy Metal Content in Impacted Third Molars from Industrial and Non-Industrial Areas and Its Effect on the Isolation, Culture, and Proliferation of Dental Stem Cells (DSCs). J. Clin. Med.* 2024, 13, 5465. https://doi.org/10.3390/jcm13185465

[1] Katedra i Zakład Farmakologii, Uniwersytet Medyczny we Wrocławiu, ul. Mikulicza-Radeckiego 2, 50-345 Wrocław
[2] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław
[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław
[4] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław
[5] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław
[6] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław
[7] Zakład Podstaw Nauk Medycznych, Uniwersytet Medyczny we Wrocławiu, ul. Borowska 211A, 50–556 Wrocław

Benita Wiatrak – B,C,D
Sadri Rayad – B,C,D
Tomasz Gębarowski – B,C,E
Jakub Hadzik – B,C
Marzena Styczyńska – B,C
Tomasz Gedrange – A,B,E
Maciej Dobrzyński – E,F
Ewa Barg – B,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Benita Wiatrak
....
czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Benita Wiatrak [1], Sadri Rayad [2], Tomasz Gębarowski [3], Jakub Hadzik [4], Marzena Styczyńska [5], Tomasz Gedrange [4], Maciej Dobrzyński [6], Ewa Barg [7], Marzena Dominiak [4]: *Comparative Analysis of Heavy Metal Content in Impacted Third Molars from Industrial and Non-Industrial Areas and Its Effect on the Isolation, Culture, and Proliferation of Dental Stem Cells (DSCs). J. Clin. Med.* 2024, 13, 5465. https://doi.org/10.3390/jcm13185465

[1] Katedra i Zakład Farmakologii, Uniwersytet Medyczny we Wrocławiu, ul. Mikulicza-Radeckiego 2, 50-345 Wrocław
[2] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław
[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław
[4] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław
[5] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław
[6] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław
[7] Zakład Podstaw Nauk Medycznych, Uniwersytet Medyczny we Wrocławiu, ul. Borowska 211A, 50–556 Wrocław

Benita Wiatrak – B,C,D
Sadri Rayad – B,C,D
Tomasz Gębarowski – B,C,E
Jakub Hadzik – B,C
Marzena Styczyńska – B,C
Tomasz Gedrange – A,B,E
Maciej Dobrzyński – E,F
Ewa Barg – B,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Tomasz Gębarowski

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Benita Wiatrak [1], Sadri Rayad [2], Tomasz Gębarowski [3], Jakub Hadzik [4], Marzena Styczyńska [5], Tomasz Gedrange [4], Maciej Dobrzyński [6], Ewa Barg [7], Marzena Dominiak [4]: *Comparative Analysis of Heavy Metal Content in Impacted Third Molars from Industrial and Non-Industrial Areas and Its Effect on the Isolation, Culture, and Proliferation of Dental Stem Cells (DSCs). J. Clin. Med.* 2024, 13, 5465. https://doi.org/10.3390/jcm13185465

[1] Katedra i Zakład Farmakologii, Uniwersytet Medyczny we Wrocławiu, ul. Mikulicza-Radeckiego 2, 50-345 Wrocław
[2] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław
[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław
[4] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław
[5] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław
[6] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław
[7] Zakład Podstaw Nauk Medycznych, Uniwersytet Medyczny we Wrocławiu, ul. Borowska 211A, 50–556 Wrocław

Benita Wiatrak – B,C,D
Sadri Rayad – B,C,D
Tomasz Gębarowski – B,C,E
Jakub Hadzik – B,C
Marzena Styczyńska – B,C

Tomasz Gedrange – A,B,E
Maciej Dobrzyński – E,F
Ewa Barg – B,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

dr hab. Jakub Hadzik prof. UMW
specjalista chirurgii stomatologicznej
PWZ: 2634982

