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Changes in Arsenic Levels in the Precambrian Oceans in Relation to the Upcome of Free Oxygen

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Abstract

Life on Earth could have existed already 3.8 Ga ago, and yet, more complex, multicellular life did not evolve until over three billion years later, about 700 Ma ago. Many have searched for the reason behind this apparent delay in evolution, and the dominating theories put the blame on the hostile Precambrian environment with low oxygen levels and sulphide-rich oceans. There are, however, doubts whether this would be the full explanation, and this thesis therefore focuses on a new hypothesis; the levels of the redox sensitive element arsenic increased in the oceans as a consequence of the change in weathering patterns that followed the upcome of free oxygen in the atmosphere at about 2.4 billion years ago. Given its toxicity, this could have had negative effects upon the life of the time. To test the hypothesis, 66 samples from drill cores coming from South Africa and Gabon with ages between 2.7 and 2.05 Ga were analysed for their elemental composition, and their arsenic content were compared with carbon isotope data from the same samples. These confirmed that a rise in arsenic concentration following the upcome of free oxygen in the atmosphere and the onset of oxidative weathering of continental sulphides. Arsenic, which is commonly found in sulphide minerals, was weathered together with the sulphide and delivered into the oceans, where it in the Palaeoproterozoic increased to over 600% compared to the older Archaean levels, at least locally. Iron had the strongest control over the arsenic levels in the anoxic (ferruginous and sulphidic) oceans, probably due to its ability to remove arsenic through adsorption. During oxygenated conditions, sulphur instead had the strongest influence upon arsenic, likely because of the lack of dissolved iron. The highest arsenic levels were found in samples recognised as coming from oxygenated conditions, although this might be due to the oxygenation state of arsenic affecting its solubility. Arsenic is toxic already at low doses, especially if the necessary arsenic detoxification systems had not yet evolved. However, the lack of correlation between arsenic and changes in $\delta^{13}\text{C}$ indicated that the increase of arsenic did not affect the primary production between 2.7 and 2.05 Ga. Thus, whether arsenic could have affected the evolution of life during the Mesoproterozoic remains to be shown.

Sammanfattning

Redan för 3,8 miljarder år sedan kan det första primitiva livet på jorden ha uppstått. Det tog dock ytterligare tre miljarder år för mer komplext flercelligt liv att utvecklas. Denna fördröjning har enligt den kanske mest vedertagna teorin berott på de låga syrenivåerna under större delen av jordens historia, särskilt då miljön samtidigt var mycket ogästvänlig med svavelhaltiga och näringsfattiga vatten. Teorin har dock kommit att ifrågasättas eftersom det visat sig att djur kan leva i miljöer med betydligt lägre syrehalt än vad som tidigare varit känt. I denna studie testades därför en ny hypotes, nämligen att uppkomsten av fritt syre i atmosfären för 2,4 miljarder år sedan ledde till att koncentrationerna av det redoxkänsliga grundämnet arsenik ökade i haven som en följd av de förändrade vittringsprocesserna. Arsenik är mycket giftigt redan i låga mängder, och en ökning av ämnet kan därför ha lett till att livet kom att påverkas negativt. Hypotesen testades genom att analysera det kemiska innehållet i 66 prover från Sydafrika och Gabon med åldrar mellan 2,7 och 2,05 miljarder år och jämföra förändringarna i arsenikinnehåll med $\delta^{13}\text{C}$ -värden i samma prover, analyserade av en annan forskargrupp. Proverna bekräftade att en ökning av arsenik skedde för runt 2,25 miljarder år sedan, och att ökningen åtminstone lokalt var över 600% jämfört med arkeiska nivåer. Trots detta verkar det enligt $\delta^{13}\text{C}$ som att den biologiska aktiviteten under denna tid inte påverkades. Järn hade störst inflytande över arsenikkoncentrationerna under de tider då haven var syrefria och rika på antingen järn eller svavel. Detta beror troligen på järnmineralens förmåga att adsorbera arsenik. I vatten där det fanns syre var det istället svavel som utövade den största kontrollen, förmodligen på grund av bristen på järn. Högst arsenikhalter uppmättes i de prover som kom från syrehaltiga vatten, vilket troligen beror på att arsenik har lägre löslighet under dessa förhållanden. Trots att arsenikens påverkan på livet inte kunde styrkas så kan det inte uteslutas att en ökning av ämnet bidrog till att ha försenat evolutionen av multicellulärt liv under i första hand Mesoproterozoikum, i synnerhet om de gener som ger viss resistans mot arsenik ännu inte hade utvecklats.

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Introduction

The very first oceans on Earth might have been formed as early as 4.4 billion years (Ga) ago (Papineau, 2010), although the initially heavy asteroid bombardment during the Hadean (4.5-3.85 Ga) could have caused them to evaporate numerous times. At 4.2 Ga, the impacts decreased in magnitude, but the Earth entered one more episode of heavy bombardments before the conditions stabilised around 3.8 Ga (Lunine, 2006). This marks the transition into the Archaean (3.85-2.5 Ga), and from hereon, the oceans remained in liquid state with the potential of housing the first anaerobic life forms (Lunine, 2006). Isotope ratios of sulphur, chromium, iron and carbon preserved in banded iron formations (BIFs) and shales are essential when studying these ancient oceans as they reflect changes in the chemical composition of the water (Wilde et al., 2001, Canfield, 2005). The by far most important such change is the emergence of free oxygen at about 2.4 Ga (Pufahl & Hiatt, 2012), which led to the shift from anoxic to oxygenated conditions. This transition is commonly referred to as the Great Oxidation Event (GOE) and although the oxygen levels remained comparatively low for another 1.7 billion years, it was still enough to have profound effects upon the Earth. In the upper parts of the oceans, an oxygenated layer developed for the first time, while on land, a new type of weathering was triggered (Pufahl & Hiatt, 2012). As the atmosphere went from reducing to mildly oxidative, sulphide minerals at the surface of the continents started being oxidised into soluble sulphates. The sulphates would readily have been washed away and brought to the seas, leading to the spread of sulphidic waters (Canfield, 1998; Poulton et al., 2004). It took over a billion more years before the sulphidic state of the ocean came to its end (Lyons & Reinhard, 2009), soon thereafter, the first clearly marked eukaryotic multicellular life made its appearance in form of the Ediacaran biota (Canfield et al., 2007).

For long, it was assumed that during most of the Proterozoic the oxygen levels were too low to enable the evolution and expansion of complex multicellular life. However, this explanation has recently been questioned as some animals can live and thrive also in low-oxygen environments (Budd, 2008). The development of the toxic and nutrient-scavenging sulphidic oceans in the Mesoproterozoic has been suggested as another reason for this delay in evolution (Poulton et al., 2004; Kendall et al., 2010), but again, it is unclear to which extent the evolution could have been hampered by these conditions (Javaux, 2011).

In this thesis, a new hypothesis on the toxicity of the Mesoproterozoic sulphidic oceans is tested: following the onset of the oxidative weathering about 2.4 Ga, the concentration of the redox sensitive and highly toxic metalloid arsenic increased in the oceans, possibly with negative effects upon life. It is already known that the upcome of free oxygen in the atmosphere led to an

increase in the sulphate flux, and since arsenic is often occurring in sulphide deposits, it might have increased in a similar way. If the levels of arsenic were persistently high throughout most of the Proterozoic, it might have been able to restrain and delay the evolution and expansion of complex multicellular life. To test the hypothesis, 66 samples from sedimentary rocks from the Transvaal Supergroup, South Africa and the Francevillian Series, Gabon, will be analysed for their elemental composition, while the results of an iron and sulphur speciation analysis from the same samples performed by another research group will be used to define the redox state under which the material were deposited. To test whether arsenic had any effect upon life, the changes in arsenic levels will be linked to the variations of $\delta^{13}\text{C}$ in organic matter. The carbon isotope analysis was performed by the same group responsible for the iron and sulphur speciation analysis, and again on the same material. The samples are between 2.7 and 2.05 Ga, thus including both the period when the oceans went from an anoxic to oxic state as well as the first appearance of sulphidic waters. Five complementary samples from the Gunflint Formation in Canada (~1.85 Ga) and the Vindhyan Supergroup in India (~1.65 Ga) will be analysed as well to give a rough estimation about how the arsenic changes proceeded under the Mesoproterozoic, which is the time when the highest arsenic levels would be expected to have occurred. The aim is to present a model of the increase, spreading and removal of arsenic in the Precambrian oceans, which, if successful and if completed with more samples of other ages and from more localities, lead to a new understanding of the late rise of multicellular life.

This thesis starts with an extensive background chapter in which the characteristic conditions of each of the three main stages of the ocean (ferruginous, oxygenated and sulphidic) are reviewed. Thereafter the biotic as well as abiotic pathways of arsenic are described, as are the arsenic detoxification mechanisms in various organisms. After sections describing the geological background, methods and results, the outcome is discussed and reconnected with the information given in the background section. The thesis ends with a few suggestions on how the research on this topic should be continued.

The biogeochemistry of the Precambrian oceans

The oceans of the Earth can be said to have existed in three stages – iron-rich during the Archaean, sulphidic-rich during most of the Proterozoic, and oxygenated during the Phanerozoic as well as in the earliest Proterozoic. In the following sections, each of these three redox stages will be reviewed, as will the mechanisms behind them, and how they relate to other important events, such as the major changes in climate, continental formation, and evolution of life (Fig. 1).

The chemical behaviour of arsenic in iron-, sulphidic-, and oxygen-rich waters will here be mentioned rather briefly, and described in detail in its own, separate chapter.

