CHAPTER 6

NITRITE METHOD FOR THE REMOVAL OF MANGANESE OXIDE FOSSIL BONE COATINGS

6.1 Procedure

Nitrite solutions were prepared from stock sodium nitrite standard solutions with the pH adjusted to the desired value using concentrated nitric acid. Each glass vessel contained one bone or tooth fragment and was sealed. As a consequence of the fact that NO_x gases are produced at pH values at which some of the experiments were performed, the seal was periodically opened to allow for the gas to escape so as to not influence other experimental conditions. All the safety precautions (use of fume hood extraction) were adhered to when performing these experiments as some nitrous oxides are harmful.

6.2 Reaction mechanism

The oxidation of NO_2^- to NO_3^- and the reduction of Mn(III) and Mn(IV) oxides to Mn(II) has been shown to be a spontaneous reaction (Bartlett, 1981). The reaction involves the consumption of protons, and occurs without the presence of oxygen. All the nitrite is oxidized to nitrate without the loss of any nitrogen as shown in 6.1.below.

$$NO_2^- + MnO_2 + 2H^+ \leftrightarrow Mn^{2+} + NO_3^- + H_2O \qquad 6.1$$

There are two main possibilities of how the redox reaction proceeds for the above reaction (6.1). Firstly, the nitrite could donate two electrons to Mn^{4+} and accept and O^{2-}

from the manganese dioxide to form nitrate. Alternatively, the second possibility could involve the loss of an oxygen atom from manganese dioxide directly to nitrite to form nitrate and the retention of two electrons from the Mn-O bond in the manganese dioxide (Luther and Popp, 2002).

6.3 Optimization

Optimization was performed using a matrix experimental design, with the varied parameters of pH and concentration of the nitrite solution. Digital photos were then taken before and after treatment of each bone fragment, and validation of the optimum concentration and pH was conducted qualitatively. This was then translated into a matrix and quadratic plot in order to determine the maximum cleaning efficiency for each parameter. The optimization was performed in stages, first using a large range of concentrations and pH values, thereafter refining the optimum value of the parameters to a smaller range. Each experimental point was done in duplicate.

Figure 6.1 Manganese oxide coated fossil bone fragment **a,** before treatment with sodium nitrite; **b,** cleaned bone fragment after soaking in a nitrite solution (1 mol L^{-1} at initial pH: 3.5); **c,** underside of cleaned bone fragment.

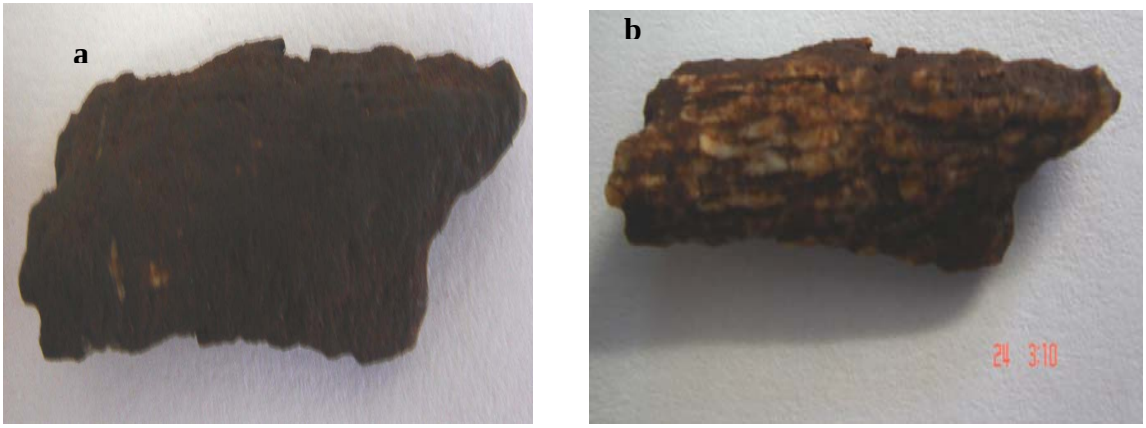

Figure 6.2 Manganese oxide coated fossil bone fragment **a,** before treatment with sodium nitrite; **b,** partial removal of manganese oxide coating after soaking in a nitrite solution (1 mol L^{-1} at initial pH: 4.0) is applied.

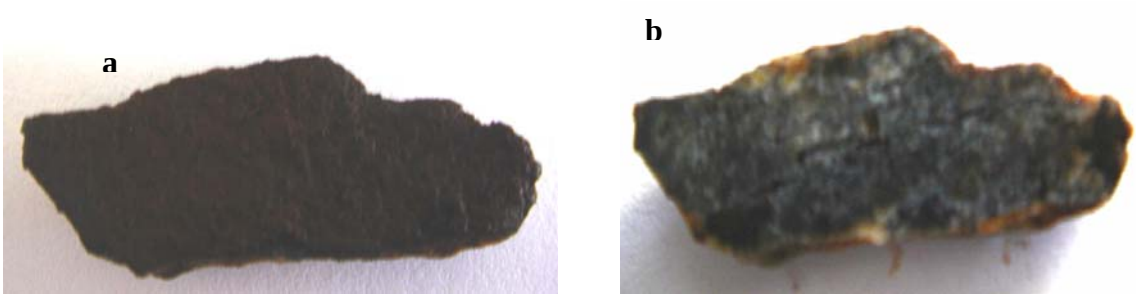

Figure 6.3 Manganese oxide coated fossil bone fragment **a,** before treatment with sodium nitrite; **b,** cleaned bone fragment after soaking in a nitrite solution (2 mol L^{-1} at initial pH: 4.5). Images are unfortunately out of focus, however, one can still observe weathering of the bone surface after cleaning (b), in accordance with bone exposed to the elements for approximately 3 years before burial.

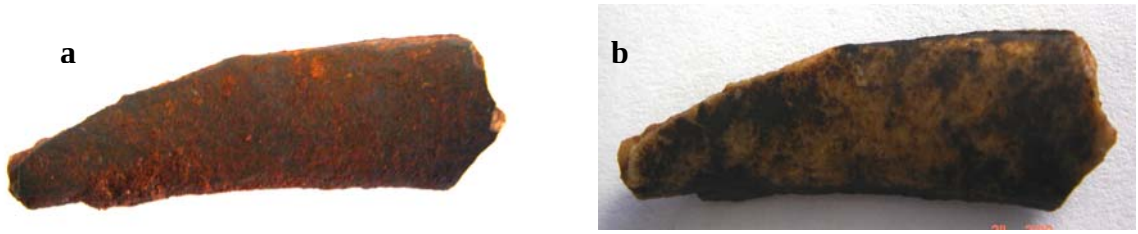

Figure 6.4 Manganese oxide coated fossil bone fragment **a,** before treatment with sodium nitrite; **b,** cleaned bone fragment after soaking in a nitrite solution (1 mol L^{-1} at initial pH: 4.5).

Figure 6.5 Manganese oxide coated fossil bone fragment **a,** before treatment with sodium nitrite; **b,** cleaned bone fragment after soaking in a nitrite solution (2 mol L^{-1} at initial pH: 4.0).

