On the road again—a review of pretreatment methods for the decontamination of skeletal materials for strontium isotopic and concentration analysis

Crista Adelle Wathen1 · Sven Isaksson1 · Kerstin Lidén1

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Abstract
Strontium isotopic and concentration results from archeological skeletons have proved useful in demonstrating human and animal mobility patterns, and dietary life-history. This initiated the movement from proxies to answer these questions. However, there remains an issue as to whether the produced isotopic and concentration values are those accumulated by an individual during life and not an analytical artifact or the result of remaining diagenetic material or other forms of contamination. Over the last 40 years, there have been a variety of protocols used with varying success to remove contaminants prior to analysis, as well as a movement from bone analysis to solely enamel. This review covers the evolution of pretreatment protocols, the role of technological advances in producing accurate and precise results, and a discussion of best practices. Archeological case studies will demonstrate the evolution of these topics as well as their limitations and potential.

Keywords Strontium isotope · Strontium concentration · Prehistoric migration · Pretreatment · Diet · Diagenesis

Introduction
Strontium isotopes are widely used in archeology to answer questions regarding human mobility patterns. This information helps reconstruct interactions between groups and the building of social and economic networks (Ericson 1985; Montgomery 2010; Szostek et al. 2015). Archeologists analyze various materials to answer these questions, e.g., human enamel and bone (Ericson 1985; Killgrove & Montgomery 2016; Price et al. 1994a), faunal bone and enamel (Lewis et al. 2017; Madgwick et al. 2017, 2019; Schulting et al. 2019; Sillen and LeGeros 1991), cereal grains (Benson 2010; Larsson et al. 2020), wood (English et al. 2001; Ostapkowicz et al. 2017), and textiles (woolen, hair, leather) (Frei 2014; Frei et al. 2009, 2017; von Carnap-Bornheim et al. 2007).

Several reviews give an overview of the various archeological applications of strontium analysis (e.g., Bentley 2006; Slovak and Paytan 2011). These reviews, however, do not describe the various pretreatment methods used prior to analysis for the removal of contamination caused by soil or post-exavation techniques. In addition, there is little research on whether post-exavation-applied adhesives are an issue in strontium isotope studies, despite there being ample research on its effects on other isotopes: radiocarbon (14C) (Devièse et al. 2018), other carbon (δ13C), nitrogen (δ15N), and oxygen (δ18O) isotopes (France et al. 2011, 2019, 2020). Furthermore, research on the effects of pretreatment methods on isotopes and trace elements has been published, particularly on δ13C, 14C, δ18O, and Sr (e.g., Garvie-Lok et al. 2004; Hopkins et al. 2016; McMillan et al. 2019; Pellegrini and Snoeck 2016; Snoeck & Pellegrini 2015; Yoder and Bartelink 2010). Analytical protocols vary by sample type and laboratory making it difficult to determine the optimal methodology to create a universal pretreatment protocol. Furthermore, in terms of human and faunal remains, enamel has become the preferred sample type, making bone analysis obsolete, and sometimes only analyzed to determine the biologically available strontium
(Francisci et al. 2020; Grupe et al. 1997; Pollard et al. 2017; Snoeck et al. 2016). In addition, there is a drift towards only publishing strontium isotopic values and not the concentration values. Fortunately, there appears to be a slight increase in recent studies that are publishing both types of values, thereby adding another layer in mobility and dietary research (e.g., Horstwood et al. 2008; Nowell and Horstwood 2009; Montgomery 2010; Montgomery et al. 2019; Reynard and Balter 2014; Snoeck et al. 2016). This review presents a timeline of strontium pretreatment methods, as well as a discussion of the technological breakthroughs beginning in the 1980s to the present.

**Strontium isotopes and concentration**

Strontium has four naturally occurring isotopes: $^{84}$Sr, $^{86}$Sr, $^{87}$Sr, and $^{88}$Sr (Brown and Brown 2011; Pollard et al. 2017). The amounts of $^{84}$Sr, $^{86}$Sr, and $^{88}$Sr in bedrock are constant and stable but $^{87}$Sr is radiogenic and its amount is dependent on the $^{87}$Rb content in the rock and how much of it has decayed into $^{87}$Sr (Brown and Brown 2011). In migration studies, the strontium ratio is expressed as $^{87}$Sr/$^{86}$Sr, which has been broadly used to map and source various archeological materials (Bentley 2006; Bentley et al. 2004; English et al. 2001; Hajj et al. 2016). The strontium pretreatment, contamination, and $^{87}$Sr/$^{86}$Sr values in modern humans (bone and teeth) is reported to be between 50 and 300 ppm; however, like their isotopes, it appears to vary according to geography, diet, bone analyzed, etc. (Montgomery 2010; Specht et al. 2017; Turekian and Kulp 1956). However, the reported values from archeological skeletal material vary greatly between populations, periods, contamination type, and can range from between 50 and 850 ppm, as demonstrated in Fig. 1 (Bentley 2006; Horstwood et al. 2008; Nowell and Horstwood 2009; Simonetti et al. 2008; Slovak and Paytan 2011); however, these numbers have been reported to be higher, likely due to contamination (Kyle 1986; Sealy et al. 1991).