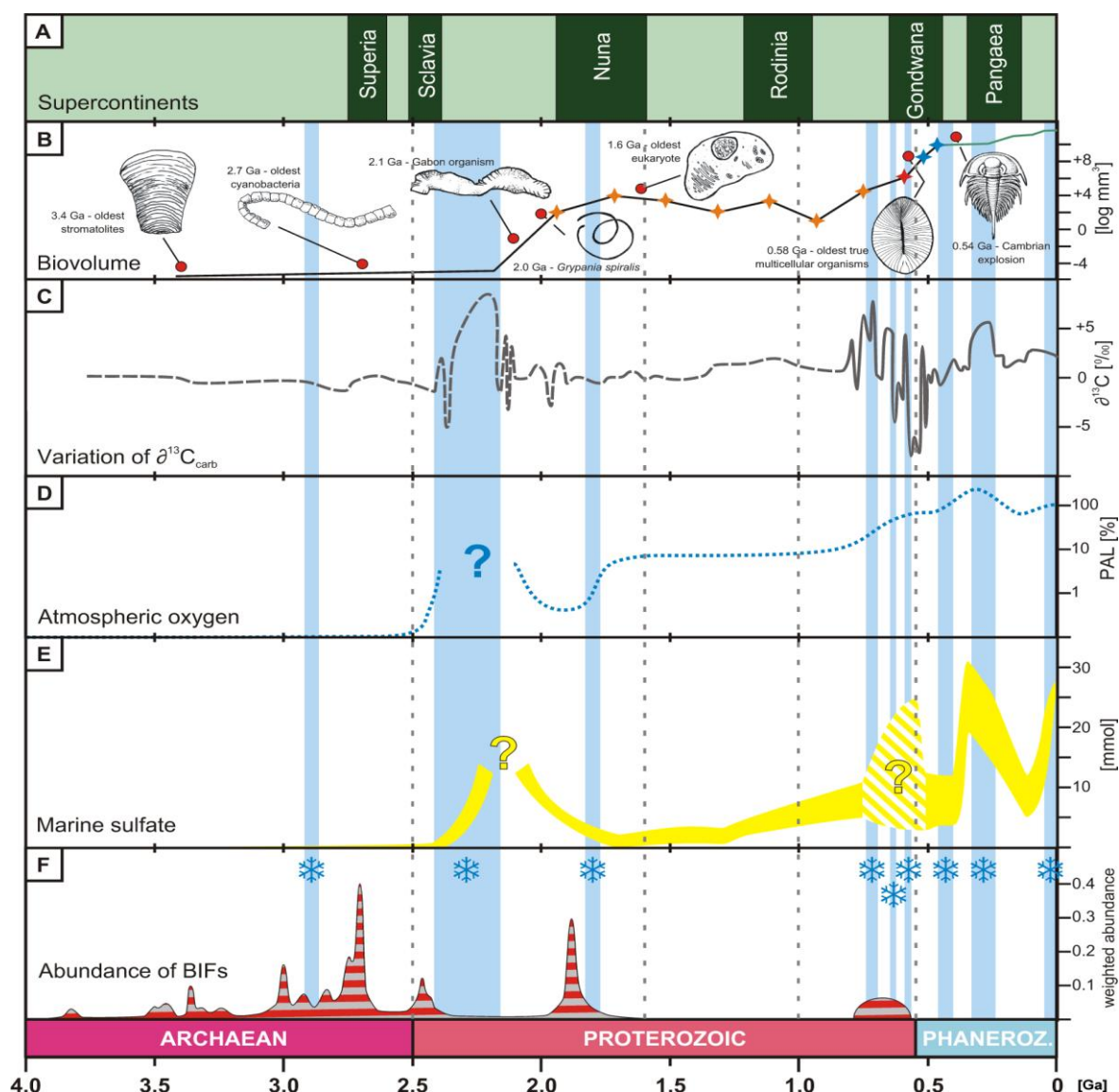


Figure 1 Important events and changes through the history of the Earth. (A) Supercontinents (B) Biovolume (orange stars: prokaryotes, red star: vendobiont, blue stars: animals, green line: plants), and the first appearance of some key taxa (C) Variations of $\delta^{13}\text{C}_{\text{carb}}$ (D) Oxygen levels through time (E) Marine sulphate levels through time (F) Deposition of iron formations. Extensive ice ages are marked with snowflakes and vertical blue lines. The vertical black dotted lines represent the boundaries between Archaean – Palaeoproterozoic – Mesoproterozoic – Neoproterozoic – Phanerozoic. Based on text and figures in Isley & Abbot, 1999; Klein, 2005; El Albani et al., 2010; Lyons & Gill, 2010, and Och & Shields-Zhou, 2012.

The Archaean ferruginous oceans

Definition of ferruginous waters and BIFs

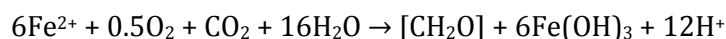
The main source of iron throughout Earth history is hydrothermal vents, around which a certain amount of the element will be deposited almost immediately together with other elements that

are commonly associated with this environment, such as vanadium, arsenic and chromium (German et al., 1991). Most of the iron will, however, spread through the oceans, particularly in the anoxic waters typical for the Archaean. The iron concentrations at this time were in the range of 40 and 120 μM , equivalent to 1000 to 10000 times the present day levels (Canfield, 2005). For this reasons, the Archaean oceans are referred to as ferruginous.

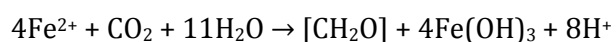
The excessive amount of dissolved iron was what enabled the worldwide depositions of BIFs during that time (e.g. Poulton & Canfield, 2011). BIFs are primarily defined as thin-bedded and/or finely laminated Precambrian sedimentary rocks, composed of alternating silica and iron-rich bands. They are estimated to contain 15-40 wt% iron (James, 1954; Klein, 2005), mainly as oxides forming magnetite and/or hematite, and to a lesser extent as carbonate BIFs (siderite), sulphides (e.g. pyrite), and phosphates (e.g. apatite) (Craddock & Dauphas, 2011).

Formations of BIFs

Several theories have been proposed to explain the mechanisms that drove the vast scale deposition of BIFs in the Precambrian oceans. The traditional view has been that BIFs are a product of photosynthetic bacterial activity whereby released oxygen reacts with dissolved Fe^{2+} to produce insoluble ferrihydrite, the diagenetic product of which are various iron oxides and carbonates (e.g. Cloud, 1965; Cloud, 1973; Decker & van Holde, 2011). However, there is much debate on the exact time when cyanobacteria evolved. Even though some argue for an early origin of cyanobacteria already by 3.8 Ga from when the first BIFs are known (Knoll, 2008), most regard them to be considerably younger (e.g. Bjerrum & Canfield, 2002), meaning that the oxidation of Fe^{2+} and deposition of Fe^{3+} must have taken place despite the lack of oxygen producing organisms (Posth et al., 2008). This could have been performed by phototrophic oxidation of iron, either by photoferrotrophs (Kappler et al., 2005; Chi Fru et al., 2013) or chemolithoautotrophs (Konhauser et al., 2002). Under low oxygen conditions, microaerophilic chemolithoautotrophic iron oxidation can yield high amounts of ferric iron, according to the equation:



Unlike microaerophilic chemoautolithotrophy, anoxygenic photoferrotrophy oxidises iron in the complete absence of oxygen by fixing CO_2 using light as energy source. As a result ferric oxyhydroxide are deposited following the equation:



Because of the anoxic nature of most of the Precambrian oceans throughout the Proterozoic, it is considered that photoferrotrophy provides the best explanation for the early production of iron formations, while chemolithoautotrophic iron oxidation probably grew in importance once free oxygen became available (Konhauser et al., 2002; Croal et al., 2004; Crowe et al., 2008; Planavsky et al., 2009; Konhauser & Riding, 2012).

The involvement of microbes in the formation of BIFs can be verified by looking at the ratio of stable iron isotopes ($^{57}\text{Fe}/^{56}\text{Fe}$) (Dauphas et al., 2004; Czaja et al., 2013). Bacteria tend to cause a fractionation in the iron sedimentary record as they favour the uptake of lighter ^{56}Fe , leaving the sediments enriched in ^{57}Fe (Beard et al., 1999; Kappler et al., 2005). Such fractionation could, however, also be produced by a purely abiological precipitation of BIFs due to photochemical oxidations. Although this theoretically could occur when UV radiation interacts with the surface water (Braterman et al., 1983), it still should be considered to be unlikely for two reasons. First, it has never been successfully demonstrated in the rather complexly composed seawater. Secondly, even if it would occur in seawater, the at the time high levels of dissolved silica would readily have reacted with iron to form amorphous gels capable of putting a serious constraint on the UV photolysis (Konhauser et al., 2002; Crowe et al., 2008). Furthermore, if the observed iron fractionation in the BIFs is combined with measured values of $\delta^{13}\text{C}$, the idea of a microbial iron respiration gains even stronger support (Craddock & Dauphas, 2011). For all these reasons, the BIFs deposited in the anoxic Archaean oceans are most likely formed through biological processes rather than abiotic.

Spatial and stratigraphic distribution of BIFs

The oldest known BIFs are part of the Isua Supracrustal Belt in Greenland, being approximately 3.7-3.8 Ga in age (Canfield, 2005; Klein, 2005; Walker et al., 1983). However, it was not until about 3.6 Ga that the more extensive deposition of BIFs began. Since then and until about 1.8 Ga, iron formations can be found on every continent and from a variety of different marine environments, ranging from near shore to deeper settings (Klein, 2005). Despite this was the deposition of BIFs not continuous, but rather occurred in pulses that have been correlated with phases of increased volcanism (Isley & Abbott, 1999). A longer anomaly occurred between 2.4 and 2.0 Ga, from when there are almost no recordings of BIFs. At 2.0 Ga, they suddenly reappear, only to disappear again at about 1.8 Ga. Thereafter they remained absent for about one billion years, until they make a brief as well as last reappearance in the Neoproterozoic (Bjerrum & Canfield, 2002; Canfield, 2005; Poulton & Canfield, 2011).

When BIFs again were deposited between 0.8 and 0.6 Ga, they differed from the older Archaean/Palaeoproterozoic BIFs in both composition and environmental setting. These younger Neoproterozoic iron formations are associated with glacial deposits, which can be identified through the presence of dropstones, as well as $\delta^{13}\text{C}$ excursions. Furthermore, the iron in the Neoproterozoic iron formations is mainly present as hematite, while Archaean and Early Proterozoic BIFs to a very large degree contain magnetite, silicates and carbonates. Also the processes responsible for their formation differ; while bacteria mediated deposition of the older BIFs, the younger iron formations appear to have been formed abiotically (Pierrehumbert et al., 2011). A fourth difference is that older BIFs are very high in rare earth elements (REEs), while the REE levels in the young iron formations are about the same levels as in modern oceans (Klein & Ladeira, 2004).