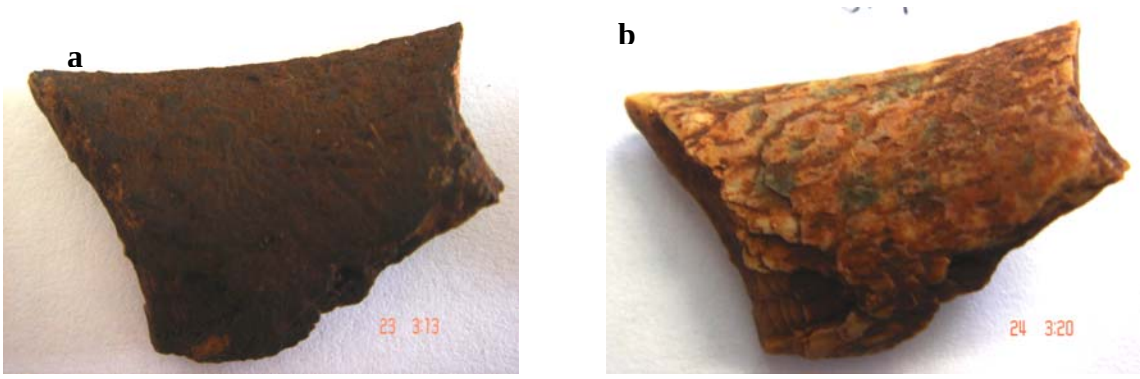

Figure 6.6 Manganese oxide coated fossil bone fragment **a,** before treatment with sodium nitrite; **b,** cleaned bone fragment after soaking in a nitrite solution (3.0 mol L^{-1} at initial pH: 4.0).

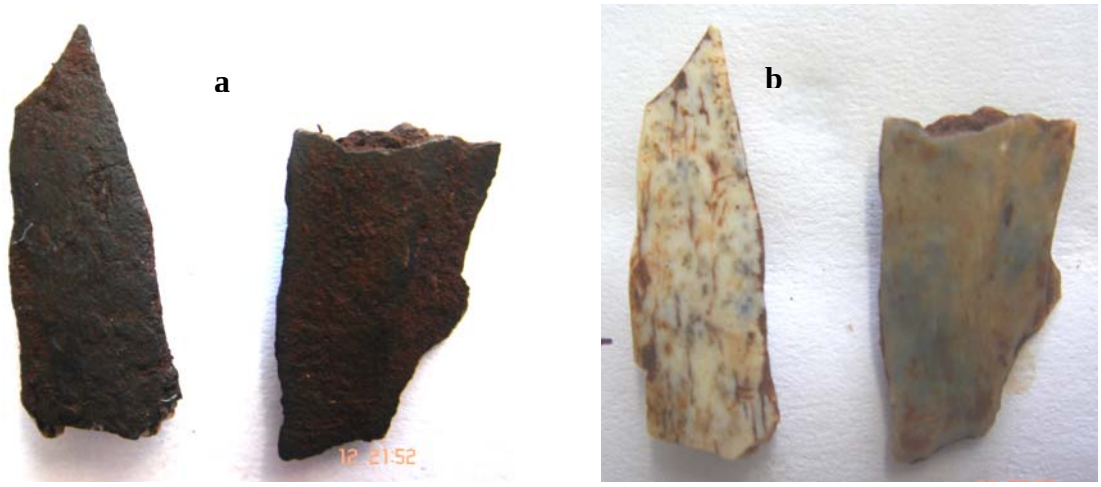

Figure 6.7 Two separate bone fragments heavily coated with manganese oxide **a,** before treatment in a nitrite solution; **b,** fragments after application of the nitrite treatment solution (0.5 mol L^{-1} at initial pH: 3.0) showing almost complete removal of the oxide coating.

The digital photographs clearly show there is a correlation between pH and efficiency of manganese oxide removal.

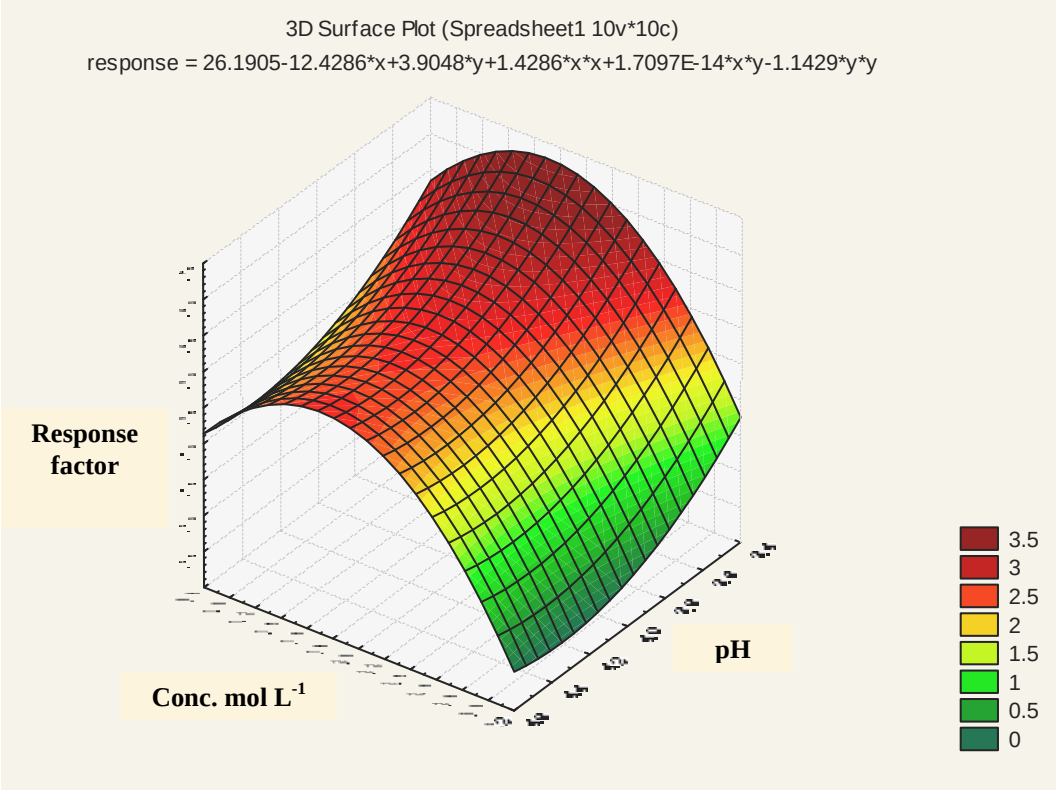

Figure 6.8 Matrix surface plot showing the optimal pH, concentration of sodium nitrite for the cleaning of manganese oxide coated fossil bone fragments.

The matrix plot in fig. 6.8 was generated with the response factor being directly related to the degree of removal of manganese oxide coating, and visible damage to the bone surface from the cleaning process. The fossil bone fragment that showed the highest degree of manganese oxide removal and least visible damage after treatment was assigned a response factor value of 3.5 while a lowest factor value of 0 was assigned for the least amount of manganese oxide removed and/or evidence of visible damage to the bone fragment. From the 3d surface plot, the maximum can be located at a concentration of 1.8 mol L^{-1} and at a pH value of approximately 3.5. The degree of bone cleaning will always increase as the pH becomes more acidic as illustrated in the matrix plot above.

This is because manganese oxides are known to be dissolved in acidic soils (Evangelou, 1998). However at a critical pH the bone matrix will be destroyed, thus two response factors have to be considered.

The pH of the nitrite solution was found to be the most dependant variable in the efficiency of the cleaning process. Luther and Popp (2002) determined that the time of completion for the reduction of manganese dioxide increases as pH increases. This decrease in the reaction rate as pH increases could be due to HNO_2 being a primary oxidant or reactant and not NO_2^- similarly demonstrated by Luther and Popp (2000).

6.4 Validation

The method was validated to determine whether damage and surface alteration occurs to the bone matrix. The validation procedure consisted of a qualitative and quantitative method, both of which proved successful in determining if any possible damage occurred through the use of nitrites as a reducing agent for the removal of manganese oxide fossil bone coatings.