The strontium concentration in modern humans (bone and teeth) is reported to be between 50 and 300 ppm; however, like their isotopes, it appears to vary according to geography, diet, bone analyzed, etc. (Montgomery 2010; Specht et al. 2017; Turekian and Kulp 1956). However, the reported values from archeological skeletal material vary greatly between populations, periods, contamination type, and can range from between 50 and 850 ppm, as demonstrated in Fig. 1 (Bentley 2006; Horstwood et al. 2008; Nowell and Horstwood 2009; Simonetti et al. 2008; Slovak and Paytan 2011); however, these numbers have been reported to be higher, likely due to contamination (Kyle 1986; Sealy et al. 1991).

Measuring concentration data or its proxies can demonstrate the systematic differences in absolute isotopic values produced in solution vs. laser ablation (LA) modes of analysis (Lewis et al. 2017; Lugli 2019; Montgomery et al. 2010; Simonetti et al. 2008). According to Simonetti et al. (2008), $^{87}$Sr/$^{86}$Sr values produced via the LA mode were more radiogenic than the solution values from the enamel of the same individual. Therefore, the isotopic data would benefit from being analyzed in conjunction with trace elements, if not to determine the dietary variation within a population but to offset any analytical artifacts left by machinery (Nowell and Horstwood 2009; Simonetti et al. 2008). These are due to isobaric interferences from other elements that share isotopes with an identical mass number, such as $^{87}$Rb and $^{87}$Sr (Lugli 2019; Nowell and...
Horstwood et al. 2009; Simmonetti et al. 2008). Ultimately, analyzing both concentration and isotopic data ensures that the data is accurate and reliable, especially with rare and important samples where it might not be possible to reanalyze at a future date. In addition, micro-sampling, especially when analyzing enamel, offers the opportunity to present a chronological timeline of dietary incorporation (Balcaen et al. 2010; Glykou et al. 2018; Montgomery et al. 2010). However, mineralization rates are individual and species-specific, meaning that intra-enamel analysis may still yield results that demonstrate a longer period of incorporation (Hoppe et al. 2004; Montgomery et al. 2010). However, mineralization rates are individual and species-specific, meaning that intra-enamel analysis may still yield results that demonstrate a longer period of incorporation (Hoppe et al. 2004; Montgomery et al. 2010). This means that LA modes of analysis demonstrate multiple long-term averages of isotopic incorporation, depending on the number of ablated lines, whereas solution modes demonstrate only one bulk value.

In archeological materials

Strontium is chemically similar to calcium and is discriminated against in the kidneys in favor of calcium synthesis (Kohn et al. 1999; Pollard et al. 2017; Pors Nielsen 2004), which is then exchanged in the inorganic fractions of the bones and teeth (Brown and Brown 2011). The inorganic fractions, bone mineral and enamel, are both calcium phosphate apatite, whereas the organic fractions, collagen and dentine, are composed of multiple helical peptide fibrils (Lee-Thorp 2008). Teeth form incrementally during early-life and upon full mineralization of the calcium fractions in the enamel, it no longer exchanges with the elements in the environment (Hillson 1996), essentially displaying a snapshot of the early-life dietary $^{87}\text{Sr}/^{86}\text{Sr}$ values (Beaumont and Montgomery 2015).