The oceans after the BIFs

By the time when the deposition of BIFs came to a final stop at about 0.6 Ga, the ocean chemistry had gone through a radical change in its composition in more ways than simply from anoxic to oxic. From being rich in iron while very low in other metals, the situation was now almost the reversed. Iron, which was only soluble in the anoxic ocean, had been precipitated and thereby largely removed from the water column. The concentration of other metals that had been low in the Archaean, such as molybdenum and zinc, began to increase (Saito et al., 2003; Scott et al., 2008; Williams, 2012). All these changes mirror the emergence of free oxygen, a process that has been studied in great detail but is yet not completely understood.

The oxygenation of the oceans and atmosphere

The emergence of free oxygen

Several lines of evidence support the emergence of free oxygen by 2.7 Ga (e.g. Barley et al., 2005). However, it was not until between 2.4 and 2.2 Ga that the levels had risen to about 0.05 atm, corresponding to 2.5% of the present atmospheric level (PAL) (Canfield, 2005; Decker & van Holde, 2011). This transition from anoxic to oxic atmospheric conditions is known as the Great Oxygenation Event (GOE) (e.g. Frei et al., 2009). Except for a potential sudden drop in the O_2 levels at about 1.9 Ga ago, the oxygen remained between 1 and 10% of PAL until 800-750 million years (Ma) ago when the levels again rose, reaching the present day values about 540 Ma ago (Lyons & Reinhard, 2009).

Atmospheric oxygen levels are usually reconstructed by using mass independent fractionation (MIF) in sulphur isotopes (^{36}S , ^{34}S , ^{33}S and ^{32}S). MIF in sulphur isotopes is produced by photodissociation, or photolysis, of gaseous sulphur compounds (Zalasiewicz & Williams, 2012). Volcanic activity releases sulphur compounds into the atmosphere, where they are split by ultraviolet light regardless of their isotopic composition. All four isotopes are eventually deposited and preserved in the sedimentary record. Conversely, microbial sulphur cycling and other near surface processes are responsible for mass *dependent* fractionation (MDF) with microorganisms selecting the lighter isotope for uptake. This causes a fractionation of the sulphur isotopes in the strata, which will be enriched in the heavier isotopes while depleted in ^{33}S and ^{32}S (Zalasiewicz & Williams, 2012). MIF is expressed in variations of $\delta^{33}\text{S}$, with large MIF signals indicating the absence of an ozone layer and low or no free oxygen. MIF signals close to zero suggest the presence of atmospheric oxygen as well as an ozone shield, which reduces the photolytic effect of UV radiation (Bekker et al., 2004). Once the GOE was initiated, MIF disappeared from the sedimentary record (Lyons & Reinhard, 2011).

The role of cyanobacteria

Responsible for the increase in oxygen levels were the cyanobacteria, the organisms credited with the invention of oxygenic photosynthesis. They split water and use the released hydrogen to fix CO_2 into organic matter, releasing O_2 as a by-product. As a consequence, they profoundly changed Earth's atmospheric and oceanic composition (e.g. Knoll, 2008). Photosynthetic organisms prior to cyanobacteria reduced CO_2 into organic matter using Fe^{2+} , H_2 and/or H_2S , likely through photosystem I (Holland, 2006). Cyanobacteria instead use both photosystem I and photosystem II, where photosystem I generates energy (ATP) and reductants (NADPH) by stripping electrons from chlorophyll, while photosystem II oxidises water for electrons, using a catalytic complex in which manganese is the central atom (Johnston et al., 2009). Cyanobacteria must have been conducting both processes by the time of the GOE, but likely they were able to do so even prior to that although without producing enough oxygen to induce noticeable changes in the atmosphere (Holland, 2006). Evidence for a biological oxygen production as early as 300 million years before the GOE is supported by enrichment of ^{53}Cr isotopes in the sedimentary records (Frei et al., 2009) as well as low amounts of molybdenum and high amounts of rhenium (Kendall et al., 2010). Biomarkers from evaporative lake sediments, stromatolites, kerogenous shales, U-Pb data (Buick, 2008), and sulphur isotopes (Canfield et al., 2000) indicate that oxygenic photosynthesis might have evolved even earlier, although this is not universally accepted (see e.g. Holland, 2006).

The role of O₂ sinks and nutrients

If cyanobacteria were present and had started to produce oxygen by 2.7 Ga or maybe even earlier, why did the environment then remain largely anoxic? Several reasons for this delay in oxygenation have been suggested. One part of the explanation could be the higher salinity and temperature (often claimed to have been between 55°C and 85°C during the Archaean), which together would be enough to keep the oceans in an anoxic state even at atmospheric oxygen levels about 70% of today's values (Knauth, 2005). However, the climate during the Archaean was far from stable, with glacial conditions towards its end (Kasting & Ono, 2006). A more plausible explanation for the postponed oxygenation would be the various sinks, reacting with any O₂ produced and thereby keeping atmospheric oxygen levels below 10⁻⁵ PAL (Canfield, 2005). The dissolved oceanic iron, as mentioned above, could have been one of these sinks (although it contradicts the interpretation of the BIFs having been formed biologically by photoferrotrophic bacteria), and biologically produced methane probably another (Saito, 2009; Peretó, 2011). A third sink could have been reducing gases emitted from volcanoes (Och & Shields-Zhou, 2012). Prior to the GOE, the SO₂/H₂S ratio in volcanic gases had in general been low, as submarine volcanoes were the sources of emission. After the GOE, this ratio became fairly high as the main source for the flux of atmospheric volcanic gases shifted from submarine to subaerial. The reducing H₂S thereby decreased on behalf of SO₂, possibly facilitating atmospheric oxygenation (Guillard et al., 2011). A problem with this explanation is, however, that the timing between these tectonic events and the GOE is not as ideal, and there are also several uncertainties regarding how the proposed high sulphate concentrations fit together with the abundance of reduced iron released from hydrothermal vents at the same time (Lyons & Reinhard, 2011). Thus, the only thing that can be confidently said is that there likely were several sinks involved in controlling Precambrian atmospheric oxygen levels, while their individual importance remains to be revealed.

Apart from the various sinks consuming O₂, there are also other possible explanations for the delayed oxygenation where the focus instead lies on either nutrients or the evolutionary history of the cyanobacteria. One important nutrient, phosphorous, could have been limited due to how it is adsorbed onto iron oxides and thereby removed from the water column (very much like arsenic behaves). This process would have been enhanced during BIFs formation, resulting in low rates of bioavailable phosphorous, with a reduced rate of photosynthesis and oxygen production as a consequence (Bjerrum & Canfield, 2002). Also nitrogen is of vital importance, and it has been suggested that early cyanobacteria had not yet developed the ability to fix nitrogen. Instead N₂ had to be fixed into NO₂⁻ and NO₃⁻ by lightning in order to be biologically available. Once cyanobacteria acquired nitrogenase (an enzyme enabling nitrogen fixation)

nitrogen would no longer have been a limiting factor for primary productivity (Grula, 2005). Another possible explanation related to the evolution of the cyanobacteria concerns their sensitivity to UV radiation, which causes damage on both DNA and proteins. Today the ozone layer shields the Earth from this harmful radiation, but as mentioned earlier, oxygen levels must be about 10^{-2} PAL for a significant ozone layer to form. Dissolved iron can provide some protection, but the formation of BIFs reduced water column turbidity, increasing the penetration efficiency of UV-radiations. Being photoautotrophs, the cyanobacteria would have needed to stay within the photic zone and therefore also find a way to deal with the increasing radiation stress. The delay in oxygenation of the atmosphere would then represent the time it took for the cyanobacteria to come up with defence and repair mechanisms (Petsch, 2004). However, once an ozone layer had formed the photosynthetic organisms could occupy increasingly shallower water and take advantage of the more abundant sunlight to flourish. This would have created a new niche, and the high productivity would have boosted oxygen production to levels seen after the GOE (Decker & van Holde, 2011). The production of oxygen has never ceased ever since, but it is known to have decreased significantly between 2.0 and 1.8 Ga (Lyons & Reinhard, 2009) for yet unknown reasons.

Effects on the atmosphere

The atmosphere before the GOE was initially considered to have been strongly reducing, with H_2O , NH_3 , CH_4 and H_2 as the major constituents (Miller, 1953). Although this view has been questioned based on photochemical evidence saying that neither methane or ammonia would last for very long in the atmosphere (Lasaga et al., 1971; Kuhn & Atreya, 1979), the theory recently got some new support when it was proposed that the early atmosphere was formed through degassing from impacting material (Schaefer & Fegley, 2007). The competing view states that the early atmosphere would more likely have reflected what was, and still is, outgassing from the Earth itself. This would have generated merely a weakly reducing atmosphere with mainly H_2O , CO_2 and N_2 , small amounts of CO and H_2 , and close to free from NH_3 and CH_4 (Holland, 1984; Zahnle et al., 2010; Decker & van Holde, 2011). However, both of these theories tend to neglect the contribution of biological activity, such as methanotrophs. Methanotrophs were likely present already in the Archaean oceans and could have produced significant amounts of methane during this time (Chi Fru et al., submitted). Thus, the gases composing the pre-GOE atmosphere would have mirrored both the emissions from the interior of the Earth as well as the activity of early anaerobic bacteria and as a consequence, the atmosphere could have been moderately reducing.

Once oxygen began to accumulate, the atmospheric composition went through a radical transformation. H₂O and N₂ remained important constituents of the atmosphere, but from now on the atmosphere was instead mildly oxidative. One of the first signs of this is the presence of terrestrial “red beds”, where the red colour is due to oxidised iron. Red beds are seen from about 2.3 Ga (Decker & van Holde, 2011). Around the same time, the two greenhouse gases CH₄ and CO₂ became less abundant; CH₄ due to oxidation (and possibly also due to decreased availability of nickel, as nickel is an essential compound in methane producing bacteria (Saito, 2009; Peretó, 2011)) and CO₂ from increased weathering of silicates as more continents had formed. This contributed to the triggering of the Huronian glaciations, and most of or all of the Earth became covered by ice sheets between 2.4 and 2.2 Ga (Kasting, 2005; Kopp et al., 2005; Melezhik, 2006).