Figure 6.9 Manganese oxide coated fossil bone fragment **a,** broken into two pieces before treatment in a nitrite solution; **b** and **c,** two bone fragments rejoined after treatment with sodium nitrate (1.4 mol L^{-1} at pH: 3.5) showing perfect alignment and sharp edges indicative of non-destructive removal of the manganese oxide coating.

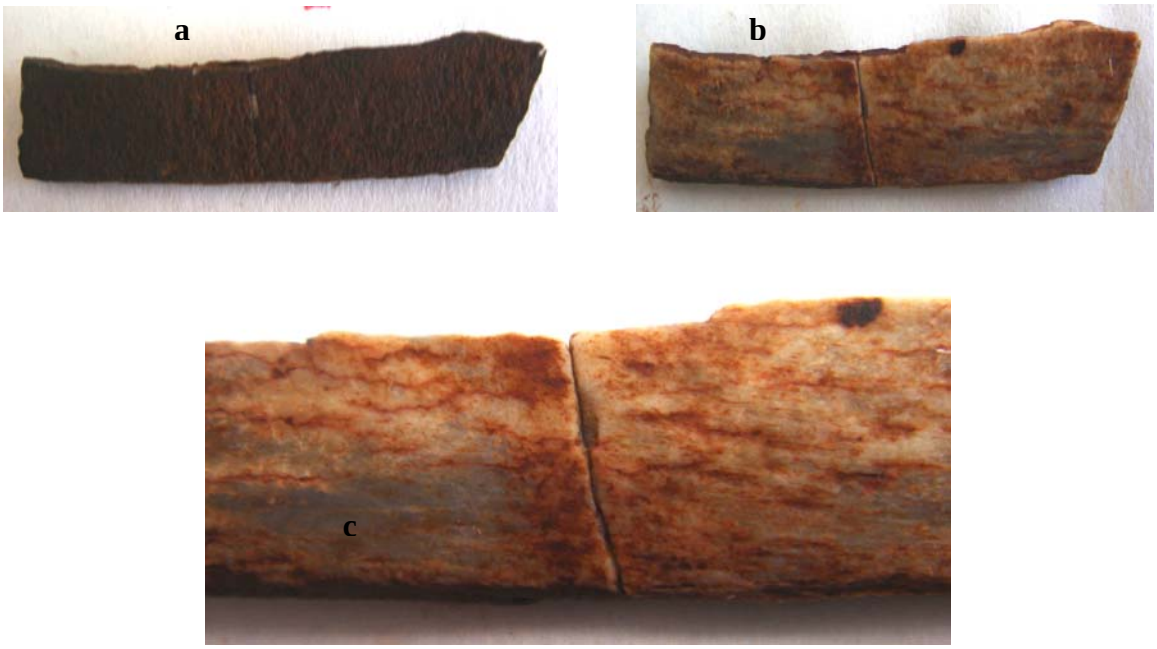

Figure 6.10 Manganese oxide coated fossil bone fragment broken into two pieces **a,** before treatment in a nitrite solution; **b** and **c,** two bone fragments rejoined after treatment with sodium nitrate (2.4 mol L^{-1} at an initial pH of 3.5) again illustrating good alignment

and sharp edges characteristic of the non-destructive removal of the manganese oxide coating.

6.4.1 Scanning electron microscopy validation for a uncoated fossil bone

This validation technique was used to determine if or to what extent cleavage or dissolution occurs on the bone surface. Conditions used were those previously determined to be optimized i.e. pH = 3.5 and Concentration = 1.8 mol L^{-1}; bone soaking of 12 hours.

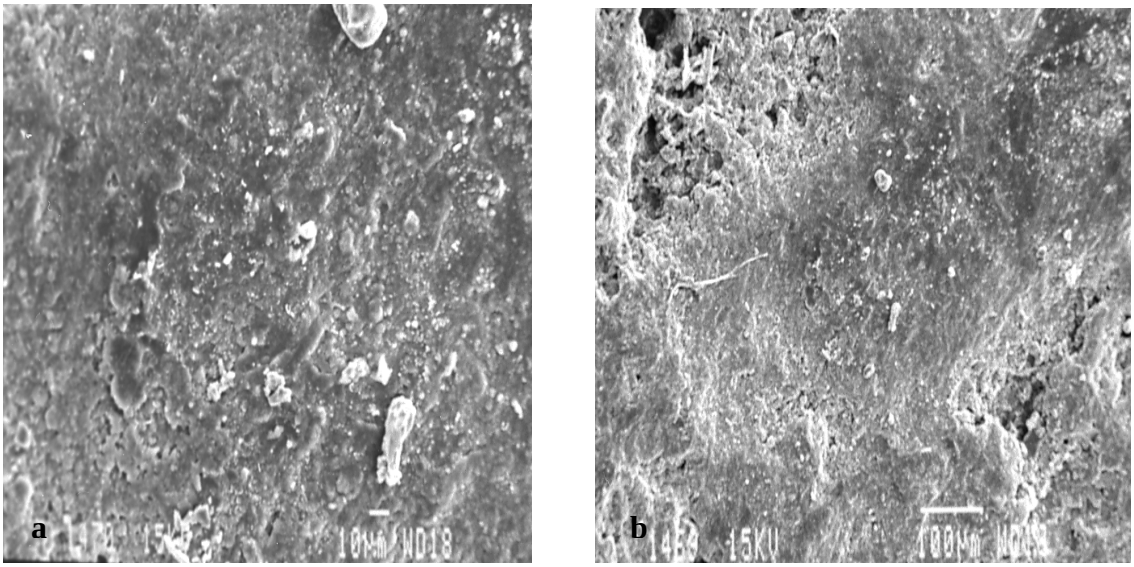

Figure 6.11 Scanning electron microscope image (500X magnification) for an uncoated fossil bone surface, **a,** before sodium nitrite treatment; **b,** after treatment in sodium nitrite.

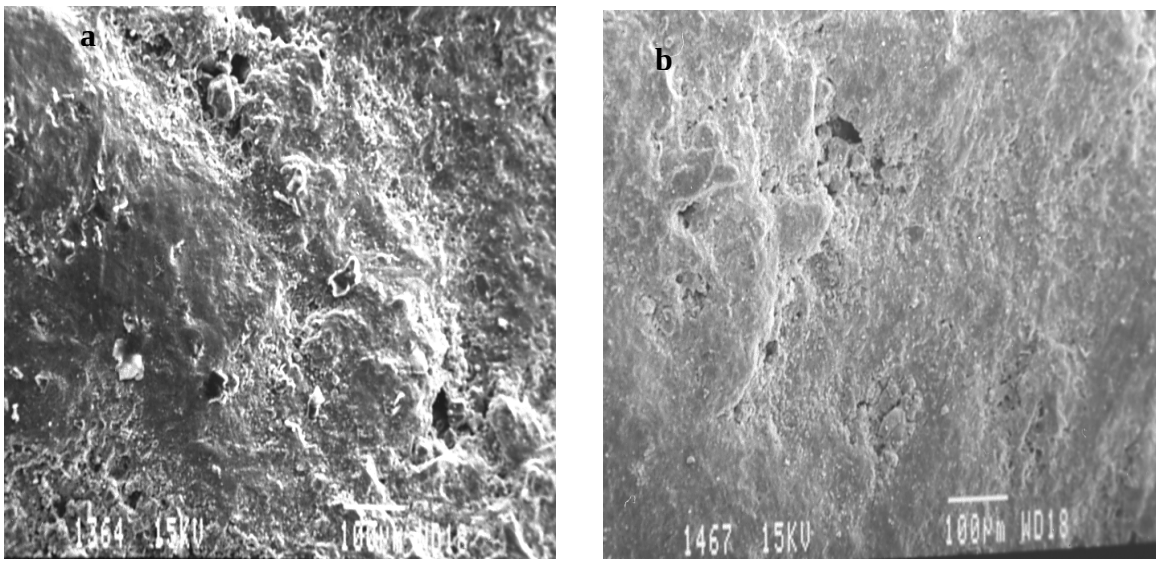

Figure 6.12 Scanning electron microscope image (150X magnification) for an uncoated fossil bone surface, **a,** before sodium nitrite treatment; **b,** after treatment in sodium nitrite.