....
czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Benita Wiatrak [1], Sadri Rayad [2], Tomasz Gębarowski [3], Jakub Hadzik [4], Marzena Styczyńska [5], Tomasz Gedrange [4], Maciej Dobrzyński [6], Ewa Barg [7], Marzena Dominiak [4]: *Comparative Analysis of Heavy Metal Content in Impacted Third Molars from Industrial and Non-Industrial Areas and Its Effect on the Isolation, Culture, and Proliferation of Dental Stem Cells (DSCs). J. Clin. Med.* 2024, 13, 5465. https://doi.org/10.3390/jcm13185465

[1] Katedra i Zakład Farmakologii, Uniwersytet Medyczny we Wrocławiu, ul. Mikulicza-Radeckiego 2, 50-345 Wrocław

[2] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

[4] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław

[5] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[6] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[7] Zakład Podstaw Nauk Medycznych, Uniwersytet Medyczny we Wrocławiu, ul. Borowska 211A, 50–556 Wrocław

Benita Wiatrak – B,C,D
Sadri Rayad – B,C,D
Tomasz Gębarowski – B,C,E
Jakub Hadzik – B,C
Marzena Styczyńska – B,C
Tomasz Gedrange – A,B,E
Maciej Dobrzyński – E,F
Ewa Barg – B,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;

B – gromadzenie danych;

C – analiza i interpretacja danych;

D – napisanie artykułu;

E – krytyczna ocena artykułu;

F – ostateczna akceptacja artykułu

Marzena Styczyńska
....
czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Benita Wiatrak [1], Sadri Rayad [2], Tomasz Gębarowski [3], Jakub Hadzik [4], Marzena Styczyńska [5], Tomasz Gedrange [4], Maciej Dobrzyński [6], Ewa Barg [7], Marzena Dominiak [4]: *Comparative Analysis of Heavy Metal Content in Impacted Third Molars from Industrial and Non-Industrial Areas and Its Effect on the Isolation, Culture, and Proliferation of Dental Stem Cells (DSCs). J. Clin. Med.* 2024, 13, 5465. https://doi.org/10.3390/jcm13185465

[1] Katedra i Zakład Farmakologii, Uniwersytet Medyczny we Wrocławiu, ul. Mikulicza-Radeckiego 2, 50-345 Wrocław

[2] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

[4] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław

[5] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[6] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[7] Zakład Podstaw Nauk Medycznych, Uniwersytet Medyczny we Wrocławiu, ul. Borowska 211A, 50–556 Wrocław

Benita Wiatrak – B,C,D
Sadri Rayad – B,C,D
Tomasz Gębarowski – B,C,E
Jakub Hadzik – B,C
Marzena Styczyńska – B,C

Tomasz Gedrange – A,B,E
Maciej Dobrzyński – E,F
Ewa Barg – B,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;

B – gromadzenie danych;

C – analiza i interpretacja danych;

D – napisanie artykułu;

E – krytyczna ocena artykułu;

F – ostateczna akceptacja artykułu

Dental Surgery Department
of Silesian Piast Medical Uniwersity
of Wrocław, Poland

prof. dr hab. Tomasz Gedrange

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Benita Wiatrak [1], Sadri Rayad [2], Tomasz Gębarowski [3], Jakub Hadzik [4], Marzena Styczyńska [5], Tomasz Gedrange [4], Maciej Dobrzyński [6], Ewa Barg [7], Marzena Dominiak [4]: *Comparative Analysis of Heavy Metal Content in Impacted Third Molars from Industrial and Non-Industrial Areas and Its Effect on the Isolation, Culture, and Proliferation of Dental Stem Cells (DSCs). J. Clin. Med.* 2024, 13, 5465. https://doi.org/10.3390/jcm13185465

[1] Katedra i Zakład Farmakologii, Uniwersytet Medyczny we Wrocławiu, ul. Mikulicza-Radeckiego 2, 50-345 Wrocław
[2] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław
[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław
[4] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław
[5] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław
[6] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław
[7] Zakład Podstaw Nauk Medycznych, Uniwersytet Medyczny we Wrocławiu, ul. Borowska 211A, 50–556 Wrocław