For many organisms other than cyanobacteria, the rise of free oxygen could have been a double-edged sword. At the same time as it opened up for much more efficient metabolism and respiration, yielding as much as 16 times more energy than the anaerobic equivalent, O₂ was also lethal to the anaerobic organisms that until then had populated the oceans (Sessions et al., 2009). Dioxygen can form several reactive and very toxic compounds, and while some organisms were able to develop defence mechanisms and eventually even take advantage of the free oxygen (such as the mitochondria), others did not respond well to the new situation. Although there are still a few strict anaerobes in modern oxygen-free environments, many must have failed in finding a refuge and consequently have faced mass extinctions due to the emergence of O₂ (Decker & van Holde, 2011). Oxygen might, however, not have been the only toxic threat; its emergence would also have initiated an increase in continental weathering of sulphide minerals into sulphates, which were subsequently washed into the oceans where they led to the rise of sulphidic conditions. It has been suggested that the toxicity of these sulphidic oceans has repressed the evolution and expansion of complex multicellular life until the Ediacaran Period/late in the Neoproterozoic (e.g. Sarkar et al., 2010).

The sulphidic oceans of the Proterozoic

Definition of euxinic waters

At about 1.84 Ga, the last of the iron formations of the older type were deposited (e.g. Poulton et al., 2010), which for a long time interpreted as the beginning of oxygenated deep oceans (e.g. Holland, 2006) and the end of the ferruginous conditions. However, an alternative scenario was proposed by Canfield (1998), in which the absence of BIFs might not necessarily be due to oxygenated deep waters, but could reflect a shift to euxinic, i.e. sulphidic and anoxic, conditions. The proposed sulphidic ocean waters together with moderately oxygenated surface waters have

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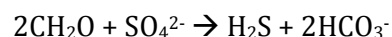
come to be known as the “Canfield ocean”, a state that persisted for at least one billion years (Johnston et al., 2009). Initially, it was believed that euxinic conditions stretched through most of the water column, including the deeper water masses (Canfield, 1998). More recently it has been suggested that the sulphidic conditions could have been restricted to the mid-depth of the oceans as well as shallower epicontinental settings (Poulton et al., 2010). Furthermore, it was first argued that euxinic mid-Proterozoic oceanic conditions could have been globally distributed (Canfield, 2005), but also this has been challenged as new evidence indicated that the sulphidic conditions might rather have been concentrated along continental margins. Although it is likely that the euxinia varied spatially over time, it appears that the deepest waters remained ferruginous and anoxic, despite the absence of BIFs (Poulton et al., 2010).

It is generally agreed on that the development of the euxinic conditions started around 1.84 Ga triggered by a major episode of within-plate anorogenic magmatism forming the supercontinent Columbia (also referred to as Nuna) (Parnell et al., 2012). The vast amounts of new crust were mainly of granitic composition, but also anomalous amounts of metallic sulphides were generated. The weathering of this new source of sulphides is considered to have contributed significantly to the transition into euxinic conditions (Parnell et al., 2012).

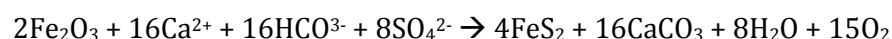
The effects of H₂S

Whether the euxinic conditions were global or only widely distributed and regardless of the exact position of the sulphidic waters within the water column, the implications would have been many. The perhaps most notable one might have been its effect upon the evolution of eukaryotes (Scott et al., 2008; Poulton et al., 2010).

The idea of a sulphidic ocean follows a model, in which the oxygenation of the atmosphere led to increased weathering of continental sulphide minerals, thereby causing sulphates (SO₄²⁻) to be delivered to the oceans (Canfield, 1998, Poulton et al., 2004). The sulphate would then be reduced by the respiration of prokaryotic microorganisms through the reaction:



The hydrogen sulphide (H₂S) released as a waste product (Kump et al., 2005) reacted with iron to form sedimentary pyrite (FeS₂), and prevented the deposition of BIFs (Canfield, 1998; Lyons & Gill, 2010). A simplified net reaction for pyrite formation (e.g. Lyons & Gill, 2010) could be:



Sulphate delivery needed to exceed iron supply by a factor of two in order to maintain euxinic conditions and prevent the formation of BIFs (due to the ratio of 1:2 between iron and sulphur in pyrite) (Poulton et al., 2010). However, the absence of iron formations has also generated alternative hypotheses, such as that the waters during this time were poor in both iron and oxygen, or that the oceans had already become oxygenated. Nevertheless, these explanations are not well supported by the sparse sedimentary record (Lyons & Gill, 2010).

H₂S is used by some chemolithotrophs in their metabolism, and is in very small amounts an essential signalling gas within the cells of mammals as well as yeast (Lloyd, 2006), and possibly also all other mitochondria-containing eukaryotic cells (Davidov & Jurkevitch, 2009). Despite this, H₂S is toxic to most organisms (five times more so than carbon monoxide) and lethal at high doses. H₂S interferes with oxygen respiration by binding to various metalloenzymes and by reducing the protein disulphide bridges within the cell, disrupting its redox environment (Lloyd, 2006). Its toxicity is further enhanced by its high diffusive power and the fact that elevated solubility in lipids allows it to pass unrestricted through biological membranes (Reiffenstein et al., 1992).

Considering how the oxygen production appears to have been fairly constant throughout the Proterozoic, one might infer cyanobacteria to be resistant against sulphide toxicity, as it is likely that sulphidic waters were able to penetrate the overlaying oxygenated surface water. This is, however, not the case for most modern cyanobacteria strains. Instead, they are highly sensitive to sulphide exposure, since already brief contacts and low concentrations cause the photoassimilation to irreversibly cease by interfering with photosynthesis II. Nevertheless, certain modern cyanobacteria living in hydrothermal and hypersaline environments have found different ways to cope with the presence of sulphur (Cohen et al., 1986). Of those cyanobacteria that are capable of tolerating sulphide, the tolerance has been found to correlate with the sulphide levels in their habitat. However, the tolerance varies even among closely related strains of cyanobacteria, and at the same time are the sulphur tolerable taxa genetically diverse. Together, this indicates that the trait of sulphur resistance has been gained several times among the cyanobacteria, and that it is an environmentally dynamic trait that can both be gained and lost (Miller & Bebout, 2004). Therefore, the sulphide sensitivity among present day cyanobacteria does not necessarily indicate that Proterozoic cyanobacteria were equally vulnerable. Instead, since the atmospheric oxygen levels remained unaffected despite the development of the sulphidic ocean, the majority of the cyanobacteria at the time must have possessed a defence mechanism against sulphide, only to lose it once it was no longer needed. For the same reason, it is difficult to know how sensitive or resistant cyanobacteria were to

arsenic during the Proterozoic. Modern day species have, however, a fairly high tolerance level and are unaffected at concentrations significantly above what other organisms can handle (Nagy et al., 2005; Bhattacharya & Pal, 2011).

The effect of sulphur upon other elements

The development of sulphidic oceans might have had more biological implications than only through the built up of H_2S . As the continental weathering advanced, the delivery of metals that commonly occur with sulphides would have increased as well. Some of these metals are biologically essential, such as zinc, copper and molybdenum, and it has been suggested that the increased flux of these would have promoted eukaryotic diversification (Parnell et al., 2012). Other metals and metalloids are, however, not essential and in some cases even toxic, such as mercury, lead and arsenic. It appears likely that also the flux of these would have increased, perhaps even to levels where their negative effects would have outweighed the positive effects from an increase of the bio-essential elements. Particularly arsenic is of interest here as it is highly toxic already at low concentrations.

An increased flux of metals might, however, not necessarily have led to elevated concentrations in the water column as they could have been removed through reactions with various sulphide compounds (Anbar & Knoll, 2002; Och & Shields-Zhou, 2012). As mentioned earlier, the sulphide would have reacted with the dissolved iron to form pyrite, but when the sulphur flux exceeded the flux of iron, iron must have become increasingly sparse. Because iron is an important micronutrient, once it became less abundant, it might have constrained biological productivity. This would also have been the case for other nutrients, e.g. molybdenum, copper and zinc, as they too would have been affected by the sulphidic conditions through similar mechanisms (Anbar & Knoll, 2002; Scott et al., 2008; Glass et al., 2009).

The same behaviour could also be expected from toxic metals and metalloids; i.e. they could have been removed from the water column through reactions with sulphide compounds. Arsenic can, for example, be incorporated into pyrite to form arsenopyrite ($FeAsS$). However, during times with high pyrite precipitation rates, arsenic can instead be excluded from the mineral and left in solution (O'Day et al., 2004). Furthermore, under reducing conditions where free sulphide is present, arsenic may have an increased solubility due to the formation of arsenate-sulphide complexes (Couture & Van Cappellen, 2011).

Around 1.4-1.3 Ga, the euxinic conditions seem to have declined somewhat. The low levels of molybdenum have been suggested as one factor behind this (Scott et al., 2008), while others argue that the main constraint upon the lingering of the euxinia would have been the subduction of sedimentary sulphides (Poulton & Canfield, 2011). Another possibility is the evolution of fungi, lichens and other land vegetation. If they had started to colonise the landmasses already at this point, it could have acted to lower the weathering rates by producing a protective cover (Shields-Zhou & Och, 2011). A third factor could be the tectonic stability between 1.8 and 1.3 Ga (Roberts, 2013), which allowed the surface of the supercontinent sulphide-rich Nuna to be constantly weathered. While this could have led to a decrease in the abundance of sulphides, more and more of the underlying bedrock would have been exposed, thus resulting in a lowered flux on sulphates into the oceans (Arvestål, unpublished). The unconformities that underlie many Cambrian deposits support a previously extensive weathering and erosion upon the older sediments (e.g. Peters & Gaines, 2012). Still, it was not until ~0.8 Ga that the next major change in the ocean chemistry took place (Och & Shields-Zhou, 2012).