Bones are continually exchanging with the elements within the environment and are turning over at an average of 7–10 years (Dent et al. 2004; Fahy et al. 2017; Hedges et al. 2007). Turnover is based on various factors such as biological age, bone type, and nutritional status (Dent et al. 2004; Fahy et al. 2017; Hedges et al. 2007; Pollard et al. 2017; Sealy et al. 1995). For instance, if an individual moves from one geological area to another, the $^{87}\text{Sr}/^{86}\text{Sr}$ values in the bones begin to equilibrate to the $^{87}\text{Sr}/^{86}\text{Sr}$ values of the new area, therefore, displaying an average between the two locations (Ericson 1985; Hedges et al. 2007; Price et al. 2015), granted that they are geologically different. This assumes that an individual only moved from point A to point B; however, metabolized strontium displays a long

Fig. 1 A portion of the ranges of archeological Sr ppm data found at various sites and periods. Those with an "*" signal that the analysis was completed on teeth as a whole and those with “+” are of bone. The darkened portion signifies the range of Sr ppm in modern humans and those that are within the range. (Buckberry et al. 2014; Evans et al. 2006, 2012; Kyle 1986; Montgomery et al. 2007; Shaw et al. 2016; White and Schwarcz 1989)
and ongoing period of an individual’s lifetime (Pollard et al. 2017). Therefore, comparing the $^{87}$Sr/$^{86}$Sr values of enamel and bone can identify migrants within an archeological population and detect subtler movements of recent or long-term residents (Ericson 1985; Pollard et al. 2017). However, this comparison can only be made if the materials are not altered through contamination.

**Diagenesis and preservation of osteological materials**

Diagenesis is an important research topic (e.g., Lambert et al. 1985, 1989, 1990; Madgwick et al. 2012; Nelson et al. 1986; Price et al. 1992; Sillen 1986), where it relies on a variety of material-specific and environment-specific parameters (Dent et al. 2004; Keenan 2016; Kendall et al. 2018; Lambert 1997; Madgwick et al. 2012). For instance, in life, the inorganic and organic portions of the bone are protected from the external environment by a closed system with controlled pH levels (Turner-Walker 2007). But once the body begins to decompose and the mineral portion becomes exposed, it is then vulnerable to dissolution by groundwater, which may cause exogenous ions in the soil to bind within the inorganic fractions of the bone (Lambert 1997; Turner-Walker 2007). Teeth, unlike bones, erupt through the soft tissues in the mouth and are frequently exposed to the external environment (Turner-Walker 2007). Structurally, enamel has a lower organic content and is compact with larger crystals than its inner dentine core or bone (Kohn et al. 1999; Kocsis et al. 2010; Turner-Walker 2007). The cementum and dentine portions are chemically similar to bone and are vulnerable to dissolution (Hollund et al. 2014, 2015), while the enamel is less susceptible. The degradation processes of this material are less well-understood than bone; however, the preservation of teeth still needs to be determined prior to analysis. According to Hollund and colleagues (2015), there is a link between bone and tooth degradation, as shown through histological analysis, further demonstrating that pre-screening teeth is a necessary step prior to biomolecular analysis.

In addition, there appears to be a link between the manner in which bones have entered the archeological record and the type of diagenesis (Jans et al. 2004). For instance, bone fragments (e.g., butchered animals) are well-preserved or only show evidence of fungal attack, unlike in complete burials (human and faunal) which is likely to indicate bacterial degradation (Jans et al. 2004). However, the degradation of skeletal materials is multi-faceted and can occur concurrently with one another based on various parameters in the burial context. Therefore, it is important to not only identify whether or not the material is contaminated but to determine the type of contamination so that the sampling and analytical strategies can be geared towards it.

The 1980s saw a series of breakthroughs in diagenetic research, its effects on elemental values in prehistoric bone and its attempts to isolate the biogenic values (DeNiro 1985; Nelson et al. 1986; Schoeninger and DeNiro 1984; Schoeninger et al. 1989; Sillen 1986, 1989; Sullivan and Krueger 1981; Tuross et al. 1989). Three methodologies have influenced modern analysis, Sullivan & Krueger’s acetic acid wash, Sillen’s solubility profile, and Nelson’s leachate procedure. These methods are demonstrated in Table 1. All three of these methodologies use acetic acid of varying degrees of acidity (pH 2.37–4.5), reaction time (1 min 10 s–24 h), and component analyzed (ex. supernatant vs. residue).

Sullivan and Krueger (1981) conducted stable carbon isotope analysis on the inorganic apatite instead of the organic portion, as was the standard. Despite their focus on stable carbon isotopes, their research had a twofold effect on strontium analysis. First, they demonstrated that archeological apatite over 10,000 years old was still viable. This was initially rejected, as it was believed that the variability in isotopic composition between the apatite and collagen portion was due to diagenesis (Ambrose and Krigbaum 2003; Nelson et al. 1986; Schoeninger and DeNiro 1982). However, Krueger and Sullivan (1984) argued that it was due to trophic level differences. Second, their published protocol (Table 1) called for two overnight washes to remove the diagenetic portion of the apatite, which has since been applied in various paleomigration studies, with minute changes.