Benita Wiatrak – B,C,D
Sadri Rayad – B,C,D
Tomasz Gębarowski – B,C,E
Jakub Hadzik – B,C
Marzena Styczyńska – B,C

Tomasz Gedrange – A,B,E
Maciej Dobrzyński – E,F
Ewa Barg – B,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Uniwersytet Medyczny we Wrocławiu
Wydział Lekarsko-Stomatologiczny
KATEDRA I ZAKŁAD
STOMATOLOGII DZIECIĘCEJ
I STOMATOLOGII PRZEDKLINICZNEJ
Kierownik
dr hab. n. med. Maciej Dobrzyński, profesor uczelni

....

czytelny podpis

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Benita Wiatrak [1], Sadri Rayad [2], Tomasz Gębarowski [3], Jakub Hadzik [4], Marzena Styczyńska [5], Tomasz Gedrange [4], Maciej Dobrzyński [6], Ewa Barg [7], Marzena Dominiak [4]: *Comparative Analysis of Heavy Metal Content in Impacted Third Molars from Industrial and Non-Industrial Areas and Its Effect on the Isolation, Culture, and Proliferation of Dental Stem Cells (DSCs). J. Clin. Med.* 2024, 13, 5465. https://doi.org/10.3390/jcm13185465

[1] Katedra i Zakład Farmakologii, Uniwersytet Medyczny we Wrocławiu, ul. Mikulicza-Radeckiego 2, 50-345 Wrocław

[2] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław

[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław

[4] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław

[5] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław

[6] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław

[7] Zakład Podstaw Nauk Medycznych, Uniwersytet Medyczny we Wrocławiu, ul. Borowska 211A, 50–556 Wrocław

Benita Wiatrak – B,C,D
Sadri Rayad – B,C,D
Tomasz Gębarowski – B,C,E
Jakub Hadzik – B,C
Marzena Styczyńska – B,C

Tomasz Gedrange – A,B,E
Maciej Dobrzyński – E,F
Ewa Barg – B,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Ewa Barg
....
czytelny podpis

Uniwersytet Medyczny we Wrocławiu
Wydział Farmaceutyczny
Katedra Podstaw Nauk Medycznych i Immunologii
ZAKŁAD PODSTAW NAUK MEDYCZNYCH
kierownik

dr hab. n. med. Ewa Barg, profesor uczelni

Wrocław, dnia 1.10.2024 roku

Oświadczenie

Oświadczam, że w poniższej pracy mój wkład stanowił zgodnie z rozdzielnikiem

Benita Wiatrak [1], Sadri Rayad [2], Tomasz Gębarowski [3], Jakub Hadzik [4], Marzena Styczyńska [5], Tomasz Gedrange [4], Maciej Dobrzyński [6], Ewa Barg [7], Marzena Dominiak [4]: *Comparative Analysis of Heavy Metal Content in Impacted Third Molars from Industrial and Non-Industrial Areas and Its Effect on the Isolation, Culture, and Proliferation of Dental Stem Cells (DSCs). J. Clin. Med.* 2024, 13, 5465. https://doi.org/10.3390/jcm13185465

[1] Katedra i Zakład Farmakologii, Uniwersytet Medyczny we Wrocławiu, ul. Mikulicza-Radeckiego 2, 50-345 Wrocław
[2] Medyczne Centrum Innowacji Wrocław Sp. z o.o., Uniwersyteckie Centrum Stomatologiczne Wrocław, ul. Krakowska 26, 50-425 Wrocław
[3] Katedra Biostruktury i Fizjologii Zwierząt, Uniwersytet Przyrodniczy we Wrocławiu, ul. Kożuchowska 1/3, 51-631 Wrocław
[4] Katedra i Zakład Chirurgii Stomatologicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50-425 Wrocław
[5] Katedra Żywienia Człowieka, Uniwersytet Przyrodniczy we Wrocławiu, ul. Chełmońskiego 47/21, 51-630 Wrocław
[6] Katedra i Zakład Stomatologii Dziecięcej i Stomatologii Przedklinicznej, Uniwersytet Medyczny we Wrocławiu, ul. Krakowska 26, 50–425 Wrocław
[7] Zakład Podstaw Nauk Medycznych, Uniwersytet Medyczny we Wrocławiu, ul. Borowska 211A, 50–556 Wrocław