The end of the euxinic conditions

At about 0.75 Ga, the vast period with euxinic conditions seems to have come to end (Frei et al., 2009), although local exceptions are known (Li et al., 2010). The oxygen levels had been rising gradually from about 0.8 Ga and onwards, perhaps as a consequence of the breakup of the supercontinent Rodinia. The breakup was initiated around 0.825 Ga, and reached its maximum dispersal between 0.75 and 0.7 Ga. The biological diversification was promoted as shallow and nutrient-rich waters typical for coastal areas could develop, although at the same time, the climate got increasingly harsh. Although speculative, there might be a connection between the rise of oxygen and climate cooling during the Neoproterozoic Oxygenation Event (NOE) just as it had been during the GOE, in both cases due to an oxygen-caused collapse of the methane in the atmosphere. During the Cryogenian period (0.85-0.635 Ga) at about 0.75 Ga, the oxygen levels started to climb above 10% of PAL (Lyons & Reinhard, 2009) and a minor glaciation occurred, but it was later during this period that the coolhouse conditions advanced into the extreme (Och & Shields-Zhou, 2012). Two separate glaciations reached equatorial latitudes during this time; the Sturtian glaciation (0.716-0.67 Ga) followed the Elatina glaciation (also known as Marinoan glaciation, 0.65-0.635 Ga) (Shields-Zhou & Och, 2011). The extensive ice sheets during both glaciations led to ocean stagnation, allowing iron to once more accumulate in the oceans. Indeed, the widespread ferruginous conditions during the Cryogenian were similar to the oceans as they had been during the Archaean and Proterozoic, and just as back then, banded iron formations started to be deposited. However, while the Archaean iron formations were formed by

anoxygenic photosynthetic bacteria as they oxidised Fe^{2+} in their metabolic process (see above), these younger Neoproterozoic iron formations instead formed abiotically when the oxygenated coastal waters came in contact with the iron-rich anoxic deeper waters (Pierrehumbert et al., 2011).

Despite the growing glaciers, the oxygen levels kept rising. This is reflected in the concentrations of molybdenum, which during the Proterozoic had remained at very low levels due to the euxinia. During the Cryogenian, at 0.663 Ga, the molybdenum concentrations started to rapidly increase and before 0.551 Ga it had reached Phanerozoic values (Scott et al., 2008; Halverson et al., 2010). Also carbon isotopes confirm the rise of oxygen, as does isotopes of chromium (Halverson et al., 2010).

When the Ediacaran period was initiated at 0.635 Ga, the glaciers had temporarily retreated, but roughly in the middle of this period, the temperature dropped again during an event known as the Gaskiers glaciation. This glaciation was, however, only of limited extension and never reached into lower latitudes (Shields-Zhou & Och, 2011). When temperatures again started to rise and put an end to the Gaskiers glaciation at 0.580 Ga, the oceans looked remarkably different. For the first time in history were the oceans now oxygenated not only at the surface but also at the deep waters, and the oxygen levels in the atmosphere keep rising out just before the end of the Ediacaran period at 0.542 Ga when it reached concentrations similar to those of today. Furthermore, when the Gaskiers had withdrawn, the ocean was no longer home to mainly (although perhaps not solely) single-celled prokaryotes and eukaryotes, but also to the clearly multicellular Ediacaran biota (e.g. Canfield et al., 2007).

As outlined above, the low oxygen levels throughout much of the Precambrian, the toxicity of H_2S in Mesoproterozoic oceans and the resulting limited nutrient availability have all been presented as potential causes for the delay in evolution of complex multicellular life, but is it enough? There might be yet another factor behind the delay, one that has not previously been recognised – the upcome, spreading, and enrichment of arsenic in oceanic waters caused by oxygen-driven weathering of sulphide minerals on continental landmasses. Arsenic could have contributed significantly to regulating the evolution and expansion of life after the rise of atmospheric oxygen.

Arsenic

The distribution of arsenic in nature

Arsenic, infamous for its toxicity, is a group 15 metalloid found in the fourth period in the periodic system, beneath nitrogen and phosphorous. In nature, arsenic has only one stable isotope, ^{75}As , and occurs in four oxidation states: arsine (As^{3-}), elemental arsenic (As^0), arsenite (As^{3+} ; $\text{H}_3\text{AsO}_{3(\text{aq})}$, H_2AsO_3^- , HAsO_3^{2-} and AsO_3^{3-}) and arsenate (As^{5+} ; $\text{H}_3\text{AsO}_{4(\text{aq})}$, H_2AsO_4^- , HAsO_4^{2-} and AsO_4^{3-}). As^{3+} and As^{5+} are the most common (inorganic) forms and both are also soluble. However, As^{5+} more easily adheres to iron oxides, and although As^{3+} might do this too, its higher solubility makes it in general more bioavailable. As a consequence, it is two to three times more toxic than As^{5+} (Hu et al., 2012). Elemental arsenic occurs only rarely, while As^{3-} exists either in strongly reducing environments or in fungal cultures (Ledbetter & Magnuson, 2010).

Of the 88 elements that are naturally occurring, arsenic ranks as the 47th most abundant (Voughan, 2006). It is slightly enriched in the Earth's crust ($1.8 \mu\text{g/g}$), in which it is the 20th most abundant element (Lièvreumont et al., 2009). The enrichment is further enhanced in sedimentary rocks, particularly shales ($13 \mu\text{g/g}$), while it is instead rather low in sandstones and carbonates (both $1 \mu\text{g/g}$) (Mason & Moore, 1982). In modern day oceans, the average concentration of arsenic is $2.6 \mu\text{g/L}$, and the residence time of the element is about 50000 years (Mason & Moore, 1982). Common arsenic sources are volcanic rocks and their weathering products, hydrothermal ore deposits, geothermal waters, and marine sedimentary rocks (Wang et al., 2006).

In minerals, arsenic can occur in three different forms, either as the anion or dianion (As_2) or as the sulpharsenide anion (AsS). Most commonly these compounds bond to metals to form metal arsenides, like FeAs_2 and NiAs_2 , or sulpharsenides, such as arsenopyrite, FeAsS (e.g. Voughan, 2006). FeAsS is the most commonly occurring arsenic mineral and is mainly found in mineral veins, which is also where most other arsenic compounds are located (Mandal & Suzuki, 2002). However, FeAsS as well as other arsenic bearing sulphide minerals are, contrary to what is sometimes claimed, not only formed in the Earth's crust under high temperature conditions. FeAsS can form as an authigenic mineral, whereas orpiment (As_2S_3) which together with realgar (AsS) is the most commonly occurring As-sulphide mineral next to FeAsS , can be formed through precipitation by microbes (Smedley & Kinniburgh, 2002). Arsenic can also be found in smaller quantities in the fairly abundant pyrite (Voughan, 2006), with the average content ranging from 0.1% in deposits with enargite (Cu_3AsS_4) to 4% as arsenopyrite and tennantite ($(\text{Cu,Fe})_{12}\text{As}_4\text{S}_{13}$) in deposits with pyritic copper (Mandal & Suzuki, 2002).

Arsenic cycling

Following mineral weathering and delivery to the ocean, the behaviour of arsenic is largely dependent on the redox conditions, pH levels, the presence of sulphur and iron, and on microbial activity (Lièvremonet et al., 2009). A reducing environment with low sulphur levels and pH values between 4 and 10 favours the formation of arsenite as H_3AsO_3 . At the same range of pH with equally low sulphur concentration but under oxidising conditions, arsenate will instead dominate, either as H_2AsO_4^- or HAsO_4^{2-} (Hu et al., 2012). Arsenate is the thermodynamically most stable arsenic species, and the ratio between As^{5+} and As^{3+} in oxygenated sea water (pH 8.1) should theoretically be about 10^{26} :1. However, the actual ratio in present day oceans ranges from 10:1 down to 0.1:1 (Mandal & Suzuki, 2002).

If free sulphide is present in the water column, it can react with the arsenic to form trivalent aqueous oxythioarsenic, including oxythioarsenite ($\text{AsO}_{3-x}\text{S}_x^{3-}$) and oxythioarsenate ($\text{AsO}_{4-x}\text{S}_x^{3-}$) (Suess & Planer-Friedrich, 2012). It has been theorised that the presence of dissolved iron would inhibit this reaction by removing the sulphide; hence oxythioarsenic should only form in low-iron environments (Wilkin et al., 2003). However, oxythioarsenates were recently found in waters with moderate iron contents, although their extent and mechanism of formation remain unclear (Suess et al., 2011). It is generally believed that under sulphide-rich anoxic conditions, oxythioarsenite is assumed to be the dominant species, but oxythioarsenate appears to be the prevalent form in both anoxic and oxygenated waters, due to the oxidation of As^{3+} by $\text{S}^0_{(\text{aq})}$ (Couture & Van Cappellen, 2011).

The cycling between As^{3+} and As^{5+} can be both biotic and abiotic. Biological reduction of As^{5+} can take place through two mechanisms; one offering detoxification but not generating energy, and one where arsenic is used in the respiration of the organism. The first mechanism is widely distributed throughout the domains of life, i.e. in Archaea and Bacteria as well as in Eukaryota (Mukhopadhyay et al., 2006; Stolz et al., 2010), while the second mechanism is found in Bacteria and Crenarchaeota (a phylum within Archaea) (Stolz et al., 2010).

Biological oxidation of As^{3+} can, unlike the As^{5+} reduction, both provide arsenite resistance and yield energy (Stolz et al., 2010). The rather high oxidation-reduction potential of the element, +139 mV, makes it suitable not only as a terminal electron acceptor (Rosen et al., 2011) but also as a source of energy (Schoepp-Cothenet et al., 2011). Most, but not all, organisms performing arsenite oxidation are aerobic, including heterotrophs as well as chemolithoautotrophs. Both use As^{3+} as the electron donor and oxygen as the terminal electron acceptor, while in the case of the anaerobic chemolithoautotrophy, As^{3+} respiration is coupled to NO_3^- reduction (Stolz et al., 25

2010). As a result, during anaerobic conditions, if NO_3^- is abundant, As^{5+} instead of the expected As^{3+} can be the dominant arsenic species (Senn & Hemond, 2002).