In 1986, Andrew Sillen published the solubility profiling method (Table 1), a method influenced by Crommelin and colleagues (1983), arguing that diagenetic carbonate is soluble, and the biogenic values should be realized during the later washes (Pollard et al. 2017; Sealy et al. 1991; Sillen 1986; Trickett et al. 2003). Andrew Sillen (1986) examined 2-million-year-old bones of fauna from the Omo Basin in Ethiopia. The bones were not mechanically cleaned prior to powdering; however, the bone powders underwent three acetone washes to remove any acetone-soluble preservatives. Supernatants from the 24 acetate-buffer washes adjusted to pH 4.5 were measured for elemental content with flame atomic absorption spectrophotometry. The diagenetic apatite appeared to be removed during the first five washes with the biogenic values appearing in the later washes.

Despite the initial success of the solubility profiling method, other researchers argued that the biogenic signal is irretrievable once the apatite has undergone diagenesis, and it is difficult to know if it is fully removed (Budd et al.
Sealy et al. (1991) demonstrated promising results when using the solubility method to analyze diagenetic archeological faunal materials with an unknown biogenic signal. The diagenetic material appeared to be removed in the early stages. Sealy et al. (1991) analyzed two archeological faunal materials, hartebeest and mammoth, which were both buried in marine-derived environments. Results were promising as the residue yield from the later washes demonstrated increasingly enriched $^{87}$Sr/$^{86}$Sr values for the hartebeest material and depleted $^{87}$Sr/$^{86}$Sr for the mammoth material (Sealy et al. 1991). This was a movement towards the perceived biogenic values and gave the researchers confidence that the results were not analytical artifacts (Sealy et al. 1991). Unfortunately, it is difficult to determine if the values were the true biogenic values.

2000; Price et al. 1992; Sealy et al. 1991; Trickett et al. 2003; Tuross et al. 1989). Tuross et al. (1989) applied the solubility profiling method to different archeological skeletal elements of eight individuals of varying preservation and those of modern fauna. Results demonstrated that the later sequential washes did not produce the biogenic values in the archeological samples. Sillen (1990) rebutted that their lack of success was due to their sampling method, as Tuross and colleagues analyzed rib bones instead of cortical bone or enamel, and the burial environment. In the response, Sillen also noted that the profiling method has had issues with isolating and ensuring the measurement of the biogenic and biologically interpretable Sr/Ca ratios, especially considering that diagenesis can vary depending on the burial environment (1990).
Nelson et al. (1986) published a protocol often cited as evidence that biogenic strontium values are irretrievable. They analyzed both modern and archeological bone from marine and terrestrial environments and reported that the marine bones excavated from terrestrial environments yielded $^{87}\text{Sr}/^{86}\text{Sr}$ values inconsistent with a marine origin. Nelson et al. (1986) published what is known as a leaching protocol (Table 1), a multi-step process that included an ultrasonic cleaning,ashing, and grinding into a powder, where a portion is saved for total $^{87}\text{Sr}/^{86}\text{Sr}$ and concentration analysis. The remaining powder is set to react with a 50:50 (v/v) of glacial acetic acid and deionized water for 3 h, and then the leachate and the residue are separately analyzed for strontium concentration and isotopic values (Nelson et al. 1986; Sillen and Sealy 1995). The preliminary ashing step is reminiscent of the methodology published by Turekian and Kulp (1956), suggesting that ashing bone samples in preparation for Sr/Ca would be beneficial, as it would set all the samples at the same baseline. However, other researchers have found that it had an adverse effect on the samples. For instance, Sillen and Sealy (1995) argued that the protocol’s lack of success in retrieving the biogenic strontium values is due to the recrystallization of apatite, either by the ashing step or by the high acidity of the glacial acetic acid wash, and not necessarily due to diagenesis.

As research continued into the 1990s, there were fewer applications of the solubility profiling method and a reappearance of variations of Sullivan and Krueger’s methodology. Despite many references to Sillen’s solubility method, Sullivan and Krueger’s methodology appears to have influenced many recent methodologies.