The two sets of micrographs recorded for different magnifications showing before and after images of an uncoated fossil bone treated in 1.8 mol L^{-1} sodium nitrite solution show little evidence of bone matrix dissolution. Although the images are not identical after treatment it is because not the same area was taken thus the appearance of the bone surface differs slightly. If the bone surface is eroded by dissolution it would have been substantially etched or in some cases the surface would take on a powder-like texture and appearance which is not evident in the above micrographs.

6.5 Nitrite method reaction features

Besides validating this method, an accurate comparison between modern and fossil bone chemical behavior is required using the sodium nitrite method. For consistency, uncoated fossil bones had to also be selected. This comparison was undertaken so as to determine

the vulnerability of modern bones to fossil bone treatment using this method. It could also demonstrate the major chemical differences between a fossil specimen compared to a modern bone fragment.

6.5.1 Clean versus coated bone specimens: kinetic investigations

The trend between the rate of evolution of calcium and manganese was considered as a validation of the reaction.

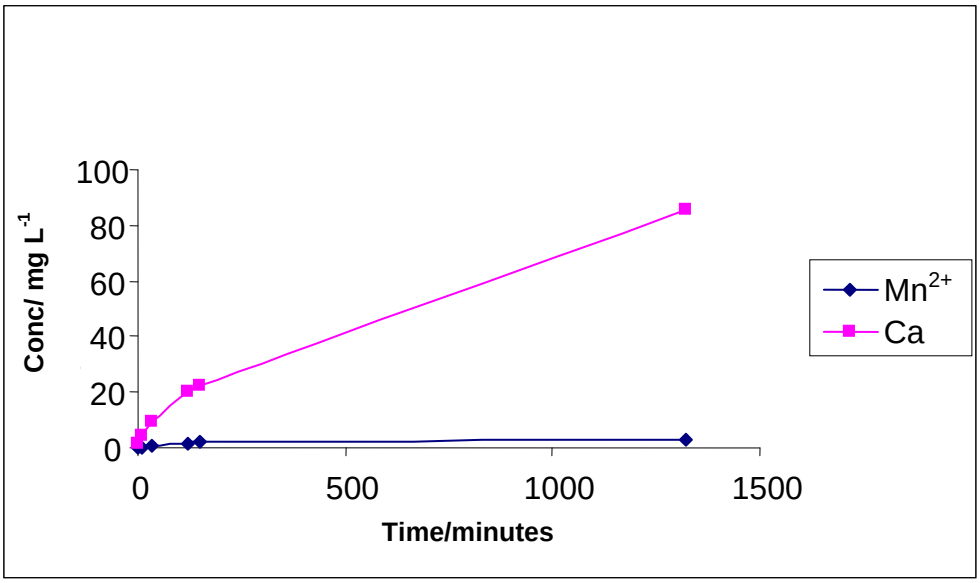

Figure 6.13 Relationship trend between manganese, calcium evolution and time for an uncoated fossil bone treated with sodium nitrite.

Although it is evident in the above figure that calcium evolution is significantly higher than that of manganese, it is not quantitatively significant relative to the total surface area and mass of the fossil bone itself. Loose debris, dust and imbedded soil particles could also be a contributing factor to the magnitude of the calcium evolution. The manganese

concentration is low as a consequence of no coating being present on the bone surface, and perhaps no loose soil embedded in or on the fossil bone.

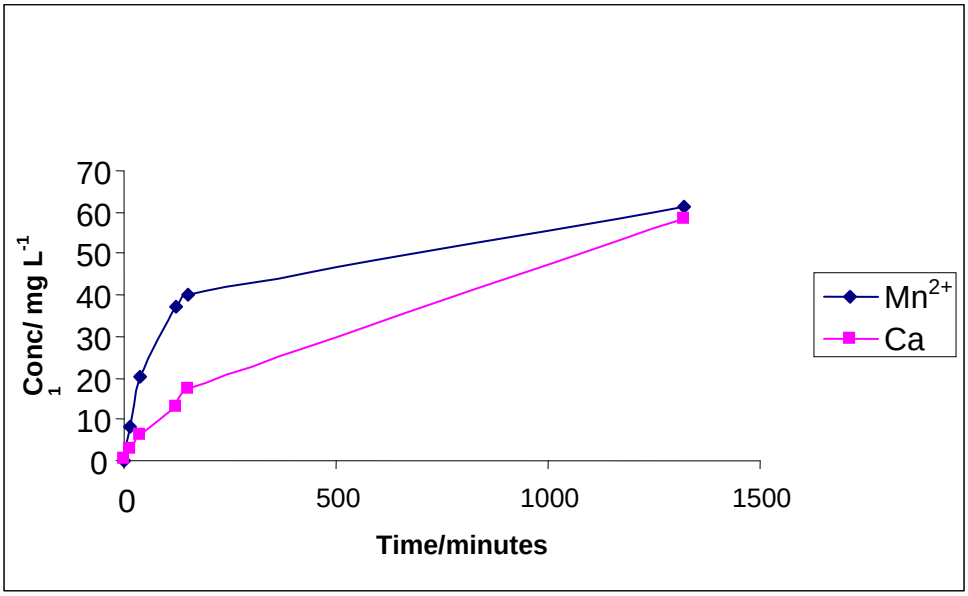

Figure 6.14 Relationship trend between manganese, calcium evolution and time for a manganese oxide coated fossil bone treated with sodium nitrite.

This evolution trend of calcium and manganese for a coated fossil bone shows a different pattern to that of the uncoated bone. The higher manganese concentrations signify the removal of the fossil bone coatings by the nitrite solution. The increase in concentration of calcium with time is expected, as the manganese oxide (which is of a typical birnessite mineral composition) coatings contain a fairly high concentration of calcium (approximately 260 mg L^{-1}) due to calcium carbonate leaching from the surrounding soils in which the coating is formed.

6.5.2 Time dependence on cleaning process using four different bone fragments

In order to try and determine the minimum time required for leaving the fossil specimens in the sodium nitrite solution to achieve optimal removal of the manganese oxide coatings, four manganese oxide coated specimens where placed into the cleaning solution for different time intervals.

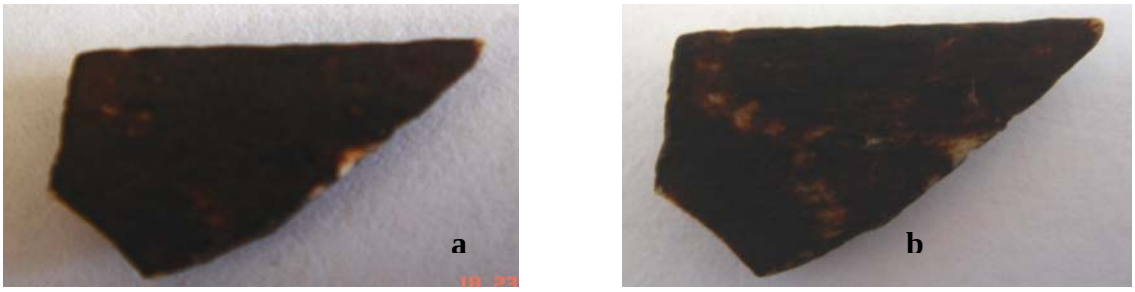

Figure 6.15 Manganese oxide coated fossil bone fragment (1): **a,** before treatment in a sodium nitrite solution, **b** after treatment for 20 minutes in 1.8 mol L^{-1} sodium nitrite solution, without sponging.