Benita Wiatrak – B,C,D
Sadri Rayad – B,C,D
Tomasz Gębarowski – B,C,E
Jakub Hadzik – B,C
Marzena Styczyńska – B,C

Tomasz Gedrange – A,B,E
Maciej Dobrzyński – E,F
Ewa Barg – B,E
Marzena Dominiak – A,E,F

A – koncepcja i projekt badawczy;
B – gromadzenie danych;
C – analiza i interpretacja danych;
D – napisanie artykułu;
E – krytyczna ocena artykułu;
F – ostateczna akceptacja artykułu

Uniwersytet Medyczny we Wrocławiu
KATEDRA I ZAKŁAD CHIRURGII
STOMATOLOGICZNEJ
kierownik
prof. dr hab. Marzena Dominiak

....
czytelny podpis

KOMISJA BIOETYCZNA
przy
Uniwersytecie Medycznym
we Wrocławiu
ul. Pasteura 1; 50-367 WROCŁAW

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 246/2019

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 133/XV R/2017 z dnia 21 grudnia 2017 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 28 z 1997 r. poz. 152 z późniejszymi zmianami) w składzie:

dr hab. Jacek Daroszewski, prof. nadzw. (endokrynologia, diabetologia)
prof. dr hab. Krzysztof Grabowski (chirurgia)
dr Henryk Kaczkowski (chirurgia szczękowa, chirurgia stomatologiczna)
mgr Irena Knabel-Krzyszowska (farmacja)
prof. dr hab. Jerzy Liebhart (choroby wewnętrzne, alergologia)
ks. dr hab. Piotr Mrzygłód, prof. nadzw. (duchowny)
mgr Luiza Műller (prawo)
dr hab. Sławomir Sidorowicz (psychiatria)
dr hab. Leszek Szenborn, prof. nadzw (pediatria, choroby zakaźne)
Danuta Tarkowska (pielęgniarstwo)
prof. dr hab. Anna Wiela-Hojeńska (farmakologia kliniczna)
dr hab. Andrzej Wojnar, prof. nadzw. (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)
dr hab. Jacek Zieliński (filozofia)

pod przewodnictwem
prof. dr hab. Jana Kornafela (ginekologia i położnictwo, onkologia)

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z projektem badawczym pt.

„Zawartość metali toksycznych w zatrzymanych zębach trzecich trzonowych u mieszkańców Legnicko-Głogowskiego Okręgu Miedziowego oraz wpływ poziomu witaminy D we krwi włośniczkowej na proces gojenia rany poekstrakcyjnej" zgłoszonym przez **lek. dent. Sadri Rayad** zatrudnionego w Stomatologicznym Centrum Transferu Technologii Sp. z o.o. we Wrocławiu oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła wyrazić zgodę na przeprowadzenie badania w: Stomatologicznym Centrum Transferu Technologii Sp. z o.o. we Wrocławiu ; PROORTO Ortodoncja i Stomatologia Estetyczna Jolanta Kardasz-Pawlik w Legnicy ; TOP DENT Prywatnym Gabinecie Stomatologicznym Michał Fuczko w Legnicy pod nadzorem prof. dr hab. Marzeny Dominiak i dr Macieja Dobrzyńskiego – promotora pomocniczego **pod warunkiem zachowania anonimowości uzyskanych danych**.