Comparison of the methods and their subsequent evolution in osteological decontamination

Uncertainty arose in the archeological community on the effectiveness of these published protocols, prompting researchers to determine the most effective protocol that could be implemented universally. Lambert et al. (1990) compared several methods to remove diagenetic material before elemental analysis. Three femurs from a Middle Woodland site in the USA and six from a Late Intermediate Estuquïña site in Peru underwent four different methods. The first was a simple brushing and washing prior to analysis by atomic absorption spectrometry as described by Szpunar et al. (1978). The second method described by Lambert et al. (1989) entailing a mechanical cleaning and removal of 1–3 mm of the outer and inner portions of the bone using aluminum oxide sandpaper, this has changed to high precision dental burrs under microscopic examination (e.g., Haverkort et al. 2008; Milella et al. 2019). The two chemical methods were the solubility profile method discussed by Sillen (1986) and a treatment with 1 N acetic acid similar to that of Sullivan and Krueger (1981). Results demonstrated that the mechanical cleaning could remove the portions contaminated with groundwater strontium and the 1 N acetic wash treatment could remove the soluble diagenetic strontium (Ezzo 1992; Lambert et al. 1990; Price et al. 1994b).

Over time, researchers begin to incorporate variations of one of the three protocols discussed above. Figure 2 demonstrates the evolution of pretreatment methods following a genealogical pattern, where researchers were directly quoting or drawing influence from another. For instance, throughout the mid-1990s to mid-2000s, Douglas Price and colleagues have applied a variant of Sullivan and Krueger’s wash. Price et al. (1994a) analyzed the compact bone and enamel of eight individuals from two Bell Beaker sites in the Bavarian region. Results demonstrated that three individuals were nonlocal due to the difference in $^{87}\text{Sr}/^{86}\text{Sr}$ values between the enamel and femur of the same individual. In addition, Price and colleagues argued that the protocol removed the soluble diagenetic strontium from bones; however, they further argued that insoluble diagenetic strontium would reflect biologically available $^{87}\text{Sr}/^{86}\text{Sr}$ values (1994a). Following this assertion, the use of bones to determine biologically available strontium began to take hold (Bentley et al. 2002; Depaermentier et al. 2020; Grupe et al. 1997). Other suggestions have been investigated, such as faunal remains (teeth, bone, and shells) (e.g., Bentley et al. 2004; Haverkort et al. 2008; Milella et al. 2019), soils, stream water, and plants (e.g., Perry et al. 2017; Snoeck et al. 2016). There are issues associated with each of these materials, for instance, bone is problematic as there is potential for the biogenic and diagenetic strontium values mixing (Madgwick et al. 2019). In addition, when using faunal remains as a proxy, there are discussions as to which type is optimal as wild vs. domesticated (Madgwick et al. 2017, 2019), small vs. large home ranges (Hedman et al. 2009; Eriksson et al. 2018), and archeological vs. modern (Maurer et al. 2012; Price et al. 2002, 2004), as each produces different data interpretations. For instance, modern environmental materials may yield problematic data due to contamination through anthropogenic or industrial activities (Christian et al. 2011; Maurer et al. 2012). In addition, research has demonstrated that domesticated animals may have been traded, meaning that the produced values may be outside of the site’s bioavailable range and would affect data interpretations (Wang et al. 2021; Arnold et al. 2016; Sharpe et al. 2018). Therefore, there remains no consensus as to the ideal proxy (Croix et al. 2020; Holt et al. 2021).

Pretreatment protocols continued to evolve well into the 2000s as researchers attempt to combat the recurring problem of diagenesis, and its effects on archeological material (Budd et al. 2000; Dent et al. 2004; Hedges 2002; Hedges et al. 1995; Smith et al. 2005; van Klinken 1999). In the span of 2 years, 2003 and 2004, three studies with similar
collaborating authors followed similar but slightly different pretreatment protocols, as can be seen from Table 2. Prior to strontium isotope analysis, Bentley et al. (2003) placed mechanically cleaned bone and enamel samples in a weak acid for an extended period, and Knudson et al. (2004) also used a weak acid prior to ashing and powdering in preparation for analysis. In the same year, Bentley et al. (2004) followed a similar protocol to the 2003 publication (Table 2). This sequential wash is minutely reminiscent of Sillen’s solubility washes; however, the extended pretreatment period is like that of Sullivan and Krueger’s wash. According to Bentley and colleagues (2004), this modification was to lower the likelihood that the diagenetic strontium would re-mineralize back into the sample. This is due to the chemical equilibrium demonstrated in Fig. 3, a chemical reaction between the sample and the reactant solution where if enough time passes, the diagenetic strontium leaches back into the sample. However, there is little evidence to demonstrate that this slight change in methodology produced more reliable and biogenic results than the authors’ previous study in 2003. Comparing the two methods on the same samples would demonstrate their effectiveness over one another. Bentley et al. (2003 and 2004) also only published the $^{87}\text{Sr}/^{86}\text{Sr}$ data for the human and faunal remains. However, Bentley et al. (2003) used previously published Sr/Ca data by Burton et al. (1999) to predict the $^{87}\text{Sr}/^{86}\text{Sr}$ for hypothetical farmers and foragers in Neolithic southwestern Germany, to produce a “bioavailable signature.” Bentley et al. (2004, pp 366) argued that it is “simpler and more direct to measure $^{87}\text{Sr}/^{86}\text{Sr}$ in local herbivore materials...in order to estimate the $^{87}\text{Sr}/^{86}\text{Sr}$ for a local area.” However, the use of faunal material to determine the local range is problematic due to the trade and movement