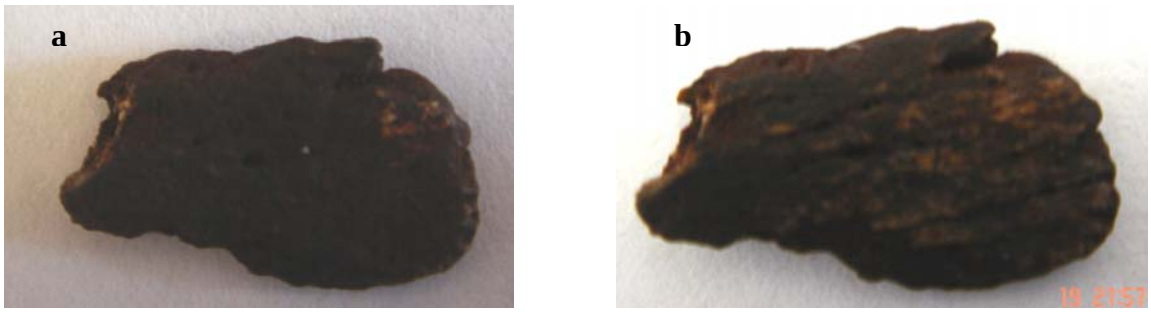

Figure 6.16 Manganese oxide coated fossil bone fragment (2): **a,** before treatment in a sodium nitrite solution, **b** after treatment for 107 minutes in 1.8 mol L^{-1} sodium nitrite solution, without sponging.

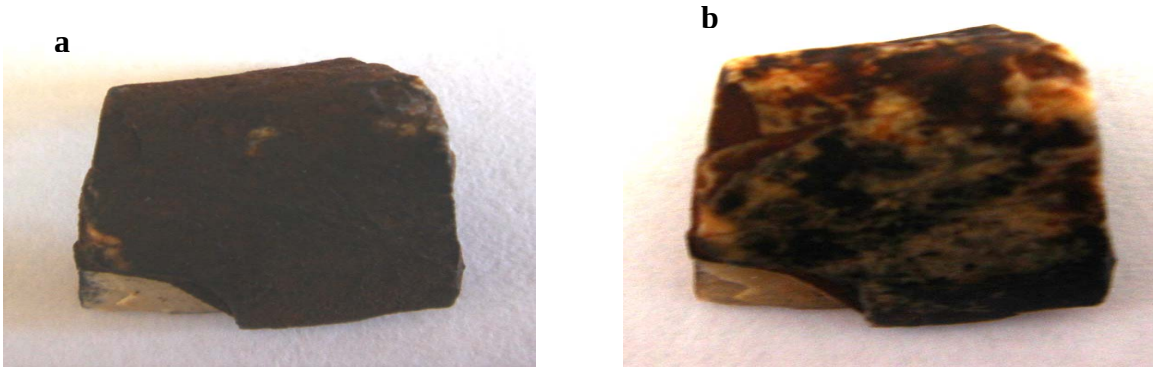

Figure 6.17 Manganese oxide coated fossil bone fragment (3): **a,** before treatment in a sodium nitrite solution, **b** after treatment for 220 minutes in 1.8 mol L^{-1} sodium nitrite solution, without sponging.

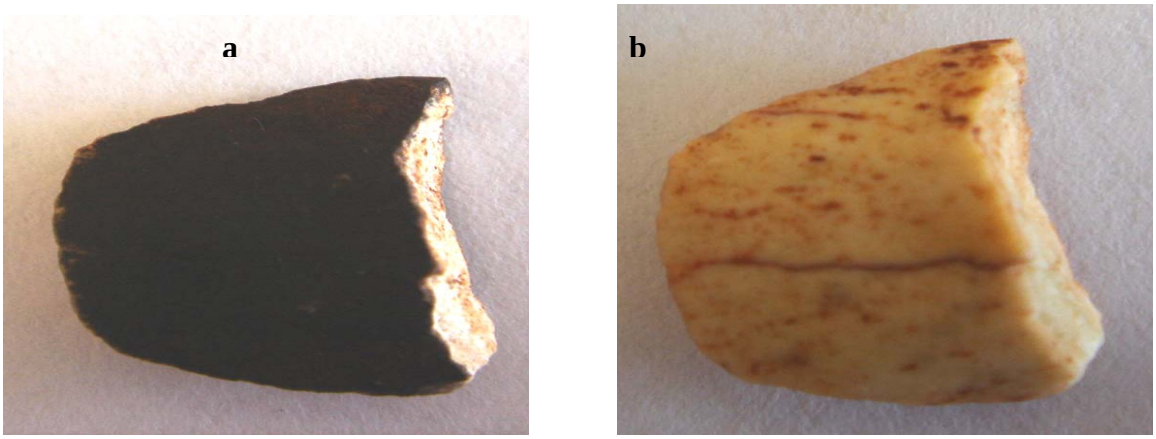

Figure 6.18 Manganese oxide coated fossil bone fragment (4): **a,** before treatment in a sodium nitrite solution, **b** after treatment for 22 hours in 1.8 mol L^{-1} sodium nitrite solution, without sponging.

The nitrite method seems to have fairly slow kinetics, as the best cleaning or removal of the manganese oxide coating is achieved for the longest treatment time of 22 hours. There does not seem to be any invasive damage on the bone surface after this treatment period, thus rendering this time interval safe. There are a number of important factors that could also play a role in the efficiency and time taken for the removal of the oxide coatings from the four fossil bone fragments used. Each bone fragment is a different size, shape and texture. These three factors have been found through experimentation and observation to influence the rate and efficiency of manganese oxide removal. Size and shape are crucial factors as the larger the bone, the greater its surface area for the reduction/oxidation reaction to occur on. Smoother non-porous surfaces have been found to clean considerably faster and more efficiently than porous, interlinked structures. Fragment 4 appears to have the smoothest surface of the four samples treated, even though the remaining samples are also fairly non-porous.

If the sample surfaces where gently sponged after soaking in the nitrite solution, most of the manganese oxide coatings were easily removed from the samples. This could be an indication that the surface reaction between the nitrite solution and the oxide coating at the bone oxide interface is fast, however the dissolution step whereby the coating is removed from the surface of the bone into solution is perhaps less kinetically favored.

6.5.3 Reaction rates and reaction comparative characteristics

Calcium and manganese evolution rates were determined for two different ages of bones: fossil bones from the Cradle of Humankind and modern cow bones. This was done to demonstrate how the influence of diagenisis and other chemical processes that occur during fossilization increase the resistance of the bone surface and matrix, thus making it less susceptible to dissolution and chemical weathering.

It has been found by Royer and Unz (2006) that in the absence of iron, reduced manganese is found only in the acid extractable fraction. This implies that in some cases the manganese oxide could also coexist as a manganese carbonate species. It was also found that in areas of high iron concentrations, absorbed manganese became the more dominant form. This could, as was suggested by Royer and Unz (2006) be due to exogenous $Fe(OH)_3$ providing additional surface area for the adsorption of manganese, and thus increasing the potential for adsorption, as opposed to solid phase precipitation of the divalent manganese species. However Wieder and Lang (1986) and Machemer and Wildeman (1992) illustrate that the adsorption of manganese is weak relative to other metals, which can lead to rapid displacement of adsorbed manganese when other metals are present.