The use of arsenic in microbe metabolism is most likely ancient in origin, since the same enzymes, Arr and Aox, are used in both Bacteria and Archaea (Stolz et al., 2010). However, at least the *aox* genes might have been transferred horizontally between different groups of bacteria and might not reflect any phylogenetic relationships among them (Heinrich-Salmeron et al., 2011). Either way, due to the reducing environment during the Archaean, As^{3+} is bound to have been the prevalent form, while As^{5+} would have increased in abundance, initially as anoxygenic photoautotrophs oxidised As^{3+} and later, once atmospheric oxygen was present, through aerobic arsenite oxidation (Stolz et al., 2010).

As mentioned already, arsenite has compared to arsenate a lower adsorption potential to oxide minerals and instead tends to remain mobile. Also reducing conditions can promote the release of arsenic, both from the mineral surface and from within the mineral structure (Lièvremonet et al., 2009). Usually, these anaerobic conditions favour the release of As^{3+} , unless there is an abundance of NO_3^- (Senn & Hemond, 2002). Microbial activity has an important role in this process, as they can change the structure of iron oxides from amorphous to crystalline and thereby decreasing the area on which arsenic would be able to adsorb (Lièvremonet et al., 2009).

Microbes, such as *Geobacter metallireducens*, also contributes to the release of arsenic from iron oxides as they reduce the iron from Fe^{3+} to Fe^{2+} . *G. metallireducens* causes aggregates of the mineral to deflocculate and while doing so, the surface charge of the mineral grains change. This change in charge is then what leads to the mobilisation of arsenic (Tadanier et al., 2005). However, the microbes affecting the behaviour of arsenic the most are those acting to change the redox conditions, either by producing O_2 or by consuming it. When going from oxic to anoxic conditions, arsenate is reduced to arsenite and thereby has an increased mobility. In fact, it is believed that the main cause for arsenic release is this shift from aerobic to anaerobic conditions (Lièvremonet et al., 2009).

Authigenic pyrite is formed in reducing environments, and if arsenite or arsenate is present, it will likely be incorporated in the mineral (Smedley & Kinniburgh, 2002). However, if the pyrite precipitation rates are high, depending on the ratio between sulphur and reactive iron, arsenic might instead be left in solution. This will allow arsenic to accumulate in the water column to even toxic levels (O'Day et al., 2004). It is also likely to accumulate under alkaline conditions, such as in “soda lakes” typical for arid environments. Under normal or acidic conditions can As^{3+}

be precipitated as e.g. orpiment or realgar, but with high pH and carbonate concentrations the precipitation of As^{3+} is prevented (Oremland et al., 2009).

The precipitation of arsenic sulphides and the incorporation into pyrite are two ways to remove arsenic from the water column. However, the main arsenic sink is its adsorption capacities onto oxide minerals, particularly iron oxides (Smedley & Kinniburgh, 2002; Borch et al., 2010). Arsenic concentrations usually have a strong correlation with iron (oxyhydr)oxides, which are capable of not only changing the oxidation state of arsenic and thus its solubility, but also binding both As^{3+} and As^{5+} depending on the ratio between these two and on the pH of the water (Dixit & Hering, 2003; Johnston & Singer, 2007; Borch et al., 2010). The adsorption onto oxide minerals is indeed what prevents arsenic toxicity problems from developing in many environments (Smedley & Kinniburgh, 2002), although how efficient the adsorption will be is affected not only by pH levels and the surface area of the oxide mineral, but also by the presence of phosphate. Belonging to the same group in the periodic system, arsenic and phosphorous share several chemical properties, and thereby compete for the same adsorption sites on the mineral (Dixit & Hering, 2003). Also oxythioarsenic adsorbs onto iron oxides, although both slower (at least compared to arsenate) and to less extent (compared to both arsenite and arsenate). Under decreasing pH levels, thioarsenic will be increasingly transformed into arsenite, although under anoxic conditions it might instead be precipitated into amorphous arsenic-sulphide minerals (Suess & Planer-Friedrich, 2012).

The toxicity of arsenic

Inorganic forms of arsenic are generally considered to be more toxic than organic species, with arsine as the far most toxic form (Vaughan, 2006). Arsine forms under strongly reducing conditions through microbiological activity (Sharma & Sohn, 2009), and differs from the other arsenic oxidation states by being a volatile gas (AsH_3) with a low solubility in water (O'Day, 2006). Among gases emitted from anoxic environments, trace amounts of arsine have been found (Oremland & Stolz, 2003), but despite its high toxicity, it seems as its role in the environment has so far been largely neglected, likely because of its volatility (O'Day, 2006).

Apart from arsine, arsenite is the most toxic form, followed by arsenate (Flora, 2011). The toxic effects of all of them are many, partly due to how chemical properties of arsenic resemble those of phosphorous. Arsenate can enter the cell through the phosphorous canals, and once inside the cell it can substitute for phosphorous in the biological systems with severe consequences, e.g. by disruption of DNA, ATP, and protein synthesis (Fekry et al., 2011). Arsenite instead enters the

cell by simple diffusion through aquaglyceroporin and interferes with the signalling transduction pathways by disrupting a number of enzymatic functions in the cell (Flora, 2011; Kaur et al., 2011). Almost all organisms are equipped with some sort of protection mechanism against arsenic (Rosen et al., 2011), but despite this, most are affected at low doses with the tolerable amounts depending upon species (Liu et al., 2001), age, and the arsenic form. For humans, the World Health Organisation recommends less than 10 µg/L arsenic in drinking water to avoid negative effects upon the health (WHO, 2010). For adults, the lethal arsenic dose ranges between 70 to 200 mg/kg/day, while small children will become seriously ill after intake of less than 1 mg/kg (Caravati, 2004). In experiments on rats, 4 mg/kg bodyweight of arsine, 80 mg/kg bodyweight of arsenite and 100 mg/kg bodyweight of arsenate resulted in the death of 50% of the test population. For killing half of the rat population with methylated (organic) arsenic, substantially higher levels were required; MMA and DMA both measured 10,000 mg/kg bodyweight (Voughan, 2006). Also plants suffer from arsenic exposure, with arsenite concentrations exceeding 1 mg/kg being able to inhibit growth of both prokaryotic and eukaryotic algae (Nagy et al., 2005). The toxicity of oxythioarsenics has not yet been thoroughly studied, but it seems to be dependent upon the type of the complex. Mono- and dithioarsenate are considerably less toxic than trithioarsenate, which will cause acute toxicity at similar levels as arsenite and arsenate (Planer-Friedrich et al., 2008).

It was recently claimed that a bacterium, GFAJ-1, living in extreme environments, such as the Mono Lake in California, is able to completely replace phosphorous with arsenic (Wolfe-Simon et al., 2011). However, this suggestion has been strongly criticised (e.g. Benner, 2011; Fekry et al., 2011; Schoepp-Cothenet et al., 2011), as such a substitution would not only lead to the arsenic-related difficulties discussed above, but also to a huge increase in kinetic instability. The half-life of the phosphate version of DNA due to non-enzymatic hydrolysis in water at 25°C is approximately 30,000,000 years, while for the arsenic counterpart mere a 0.06 s, an issue that likely would be problematic to overcome (Fekry et al., 2011). The results were also questioned based on uncertainties regarding phosphate contamination in the culture media. Instead of replacing phosphate with arsenate, GFAJ-1 might be capable of extreme phosphorous scavenging, a scenario that appears more likely, at least as long as the contamination levels remain unknown (Benner, 2011; Schoepp-Cothenet et al., 2011). A mass spectrometry of GFAJ-1 DNA confirmed this suspicion; the microbe had not included any detectable amounts of arsenate (Reaves et al., 2012). However, computer modelling has shown that adenosine triphosphate might not be as unstable as previously thought (Nascimento et al., 2012), but until more evidence is presented, the results remain inconclusive.

Although some enzymes that usually catalyse the formation of phosphate esters also can create arsenate esters, the esters themselves behave differently from each other due to differences in bond length as well as bond angles (Rosen et al., 2011). Phosphate esters are an essential part of biomolecules, such as in ATP or DNA, and there are many reasons why the use of phosphate is more advantageous than arsenate. ATP is used for energy transfer within the cell and during its synthesising in mitochondria, one of the three phosphate groups can accidentally be replaced by arsenate. The end product will then be the unstable ADP-arsenate, which undergoes non-enzymatic hydrolysis about 100 000 times faster than ATP (Mandal & Suzuki, 2002; Rosen et al., 2011). Although not yet seen, also the second or all three of the phosphates in ATP could theoretically be replaced. However, as these products are likely to be highly unstable, they would probable hydrolyse faster than their existence could be verified (Mandal & Suzuki, 2002).

The capability of arsenate to impede the energy metabolism inside the cell is considered as one of the major problems associated with exposure of the element (Lièvremonet et al., 2009). Arsenate replacing phosphate in the DNA is another problem, as it could inhibit the DNA repair mechanism (Mandal & Suzuki, 2002), causing cancer, mutations and teratogenesis (Lièvremonet et al., 2009). Damage upon the DNA can also be inflicted by free radicals, which are being generated as arsenate is reduced to arsenite in the plasma of the cell and causes the DNA to degrade (Flora, 2011). Arsenate can also interfere with the enzyme pyruvate dehydrogenase, which leads to blocking of oxygen respiration (Tamás & Wysocki, 2001).

Arsenic detoxification mechanisms

Arsenic detoxification usually involves two steps; a redox process within the cell, and a subsequent transportation of the As-compound out of the cell (Bhattacharjee & Rosen, 2007).

As mentioned earlier, bacteria can either oxidise or reduce arsenic as a detoxifying mechanism. Shared by all As³⁺-oxidising organisms is the enzyme arsenite oxidase (Aox), which is a heterodimer (a large complex consisting of two different macromolecules) that performs the arsenic oxidation. Although Aox is highly conserved, heterogeneity is seen in the *aox* operon, which is the part of the DNA coding for the enzyme (Stolz et al., 2010). Organisms that reduce arsenate through respiration share another core enzyme, ArrAB, but also in the *arr* operon is there some heterogeneity (Stolz et al., 2010).