**Fig. 2** Flow chart demonstrating the genealogical influence of three primary protocols represented in red. Orange represents a reference to the protocol, blue signifies the authors drawing influence by an original protocol, and green represents a reference to the previous author in blue. Chart only showcases a small selection of studies. § Includes secondary protocol. (Beard and Johnson 2000; Bentley et al. 2003, 2004; Cox and Sealy 1997; Ezzo 1991; Ezzo et al. 1997; Hoppe et al. 1992; Lamb et al. 1990; Maurer et al. 2011; Nielsen-Marsh and Hedges 2000; Price et al. 1992, 1994, 2000; Renson et al. 2015, 2016, 2018; Sullivan and Krueger 1981; Thornton 2011; Trickett et al. 2003; Tuross et al. 1989)
of domesticated animals, which may only be visible once isotopic analysis is completed.

In most paleomigration studies, bone and enamel underwent similar pretreatment protocols (e.g., Bentley et al. 2003, 2004). However, a shift occurred in the mid to late 2000s where publications only demonstrated enamel strontium isotopic values, as it is less susceptible to diagenesis and would still indicate nonlocal individuals (Hillson 1996; Lee-Thorp 2008; Pollard et al. 2017). The pretreatment for enamel becomes simple and reminiscent of the mechanical cleaning originally described by Lambert et al. (1989). However, mechanically cleaning the enamel portion to remove the dentine portion is not enough to ensure its full removal, additional chemical steps are necessary, such as ultrasonically bathing the tooth in deionized water, followed by HNO₃, and then a final rinse with deionized water (Hedman et al. 2009; Trickett et al. 2003). These additional steps ensure the removal of the dentine portion, which is susceptible to diagenesis and can cause values that are difficult to document if mixed with those of the enamel (Budd et al. 2000; Glykou et al. 2018; Kyle 1986). According to Budd et al. (2000), dentine could be used as a proxy for pretreatment effectiveness as successful methods would retrieve similar values. However, dentine forms slightly before the enamel, therefore creating some isotopic variation (Fehrenbach and Popowics 2015; Hillson 1996).

While there appears to be an interest in applying bone to mobility studies, there is an important distinction between bones that are unburnt and those that are

![Fig. 3](image-url) Sample and solution reactant reaching chemical equilibrium based on time and speed of reaction. The solution change should happen before chemical equilibrium to minimize the exchange of ions back into the sample.

| Author            | Method                                      | Sample                        | Isotope/concentration | Assessment                                      | Influence? |
|-------------------|---------------------------------------------|-------------------------------|-----------------------|-------------------------------------------------|------------|
| Price et al. 1994a | Mechanical cleaning and overnight in 1 N acetic acid | Bone and enamel              | Both                  | Protocol can remove soluble diagenetic but insoluble would be bio-available | Sullivan and Kreuger |
| Bentley et al. 2003 | Mechanical cleaning and overnight in 5% acetic acid at 8 h | Bone and enamel              | Isotope               | Sullivan and Kreuger                             |
| Knudson et al., 2004 | Sonicate in deionized H2O and 5% acetic acid at 30 min and adding a 2nd aliquot for 5 min before ashing and powdering | Bone and enamel              | Isotope               | Sullivan and Kreuger                             |
| Bentley et al. 2004 | Mechanical cleaning and 5% acetic acid for 1 h, rinse in MilliQ H2O, and placed in fresh 5% acetic acid for 7 h | Bone and enamel              | Isotope               | Modification reduced likelihood of re-mineralization of diagenetic strontium | Sullivan and Kreuger |
| Snoeck et al. 2016 | Bone- 1 N acetic acid and sonicated for 3 min and rinsed 3 x Enamel- ultrasonic wash in acid for 30 min and then rinsed similarly | Cremated bone and unburned enamel | Both                  | Sullivan and Kreuger                             |
| Pokutta et al. 2019 | Similar to root canal method—NaOCl (5.25%) and EDTA (17%) At 60 °C | Poorly preserved enamel       | Isotope               | N/A                                             |
calcined. In 2014, Harbeck et al. published a groundbreaking study that showed that calcined bones retain their original isotopic composition, even after exposure to high temperatures. This is especially important as enamel rarely survives cremation, but the petrous portion of the temporal bone generally survives (Croix et al. 2020; Harbeck et al. 2014). Snoeck et al. (2016) analyzed 17 cremated bone fragments alongside three unburned enamel samples from five sites dated to the Neolithic and Bronze Age in Northern Ireland. The study found that reliable strontium (isotopic and concentration) values were reproducible from calcined bone and that it is possible to identify isotopic differences between individuals at the same site. Out of the 17 individuals, six underwent analysis for both strontium isotopes and strontium concentration. Four out of these six individuals had isotopic values close to seawater, likely from marine resource consumption. In addition, these individuals had low strontium concentrations (ranging 74.5–79.9 ppm). Snoeck and colleagues argued that it is unlikely these individuals consumed large amounts of salts and algae as there is no indication of food preservation techniques during the British or Irish Neolithic (2016). Additionally, the sample types (calcined bone and unburnt enamel) underwent different pretreatment protocols. The differentiation of protocols between the calcined bone and the enamel is likely to prevent further recrystallization and that calcined bone appears to be more resistant to diagenetic alteration than enamel (Snoeck et al. 2015, 2018).