6.5.4 Manganese and calcium evolution rates for two different nitrite concentrations

A comparative trend was determined between manganese and calcium for a 1.4 mol L^{-1} and 2.4 mol L^{-1} nitrite treatment solution in order to observe the effect it has on the fossil specimen.

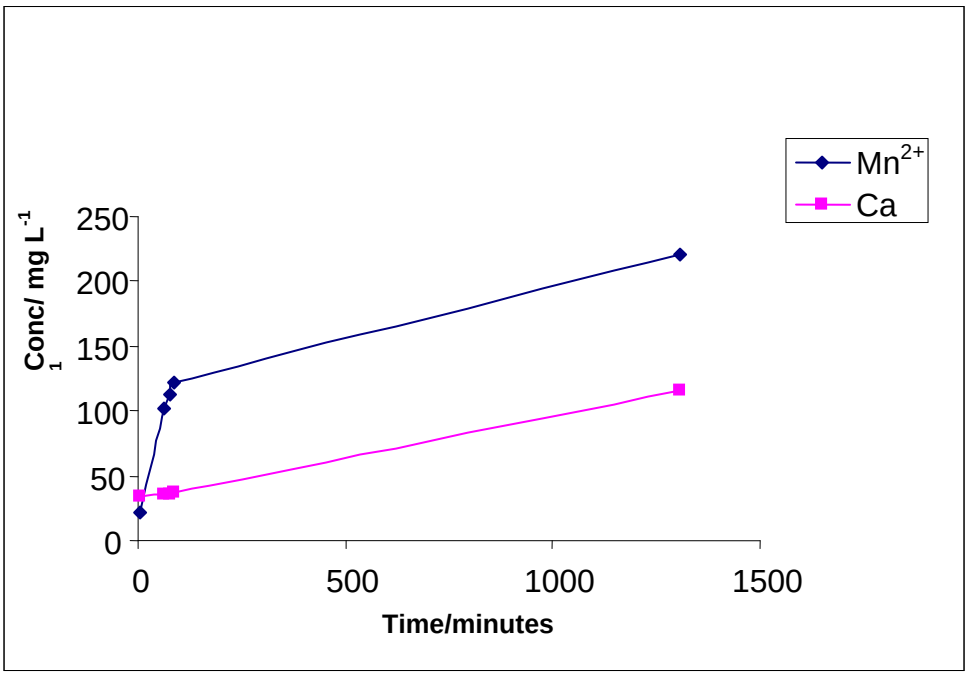

Figure 6.19 Manganese and calcium evolution trends as a function of time for a manganese oxide coated fossil bone treated with 1.4 mol L^{-1} sodium nitrite at an optimum pH of 3.5.

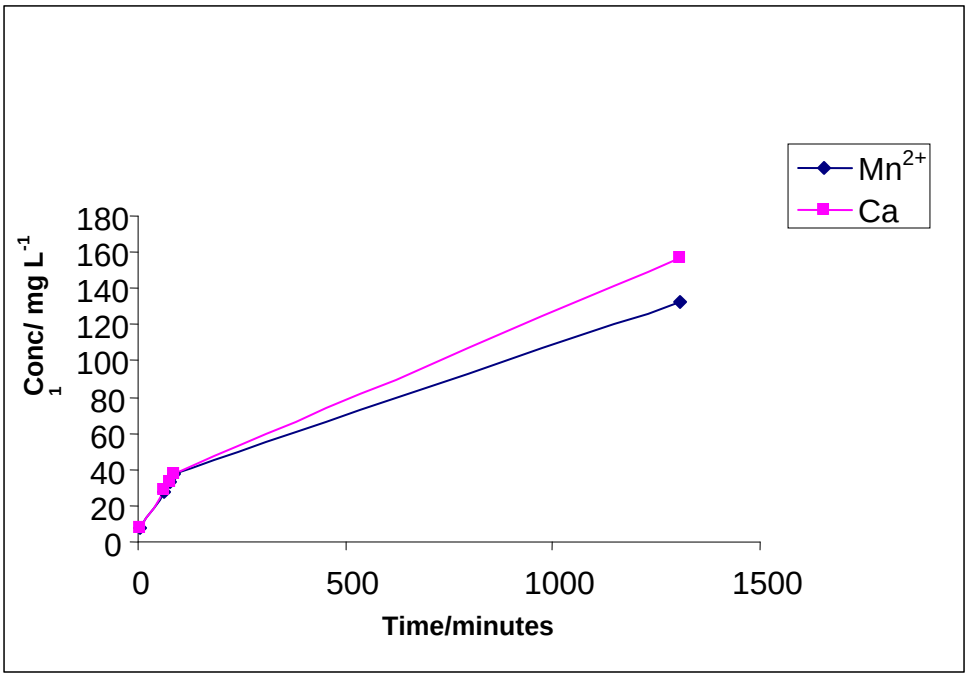

Figure 6.20 Manganese and calcium evolution trends as a function of time for a manganese oxide coated fossil bone treated with 2.4 mol L^{-1} sodium nitrite.

The rate of calcium and manganese evolution seems to also depend considerably on the concentration of the sodium nitrite solution used from the trends in fig. 6.19 and fig. 6.20. In the above figures, the two different concentrations of nitrite produce opposite trends with respect to calcium and manganese evolution. In fig. 6.19 the calcium evolution rate is significantly lower than the manganese. In fig. 6.20 the calcium evolution marginally exceeds the rate of manganese evolution. Since the initial pH of both solutions is the same, therefore the pH value does not seem to have an influence on the above trend. A possible reason for this trend could thus be that only concentration of the nitrite has a direct influence on the surface binding and electron transfer capabilities of the oxide layer, therefore at higher concentrations the residual calcium present is dissolved into solution at a faster rate.

6.5.5 Rate dependence on pH

Two bones of similar sizes were selected of similar sizes and broken into three roughly equal fragments. Each fragment was then treated with 1.8 mol L^{-1} sodium nitrite under identical conditions. Each pH value was tested in duplicate.