Bacteria that reduce arsenate only for detoxifying purposes use the *ars* systems. The *ars* operon can consist of either three or five genes, ArsRBC and ArsRDABC, respectively. ArsR codes for a

transcriptor repressor, ArsB for a protein acting as a specific arsenite efflux pump situated in the cell membrane, and ArsC for an arsenate-reducing enzyme. The ArsA in the longer version of the operon codes for an ATPase yielding energy for the efflux pump, while ArsD improves the efficiency of the ArsAB pump (Lièvremonet et al., 2009). Such arsenate detoxifying systems are present also in other kingdoms of life, including archaeans as well as eukaryotes. However, despite the similarities in function (and despite also the use of the same terminology), it appears as the arsenate reducing systems have evolved separately through convergent evolution and are not necessarily homologues (Mukhopadhyay et al., 2006).

Bacterial arsenate reductases, ArsC, include two separate enzyme families; one using glutaredoxin and glutathione as reductants, the other one thioredoxin (Bhattacharjee & Rosen, 2007). The latter of these is also present in a fairly well conserved version in all other kingdoms of life, including Archaea and Eukaryota. In humans as well as in other mammals, the enzyme has a dephosphorylating capacity, but the physiological function of it remains unknown (Alonso et al., 2004; Bhattacharjee & Rosen, 2007; Moorhead et al., 2009). There is also a third family of arsenate reductases, Acr2p, found solely in eukaryotes. This enzyme group appears to be unrelated to the bacterial reductases and is therefore likely a later invention (Mukhopadhyay et al., 2006; Bhattacharjee & Rosen, 2007). The bacterial ArsC (and also ArsB) systems are, on the contrary, believed to have evolved in the very beginning of the history of life (Mukhopadhyay et al., 2006).

An arsenite efflux pump is present in many organisms, but as for ArsC, it has evolved more than once. ArsB, the gene responsible for arsenite transport, is only present in prokaryotes, while another gene with the same function, Acr3, is found in bacteria, archaeans, and some eukaryotes, such as fungi (except yeast) and lower plants. Other eukaryotes lack a specific efflux pump and instead rely either on ABC transporters or on sequestration of the arsenite followed by translocation of the complex into the vacuole. ABC transporters are membrane proteins and occur in abundance in eukaryotes as well as prokaryotes. They require ATP for energy and are not specific for arsenite or any other metalloid, but are capable of transporting a variety of substances (Maciaszczyk-Dziubinska et al., 2012).

Although not all bacteria have the ArsA gene, homologues of it have been found in both archaeans and eukaryotes. There are, however, a few differences; bacteria (and some Archaea, such as Halobacteria) have a duplicated version of the gene, A1-A2, while eukaryotes (and other archaeans, such as methanogenic species) have only a single A structure. ArsA homologues in eukaryotes have been verified by genomic sequencing in plants (*Arabidopsis thaliana*), yeast

(*Saccharomyces cerevisiae*), soil nematodes (*Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*), house mice (*Mus musculus*), and humans (Bhattacharjee et al., 2001).

To summarise, arsenic detoxification mechanisms are ancient in origin, but the need for an efficient protection against the arsenic has resulted in some modifications as well as in later complementary inventions. Most organisms are in fact equipped with these more recent adaptations, but although they are very similar in their function, they have evolved independently.

The widespread distribution of arsenic resistance genes implies that all now living organisms at some point in their evolution were frequently exposed to the metalloid, enough to evolve and maintain the detoxification systems. However, has arsenic affected the course of evolution in more ways than so? Before answering this question, it is necessary with an overview of some key evolutionary events in the history of life itself.

Evolution of life

Signs of early life

When did life on Earth begin? What are the first indications of life? These are questions to which so far no uncontroversial answers can be given. Carbon isotope data of sedimentary organic matter suggest possible life already at 3.8 Ga (Schidlowski et al., 1979; Schidlowski, 1988) although these signatures could have been formed abiotically (van Zuilen et al., 2002; McCollom & Seewald, 2006). In Australian sandstones, formed at 3.4 Ga, carbon isotope signals again indicate the presence of life, now also accompanied by sulphur isotope fractionation and microstructures interpreted as biological in origin (Wacey et al., 2011). From the same time and locality are also the oldest stromatolites known. Stromatolites are laminated structures formed by the accretion of sediments on microbial mats (Allwood et al., 2009), but it should be noted that their biological origin sometimes is questioned as they lack several morphological features recognised in modern cyanobacteria-formed ones (Brasier et al., 2002).

The oldest known microfossils come from the 3.4 Ga old Strelley Pool Formation in Australia (Awramik et al., 1983; Schopf & Packer, 1987) and have been interpreted as the cellular remains of sulphur-metabolising bacteria (Wacey et al., 2011). It has been speculated whether the first cyanobacteria would be of the same age due to the presence of stromatolites (Hofman et al., 1999), but chemical signals characteristic of cyanobacteria do not appear in the sediment record

until 2.7 Ga (Summons et al., 1999). A possible eukaryotic cell has been described from 2.7 Ga, although most regard the origin of eukaryotes to be closer to 1.6 Ga (e.g. Knoll, 2011). Therefore problematic are the findings of two macroscopic fossils of unknown affinity; some up to 12 cm long wrinkly organisms from 2.1 Ga old rocks in Gabon (El Albani et al., 2010), and the slightly younger coiled fossil known as *Grypania spiralis* (Han & Runnegar, 1992). Especially the former is of interest as the formation in which it makes its short appearance is one of those that has been sampled and analysed herein. The fossil has been interpreted as showing an organisation typical for multicellular organisms, thus triggered speculations about its possibly eukaryotic origin (El Albani et al., 2010). From the same basin in Gabon are the oldest steranes found, a chemical compound formed solely produced by eukaryotes (Dutkiewicz et al., 2007). However, despite the presence of steranes it is just as likely that the Gabon organisms are colonial instead of truly multicellular and bacterial instead of eukaryotic. It also cannot be excluded that the fossil is an abiotically formed structure (Knoll, 2011).

True multicellular organisms appear in the fossil record for the first time after the Gaskiers glaciation at 580 Ma (e.g. Canfield et al., 2007), and these are generally considered as animals and not as plants as the depth they inhabited would have prevented photosynthesis (Clapham & Narbonne, 2002). Trace fossils, calcified tubes and the remains of sponges represent the earliest first modern animals and are known from as early as 555 Ma. Shortly thereafter, in the lowermost Cambrian ~540 Ma, these early animals rapidly increased in numbers as well as diversity during the event commonly referred to as the Cambrian explosion (e.g. Shields-Zhou & Och, 2011).

When it comes to the first colonisation of land, molecular evidence grows in importance due to the lack of appropriate sedimentary deposits from the Precambrian that would preserve unequivocal land living life forms. Mosses, fungi, liverworts and various protists were likely to have been the first ones to leave the oceans for land, and probably had done so by 1.0 Ga (Knauth & Kennedy, 2009). Together with lichens and green algae, they could have invaded Rodinia to create the first inhabited, green supercontinent, with extensive effects upon the weathering patterns (Shields-Zhou & Och, 2011).

$\delta^{13}\text{C}$ as a proxy of biological activity

Variations in biological activity through time are usually recorded by carbon isotope ratios preserved in carbonates (C_{carb}) as well as in organic carbon (C_{org}) (e.g. Bekker et al., 2008). During times of increased productivity, the uptake of the lighter ^{12}C isotope will be intensified,

leaving the sediments enriched in the heavier ^{13}C . In modern oceans, C_{carb} and C_{org} are generally coupled because of the unstratified, oxidised waters. The redox-stratified oceans of the Precambrian, however, produced significantly different carbon isotope trends between these two sources (Bekker et al., 2008). The most extreme example might be the so-called Lomagundi Event, which took place between 2.2 and 2.1 Ga. During this time, the largest worldwide positive C_{carb} excursion ever recorded was produced in carbonates from shallow marine environments, while the C_{org} from deep marine depositions reveal no such positive anomaly but rather a negative one (Bekker et al., 2008).

When conducting a whole rock and kerogen analysis, the “normal” range for $^{13}\text{C}_{\text{org}}$ will be between -20 and -30. More negative readings of $\delta^{13}\text{C}$ are interpreted as reflecting an increase in biological activity, although the burial rate, oxidation and, in younger deposits, type of vegetation also will have an effect upon the carbon isotope ratios (Wallmann & Aloisi, 2012). Values between -25 and -30 are usually indicative of the activity of photosynthesising cyanobacteria, using the enzyme rubisco. If the $\delta^{13}\text{C}$ falls down to -40 or below, it supports the activity of methanogenic and/or methanotrophic bacteria (Rollinson, 2007).

Geological background

The 73 samples studied herein originate from four different localities in South Africa (30 samples), Gabon (36 samples), Canada (five samples), and India (two samples) ranging in age from 2.7 to approximately 1.6 Ga. Those from South Africa and Gabon came from drill cores, whereas samples from Canada and India were collected from outcrops. The material had been obtained by other research groups for different purposes, meaning that it was not specifically selected for this study but more depending on what was available. Details regarding when and by who the samples had been collected is unclear.

South Africa

The 30 samples from South Africa come from two different supergroups; the Transvaal and the Ventersdorp Supergroup. The Ventersdorp Supergroup reaches a maximum thickness of 8 km and consists of three groups, the Klipriviersberg, Platberg, and Pniel Group, with the latter divided into two formations based on their composition. While the younger Allandridge Formation is dominated by volcanic rocks, the older Bothaville Formation, to which the four oldest samples belong, consists of predominantly sedimentary deposits, including conglomerates, dolomites/carbonates, sandstones and shales (Fig. 2A). The environment in

which the Bothaville Formation was deposited is sometimes interpreted as alluvial plains with discharge levels varying between low and high (Buck, 1980; Wronkiewicz & Condie, 1990), but geochemical data has instead suggested the environment to be near-shore marine (Watanabe et al., 1997). The presence of stromatolites within the shale layers (Buck, 1980) also favours a marine setting, since non-marine stromatolites remained rare until the late Mesozoic (Buck, 1980 and references therein; note that Buck comes to a different conclusion regarding the environment). Using U-Pb dating, the age of the formation has been determined as 2699 ± 16 Ma (Wronkiewicz & Condie, 1990; Catuneanu & Eriksson, 2002).