Enamel is relatively stable and less susceptible to diagenesis, but it is not immune, this means that a pretreatment method may be necessary (Francisci et al. 2020; Kohn et al. 1999; Makarewicz and Sealy 2015; Pokutta et al. 2019). Other research fields continue to influence archaeological research, for instance, Pokutta et al. (2019) (Table 2) used a protocol regularly used by dentists to clean teeth during root canal treatments. Pokutta et al. (2019) applied the irrigation procedure discussed by Castagnola et al. (2014) to decontaminate poorly preserved teeth of individuals from the Altai region, where results demonstrated high female mobility in comparison to the males. However, considering the success of the method for poorly preserved teeth, it would be interesting to apply it to bones as well.

## Technical developments

As previously mentioned, there was a large focus in the 1990s to determine an optimal pretreatment method for diagenetic samples. Several other breakthroughs occurred during this decade, particularly technical developments in mass spectrometry. This allowed an increase in sample analysis while also becoming cost-effective (Copeland et al. 2010; Makarewicz and Sealy 2015), as many materials were analyzed using atomic absorption spectrophotometry (Sillen 1986). Two technological developments that are still in practice are Thermal Ionization Mass Spectrometry (TIMS) and Multi-Collector Inductively Coupled Plasma Mass Spectrometry (MC-ICP-MS). Table 3 demonstrates these differences between TIMS and MC-ICP-MS and with it coupled with Laser Ablation (LA-MC-ICP-MS). While these methods produce reliable strontium values, their use is dependent on the archeological question and the sample type undergoing analysis.

## Discussion

Diagenesis is a dynamic process, and it remains an ongoing topic of research. Further interdisciplinary research is needed as the geochemical and biochemical information found in archeological material continues to be relevant. In addition, further research is required to determine the effects of museum-applied contamination in the form of adhesives.

|                      | MC-ICP-MS                  | LA-MC-ICP-MS                | TIMS                        |
|----------------------|----------------------------|----------------------------|-----------------------------|
| Sample type          | Organic and inorganic      | Inorganic                  | Organic and inorganic      |
| Precision of values  | Up to the 5th decimal      | Up to the 4th decimal       | Up to the 6th decimal       |
| Concentration?       | Can analyze low concentrations | > 300 ppm has successful results | Can analyze low concentration items |
|                      | > 50 ppm has successful results | < 200 ppm inaccurate results | > 50 ppm has successful results |
| Measurement          | One bulk value             | Multiple values on the same material | One bulk value |
| Benefits             | Can analyze low concentration materials | In situ, less time consuming | Can analyze low concentration materials |
| Issues               | Lacks isobaric interferences | Less destructive           | Less susceptible to isotopic mass bias |
|                      | Can only produce bulk values/destructive | Isobaric interferences and less precise values | Can only produce bulk values/destructive |
|                      |                            |                            | Time consuming              |
on strontium values, as some treatments may cross-link with the material, and may not be easily removed due to aging, additional coatings, etc. (Horie 1987). There is evidence that some post-excavation treatments affect radiocarbon (Devièse et al. 2018) and DNA analysis (Nicholson et al. 2002; Odgaard and Cassman 2007). However, other studies have found that some treatments have little effect on isotopic values (France et al. 2011; Moore et al. 1989). The effects of these treatments on strontium isotopes have not been well-studied, partially due to the gradual disuse of strontium analysis in bones. In addition, a wide range of adhesives has fallen out of disuse (Johnson 1994), and the exact adhesive and methodology used to preserve the specimen were seldom documented, such as type, viscosity, and the application amount (Horie 1987). Therefore demonstrating the need for further interdisciplinary research in identifying the adhesives and treatments and their subsequent removal to ensure that the biogenic strontium signal is produced.