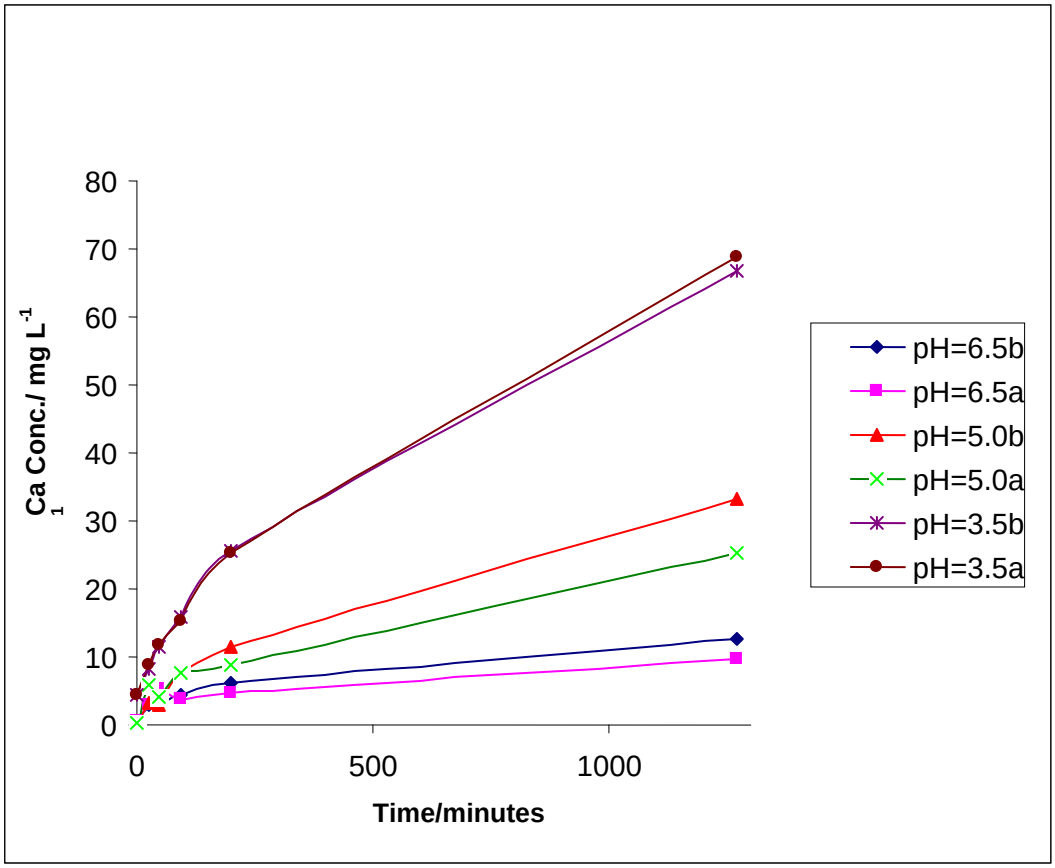

Figure 6.21 The relationship trends between calcium evolution from the bone surface and time at three different pH values (3.5, 5.0, and 6.5) for duplicate manganese oxide coated fossil bone fragments.

The rate of calcium evolution increases with decreasing pH. This is expected for most calcium carbonates which are also present in soil that are inside the oxide coating. As the

pH value increases the surface potential becomes more negative, thus repelling the nitrite anion preventing it from binding to the oxide and electron transfer is decreased.

The initial increase in the concentration of the manganese and calcium concentration is extremely rapid followed by a slower increase toward the equilibrium state. In a similar reaction studied by Luther and Popp (2002), two K_{obs} where calculated for the reaction of synthetic polymeric manganese oxide, and nitrite solution. Similarly for the reactions in fig. 6.21 and fig. 6.22, Luther and Popp (2002) observed a rapid initial rate, followed by a slower reaction rate nearing an equilibrium state. It was suggested that the second slow reaction rate could be due to a change in the manganese dioxide particle size (or surface area on the bone) and/or greater adsorption of the manganese(II) ion due to the manganese dioxide surface having a more negative charge as the pH increases thus repelling the nitrite anion and promoting stronger adsorption of the manganese ions.

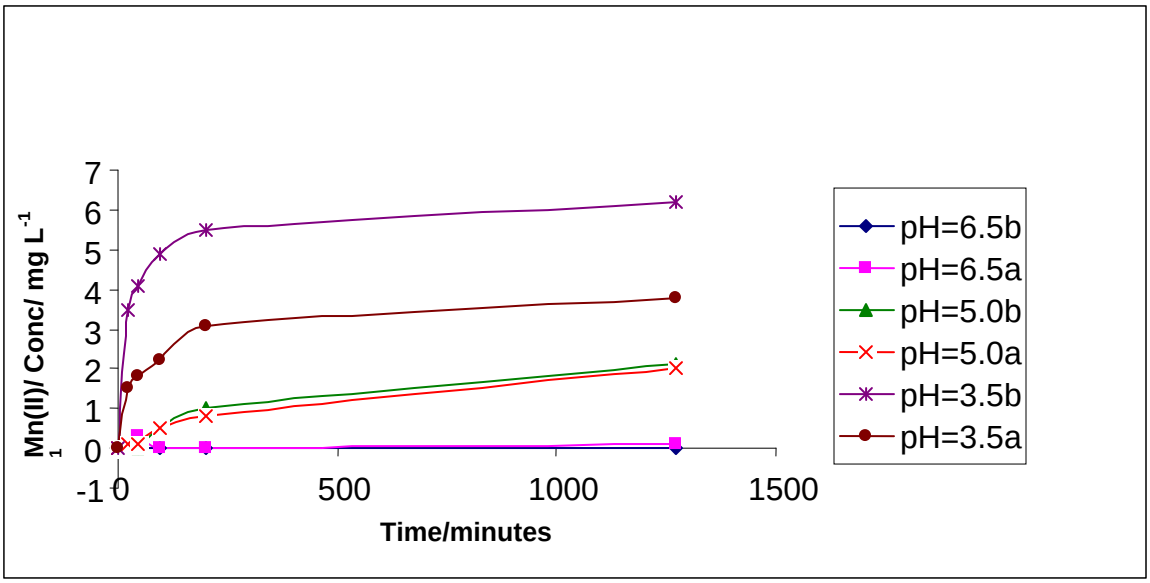

Figure 6.22 Manganese evolution trends as a function of time for three different pH values (3.5, 5.0, and 6.5) in 1.8 mol L^{-1} nitrite solution, performed in duplicate for manganese coated fossil bone fragments.

As expected and observed for synthetic manganese oxides by Luther and Popp (2002), as the pH of the nitrite solution increases, the rate of manganese evolution is seen to decrease. This shows that pH also has a direct influence on the dissolution rate of manganese oxide located on non-synthetic samples. The Mn(II) evolution rate for an initial pH value of 5.0 appears to take a greater time to reach equilibrium This can be observed as the Mn(II) increase in concentration for the time interval between approximately 200 minutes and 1300 minutes for a pH value of 5.0 is greater than the rate for a pH value of 6.5.

6.6 Comparison of the evolution rate of calcium and manganese for a modern cow and a clean uncoated fossil bone

A 50 mL, 1.8 mol L^{-1} sodium nitrite treatment solution was used and adjusted to pH value of 3.5. Each duplicate set was obtained from a single bone of the same age. Both bones used contained no visible manganese oxide coatings.

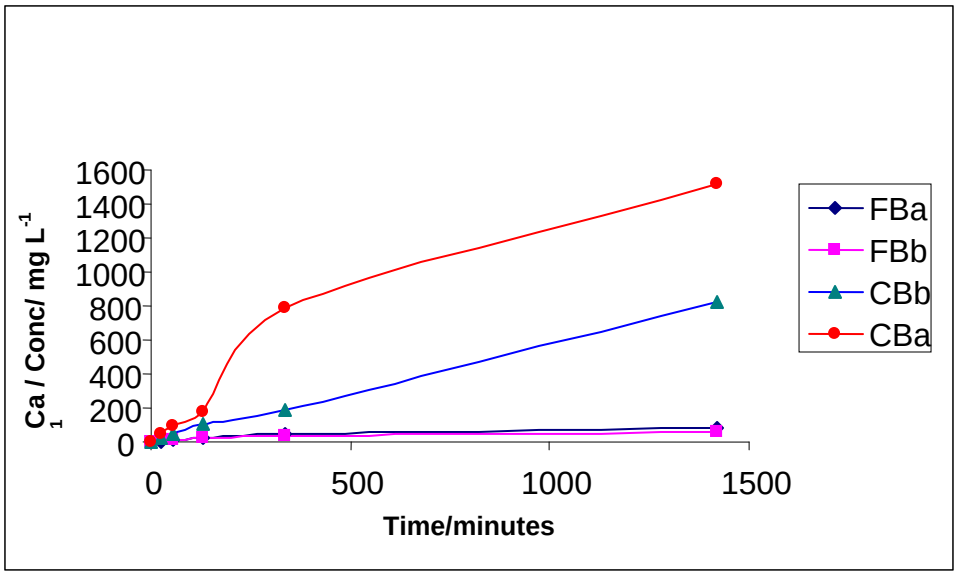

Figure 6.23 Calcium evolution trends as a function of time for an unfossilized, non-mineralized cow bone (CB) vs. an uncoated fossilized bone (FB) treated in a nitrite solution and performed in duplicate.