Pouczenie: W ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej UM we Wrocławiu

Opinia powyższa dotyczy: projektu badawczego będącego podstawą rozprawy doktorskiej

Wrocław, dnia 18 marca 2019 r.

BW

Uniwersytet Medyczny we Wrocławiu
KOMISJA BIOETYCZNA
przewodniczący
prof. dr hab. Jan Kornafel

Sadri Rayad

Publikacje stanowiące cykl w ramach postępowania doktorskiego

Lp.	Opis bibliograficzny	IF	Punkty
1.	**Rayad Sadri**, Dobrzyński Maciej, Kuźniarski Amadeusz, Styczyńska Marzena, Diakowska Dorota, Gedrange Tomasz, Klimas Sylwia, Gębarowski Tomasz, Dominiak Marzena: An in-vitro evaluation of toxic metals concentration in the third molars from residents of the Legnica-Głogów Copper Area and risk factors determining the accumulation of those metals: a pilot study, Applied Sciences-Basel, 2023, vol. 13, nr 5, art.2904 [15 s.], DOI:10.3390/app13052904	2,5	100
2.	**Rayad Sadri**, Dobrzyński Maciej, Kuźniarski Amadeusz, Styczyńska Marzena, Diakowska Dorota, Gedrange Tomasz, Klimas Sylwia, Gębarowski Tomasz, Dominiak Marzena: Mercury content in impacted wisdom teeth from patients of the Legnica-Głogów Copper Area - an in vitro pilot study, Journal of Xenobiotics, 2023, vol. 13, nr 3, s. 463-478, DOI:10.3390/jox13030029	6,8	20
3.	Wiatrak Benita, **Rayad Sadri**, Gębarowski Tomasz, Hadzik Jakub, Styczyńska Marzena, Gedrange Tomasz, Dobrzyński Maciej, Barg Ewa, Dominiak Marzena: Comparative analysis of heavy metal content in impacted third molars from industrial and non-industrial areas and its effect on the isolation, culture, and proliferation of dental stem cells (DSCs), Journal of Clinical Medicine, 2024, vol. 13, nr 18, art.5465 [14 s.], DOI:10.3390/jcm13185465	3*	140
4.	**Sadri Rayad**, Sylwia Klimas, Maciej Janeczek, Agata Małyszek, Marta Bort, Andrzej Małysa, Marzena Dominiak, Maciej Dobrzyński. Studies on the content of toxic metals in teeth: A narrative review of literature [published online as ahead of print on September 26, 2024]. Dent Med Probl. doi:10.17219/dmp/193406	2.7*	70