In addition, it is widely agreed that enamel is less susceptible to diagenesis and contamination. However, the practice of gluing teeth into the maxilla and mandibles may affect isotopic analysis. Further research on how glue may affect enamel analysis is necessary, especially due to the reliance on this sample material. Despite the current protocol of abrading the surface prior to analysis, some enamel is not well-preserved, further necessitating some chemical pretreatment (Pokutta et al. 2019). In addition, as Hollund and colleagues (2015) demonstrated, there is a link between the level of bone and tooth degradation. Since teeth are invaluable to isotopic analysis, further research is needed into tooth diagenesis. Therefore, further research into the effects glues and tooth diagenesis may be required.

Currently, researchers are building robust isoscapes of various localities by aggregating previous isotopic data and by analyzing new materials (e.g., Blank et al. 2018; Evans et al. 2010; Snoeck et al. 2020). These isoscapes not only benefit archeologists but are multi-disciplinary as other researchers from ecological and forensic backgrounds will be able to contextualize strontium isotopic ratios in a particular region (Snoeck et al. 2020). There will likely come a time when isoscapes will be used exclusively to determine the local ranges of various sites by averaging a multitude of sample materials and possibly isolate those from certain periods. The only issue is that there is no consensus as to which material is preferable to build these isoscapes. For instance, several researchers (e.g., Holt et al. 2021; Snoeck et al. 2020) adhere to the recommendation of measuring plants to characterize the local signal. The benefit of using plants is that they are readily available and there is less chance for diagenesis; however, other forms of contamination, especially anthropogenesis, are a concern (Holt et al. 2021; Maurer et al. 2012). For this reason, the authors adhere to the recommendation of incorporating available isoscapes as well as sourcing other materials to calculate the local range.

This review covers the evolution for pretreatment methods for preparing osteological materials; however, research on textiles, wood, and cereal grains, and other non-osteological materials, is necessary. These items undergo their own forms of diagenesis, contamination, and subsequent pretreatment methods (e.g., Frei et al. 2009; Kempson et al. 2010; Rich et al. 2016a, b; van Ham Meert et al. 2020; von Carnap-Bornheim et al. 2007), all of which could not be included in this review. However, these materials have a less robust history in terms of the methodological development to decontaminate these materials. Therefore, it appears that continued research into the effects of diagenesis and pretreatments on these types of materials is necessary.

Conclusion

Analytical development of pretreatment protocols for \(^{87}\text{Sr}/^{86}\text{Sr}\) and concentration values during the last few decades remains relevant. During the 1980s, there was an increase in preservation research, including breakthroughs in pretreatment protocols, and diagenetic research. The 1990s ushered in a decade of technological developments as well as a continued desire to determine an optimal pretreatment method. The development of mass spectrometers allowed reliable sample analysis while keeping costs low, which allowed further research into the topic of diagenesis and a better understanding of post-mortem processes. During the 2000s, discussion arose on whether bone should be analyzed, and this gave way to only enamel being analyzed, as it is less susceptible to diagenesis than bone. In addition, the decline in bone analysis occurred in the same period as a decline in the publication of strontium concentration data alongside strontium isotope values. This lack of strontium concentration data has hindered further discussions on the incorporation of dietary strontium. At the same time, we see an increase in the analysis of other archeological materials, particularly wooden and woolen textiles, which also elucidate information of mobility, and trading routes. In the last few years, there is a slow rise in the publication of strontium concentration data and that data being plotted against the strontium isotopic values, as well as a slight increase in bone analysis, particularly in calcined bone. Moving forward, it is desirable that research into a universal pretreatment continues, as such a standard would ensure reliable results, especially in materials that cannot be re-analyzed. In consideration of the difficulty in reanalyzing and locating some materials to
calculate the local bioavailable range, it is important that isoscapes continue to be created through aggregated data. Overall, the continued building of isoscapes, combined strontium isotopic and concentration analysis, and further pretreatment research would allow researchers to better determine dietary variation within populations and time periods, and better identify short or long-term migrants.

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