It has been observed that there is a big difference in evolution rates for modern bone material compared to fossil bones from in the Cradle of Humankind. As seen in fig. 6.23 the rate of calcium evolution is significantly greater for the modern cow bone (CB) specimen compared to the much older fossil bone. The main reason for this is that the two specimens have different chemical compositions. The fossil bone (FB) which has been exposed to soils and other environmental factors has undergone diagenisis and mineral leaching and other changes that render it more resistant to dissolution. During these processes the less resistant minerals of the bone matrix are either encapsulated or removed and replaced by silicious and other soil minerals that protect the bone matrix.

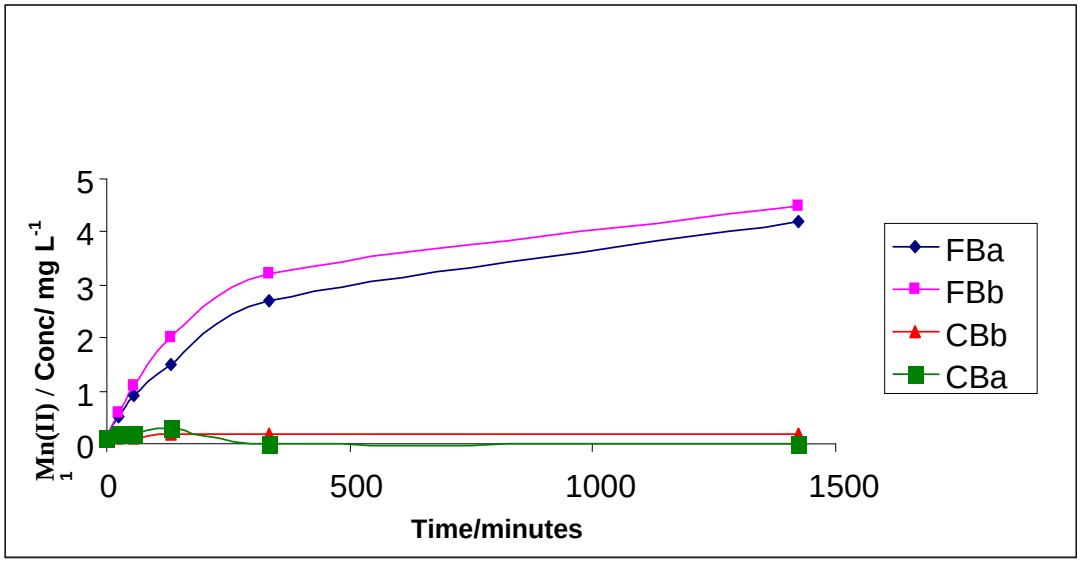

Figure 6.24 Manganese evolution trends as a function of time for a comparison between an uncoated unfossilized cow bone (CB) vs. clean uncoated fossilized bone (FB) treated in a nitrite solution and performed in duplicate.

Although no manganese oxide coatings were present or visible on the surface of the modern cow and fossil bones used, the manganese source is most likely to arise from loose soil debris not removed from the surface and bone matrix. The higher rate of evolution of manganese observed for the fossil bone specimens is expected, as these bones have been exposed to soil which contains high concentrations of manganese, some of which either remains as loose debris inside the porous bone structure, or is transferred diagenetically and through precipitation is transferred onto the surface of the bone and cannot be seen with the naked eye, only microscopically.

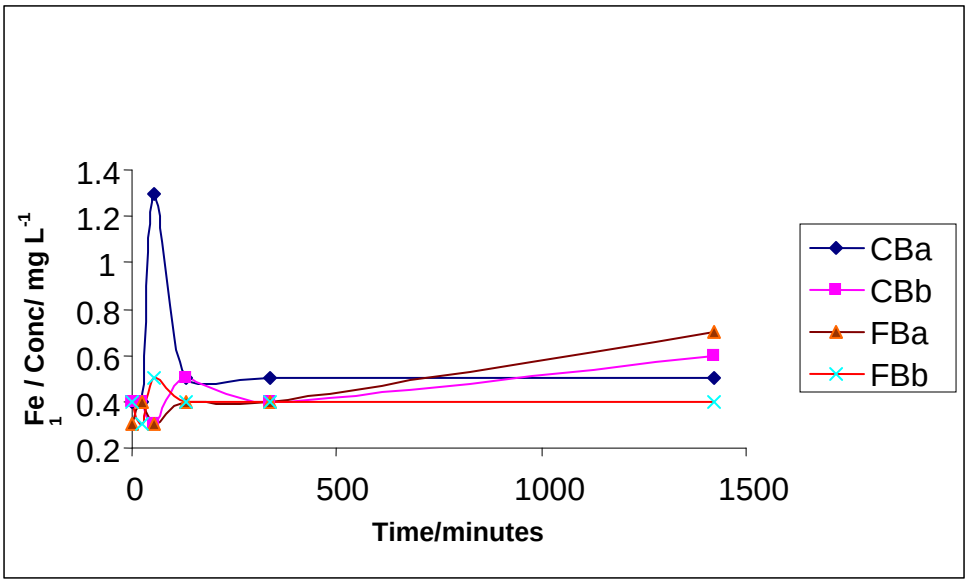

Figure 6.25 Iron evolution trends as a function of time for comparison between unfossilized cow bone (CB) vs. fossilized bone (FB) in a nitrite solution and performed in duplicate.

The trend for iron evolution regarding modern and fossil bones is not so distinguishable, and does not follow a specific trend. This could be due to the fact that iron penetrates deeper into the matrix of the bone, thus protecting it from being released via dissolution at the surface. The concentrations are also very low, which could also indicate that this is not a surface dissolution as compared to the manganese process.

6.6.1 Gravimetric analysis of modern cow vs. fossil bone

A gravimetric analysis test was performed to compare the mass % loss due to dissolution for comparison between samples of different ages.

Table 6.1 Gravimetric data pertaining to a comparison between the loss of mass for a modern cow bone vs. uncoated fossil bone specimen.

Sample type	**Initial mass/g**	**Final mass/g**	**% mass loss**
Modern cow bone A	0.6464	0.5658	12.5
Modern cow bone B	0.2985	0.2517	15.7
Fossil bone A	0.4928	0.4854	1.50
Fossil bone B	0.5133	0.5032	1.97

In table 6.1 above, the masses before and after nitrite treatment for uncoated fossil and modern cow bone fragments were recorded. The modern cow bone showed a greater loss in mass as a result of the treatment process as compared to the fossil bone. This again shows that fossil bones show more of a resistance to pH change and chemical treatment than modern unfossilized bones. The difference in mass loss for the modern cow and fossil bones could be due to the well-known increase of the fluoroapatite percentage in old bones and the selective leaching of different fractions of the collagen. The variation amongst each duplicate set could be due to environmental factors and variation in organic, collagen and protein content as well as surface area differences.

6.7 Method overview

The use of nitrites for the removal of manganese oxide fossil; bone coatings was successfully achieved and this has been shown to be non invasive. This method does however require some that the pH is adjusted to 3.5 which is the optimum value at which the manganese oxide coating is reduced. Once the fossil specimen (s) is placed into the nitrite solution, continual monitoring is not essential, however due to the generation of nitrous oxides all necessary precautions should be taken during specimen changing.