IF 2023

Impact factor: **15,0**

Punkty ministerialne: **330,0**

OSOBA SPORZADZAJĄCA: PIOTR ORNAWKA
DZIAŁ BIBLIOGRAFII I BIBLIOMETRII BG UMW

Sadri Rayad

Wykaz publikacji

1. Publikacje w czasopismach naukowych

1.1 Publikacje w czasopiśmie z IF

Lp.	Opis bibliograficzny	IF	Punkty
1	Sarzyńska Kathie, Czwojdziński Eddie, Kuźniarski Amadeusz, **Rayad Sadri**, Piwowar Agnieszka, Jankowska-Polańska Beata: Medical students' knowledge about COVID-19 and evaluation of the effectiveness of the applied preventive strategies, Archives of Public Health, 2022, vol. 80, art.122 [13 s.], DOI:10.1186/s13690-022-00873-8	3,3	100
2	**Rayad Sadri**, Dobrzyński Maciej, Kuźniarski Amadeusz, Styczyńska Marzena, Diakowska Dorota, Gedrange Tomasz, Klimas Sylwia, Gębarowski Tomasz, Dominiak Marzena: An in-vitro evaluation of toxic metals concentration in the third molars from residents of the Legnica-Głogów Copper Area and risk factors determining the accumulation of those metals: a pilot study, Applied Sciences-Basel, 2023, vol. 13, nr 5, art.2904 [15 s.], DOI:10.3390/app13052904	2,5	100
3	**Rayad Sadri**, Dobrzyński Maciej, Kuźniarski Amadeusz, Styczyńska Marzena, Diakowska Dorota, Gedrange Tomasz, Klimas Sylwia, Gębarowski Tomasz, Dominiak Marzena: Mercury content in impacted wisdom teeth from patients of the Legnica-Głogów Copper Area - an in vitro pilot study, Journal of Xenobiotics, 2023, vol. 13, nr 3, s. 463-478, DOI:10.3390/jox13030029	6,8	20
4	Wiatrak Benita, **Rayad Sadri**, Gębarowski Tomasz, Hadzik Jakub, Styczyńska Marzena, Gedrange Tomasz, Dobrzyński Maciej, Barg Ewa, Dominiak Marzena: Comparative analysis of heavy metal content in impacted third molars from industrial and non-industrial areas and its effect on the isolation, culture, and proliferation of dental stem cells (DSCs), Journal of Clinical Medicine, 2024, vol. 13, nr 18, art.5465 [14 s.], DOI:10.3390/jcm13185465	3*	140
5	**Sadri Rayad**, Sylwia Klimas, Maciej Janeczek, Agata Małyszek, Marta Bort, Andrzej Małysa, Marzena Dominiak, Maciej Dobrzyński. Studies on the content of toxic metals in teeth: A narrative review of literature [published online as ahead of print on September 26, 2024]. Dent Med Probl. doi:10.17219/dmp/193406	2.7*	70
	Podsumowanie	18,3	430

***IF 2023**

1.2 Publikacje w czasopiśmie bez IF

Lp.	Opis bibliograficzny	Punkty
1	Łada Jakub, **Rayad Sadri**, Żyła Tomasz, Skośkiewicz-Malinowska Katarzyna: Metody zakładania koferdamu oraz jego zastosowanie w praktyce stomatologicznej w ocenie studentów stomatologii, Magazyn Stomatologiczny, 2014, vol. 24, nr 4, 131-138 (on-line)	4

2	Dominiak Marzena, Różyło-Kalinowska Ingrid, Gedrange Tomasz, Konopka Tomasz, Hadzik Jakub, Bednarz Wojciech, Matys Jacek, Lella Anna, **Rayad Sadri**, Maksymowicz Radosław, Kuźniarski Amadeusz: COVID-19 and professional dental practice. The Polish Dental Association Working Group recommendations for procedures in dental office during an increased epidemiological risk, Czasopismo Stomatologiczne, 2020, vol. 73, nr 1, s. 1-10, DOI:10.5114/jos.2020.94168	20
	Podsumowanie	24

2. Monografie naukowe

2.1 Książka autorska –
2.2 Książka redagowana –

2.3 Rozdziały

Lp.	Opis bibliograficzny	Punkty
1	Kuźniarski Amadeusz, **Rayad Sadri**, Dobrzyński Maciej: Programowane działania na rzecz ochrony zdrowia jamy ustnej realizowane w wymiarze regionalnym, ponadregionalnym i międzynarodowym, W: Stomatologia społeczna, (red.) Krystyna Pawlas, Tomasz Konopka, Warszawa 2021, Wydawnictwo Lekarskie PZWL, s. 443-454, ISBN 978-83-200-6132-1	20
2	Kuźniarski Amadeusz, **Rayad Sadri**: Miejsce stomatologii w systemie ochrony zdrowia, W: Stomatologia społeczna, (red.) Krystyna Pawlas, Tomasz Konopka, Warszawa 2021, Wydawnictwo Lekarskie PZWL, s. 455-462, ISBN 978-83-200-6132-1	20
	Podsumowanie	40

Impact factor: **18,3**

Punkty ministerialne: **494,0**

OSOBA SPORZADZAJĄCA: PIOTR ORNAWKA
DZIAŁ BIBLIOGRAFII I BIBLIOMETRII BG UMW