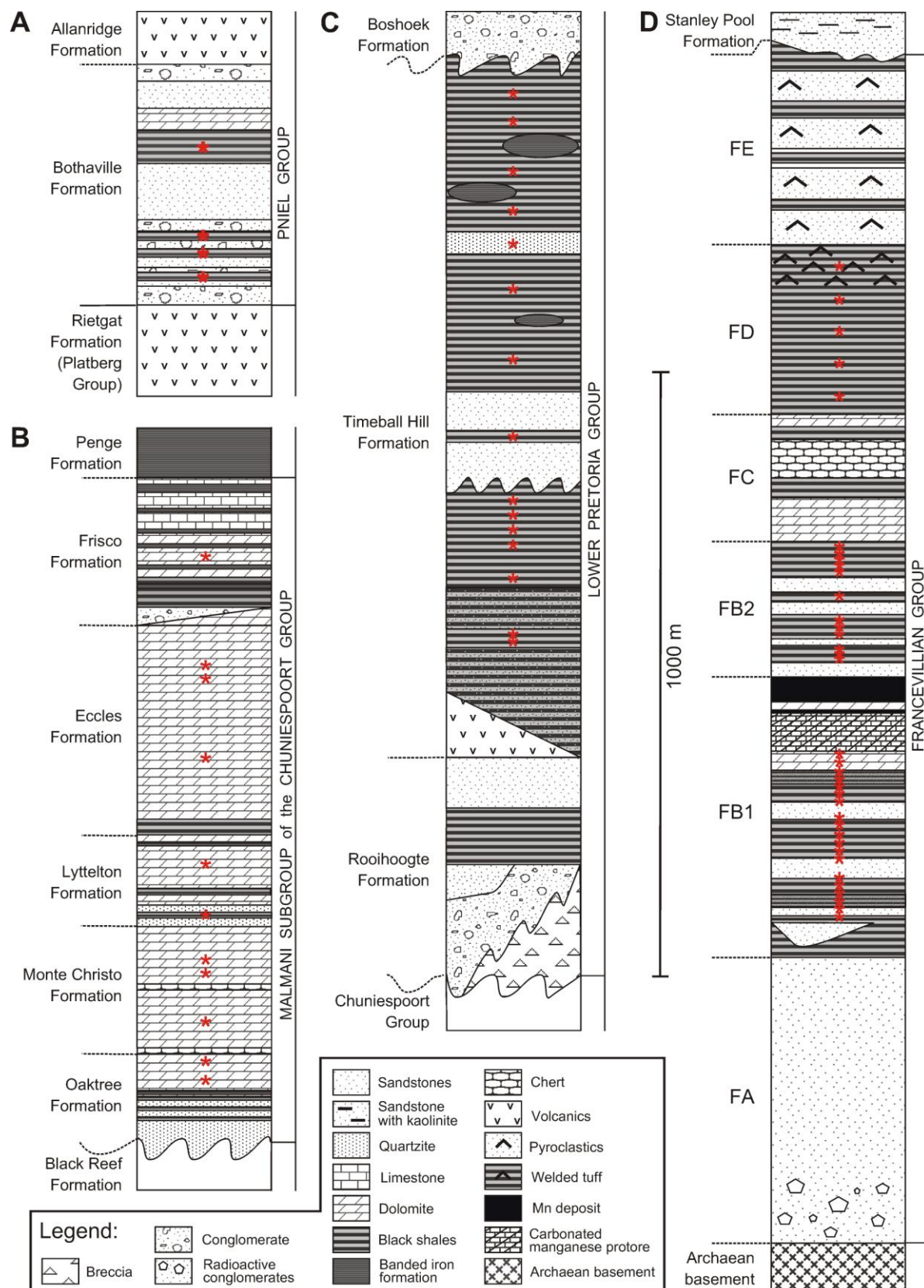


Figure 2 Generalised stratigraphy with red stars marking the approximated level of each sample. **A:** Bothaville Formation, Pniel Group, Ventersdorp Supergroup, South Africa **B:** The five formations of the Malmani Subgroup, Chuniespoort Group, Transvaal Supergroup, South Africa. **C:** Timeball Hill Formation, Pretoria Group, Transvaal Supergroup, South Africa. **D:** The five formations of the Francevillian Group, Gabon. Based on figures in Buck, 1980; Crow & Condie, 1988, Wronkiewicz & Condie, 1990; Tyler & Tyler, 1996; Catuneanu & Eriksson, 1999; Catuneanu & Eriksson, 2002; Gauthier-Lafaye, 2006, and sample reference information.

The remaining 26 South African samples come from the 12 km thick, relatively unmetamorphosed Transvaal Supergroup, which is subdivided into the Chunispoort Group and the younger Pretoria Group. The Chunispoort Group includes the Malmani Subgroup, the Penge Formation and the Duitschland Formation (e.g. Catuneanu & Eriksson, 2002). The Malmani Subgroup is about 2.0 km thick and from here, eleven samples were collected (see Fig. 2B). The supergroup consists of five formation of which the oldest, the Oaktree Formation, contains ferruginous dolomite, quartzite, and carbonaceous shale, while the overlaying Monte Christo Formation consists mainly of oolitic carbonates and chert-rich dolomites. This contrasts to the succeeding Lyttleton Formation, which, apart from shales and quartzites, is characterised by chert-poor dolomites. Next comes the Eccles Formation, which is again a chert-rich dolomite. A disconformity separates the Eccles Formation from the last part of the Malmani Subgroup, known as the Frisco Formation. Here, a variety of rock types is found; chert breccias at the bottom are followed by carbonaceous shales, dolomite and limestone. Iron depositions appear to have been a reoccurring phenomenon throughout this formation, while stromatolites have been found in all of the Malmani Subgroup formations (Eriksson & Clendenin, 1990). All carbonates from this location were formed in a subtidal environment by inorganic chemical precipitation, with most of them becoming dolomitised shortly thereafter (Eriksson et al., 1975).

The Pretoria Group is separated from the Chuniespoort Group by a disconformity representing about 80 Ma. The Rooihogte Formation marks the beginning of the Pretoria group and is interpreted to have been deposited in an alluvial environment. The overlaying Timeball Hill Formation instead has the characteristics of both deep and shallow marine environments (Fig. 2C). Here are laminated, carbonaceous shales progressing into coarser grained ferruginous mud, silt- and fine sandstones with various sedimentary structures. Higher up in the formation, shales are dominating, with intermissions of iron lenses and quartzite. The age of the Timeball Hill Formation has been determined as 2263 Ma, using the Rb-Sr dating method (Eriksson & Clendenin, 1990). The 15 samples from the Pretoria Group derive from the Timeball Hill Formation only.

Gabon

The 36 samples from Gabon come from the Francevillian Group, which consists of five different formations (Fig. 2D). The five formations, informally named FA to FE, stretch across the southeastern part of the country with a maximum thickness of 2.0 km (El Albani et al., 2010). Despite being over two billion years in age, the formation has undergone a minimal amount of metamorphose, leaving the sedimentary structures largely undeformed and presumably

chemically unaltered (Gauthier-Lafaye, 2006). The group rests upon the Archaean basement, with its youngest sequence, the FA formation, consisting of sandstones from coastal and onshore environments, sometimes intervened by fluvial deposits (El Albani et al., 2010).

The overlaying FB Formation can be further divided into two parts, FB1 and FB2, with the former having samples referred to as BA37 (19 samples), while samples from the latter are labelled Comilog (12 samples). Both parts of the formation are considered to be from a marine delta environment, but with some differences in composition. The older FB1 consists mainly of black shales, with occasional sandstone layers in between. Towards the upper part, dolomite has been precipitated before the formation ends with a layer of manganese deposits (El Albani et al., 2010). The FB1 Formation has been roughly dated as 2099 ± 115 Ma, using Sm-Nd isochrones (Gauthier-Lafaye, 2006). Also in the FB2 Formation are layers of black shales, but the dominating rock type is instead sandstone. This formation has been more precisely dated to 2100 ± 30 Ma, and it is within the black shale layers that the potential multicellular organisms have been found (El Albani et al., 2010). The FC Formation consists mainly of dolomite and chert, and is interpreted as representing a shallower environment (El Albani et al., 2010). From this formation, stromatolites as well as numerous microfossils have been reported (Bertrand-Sarfati & Potin, 1994). Next comes the FD Formation which is also the name of the samples from this succession (five samples). The FD Formation consists almost exclusively of black shale, except for its top where the shales are intercepted by layers of welded tuff. In the FE Formation, pyroclastic material frequently occurs within units of shales and sandstones. The deposition of these layers continued until the basin eventually was filled. The FD and FE formations have been dated to 2083 ± 6 Ma, using the U-Pb method (Gauthier-Lafaye, 2006).

Complementary samples

In addition to the samples from South Africa and Gabon, samples from localities in Canada and India were analysed for comparison. The five samples from Ontario, Canada come from the about 190 m thick Gunflint Formation, which is part of the Animikie Group. The formation consists of iron formations, carbonates and cherts, and was deposited between 1878.3 ± 1.3 Ma and 1849.53 ± 0.21 Ma in a back-arc basin (Poulton et al., 2004; Kendall et al., 2011). Upwelling of anoxic, ferruginous waters at this location resulted in the precipitation of iron formations once the deep water came in contact with the oxygenated surface layer in the mid to outer parts of the shelf. The source of the iron was likely hydrothermal and continuously delivered from the ocean, to which the basin stood in direct contact with (Poulton et al., 2004). All of these five samples were recognised as chert.

The two samples from India come from the 3 km thick Vindhyan Supergroup, found in the central area of the country. The Vindhyan Supergroup consists of four groups, each comprising several formations. Sandstone, limestone and shale are the dominating rock types throughout the section, representing depositional settings spanning from alluvial fans, eolian patches, deltas and lagoons to carbonate platforms and outer shelf environments (Chakraborty, 2006). The lower part of the Vindhyan sediments were formed 1650 ± 89 Ma, while the age of the upper part of the Supergroup has been determined to about 1000-1070 Ma (Bengtson et al., 2009). The samples herein were both shales that appeared to have been subjected to some weathering.

More precise details regarding the stratigraphic level and lithology were not available, neither for the Indian nor Canadian samples. As a consequence, they were excluded from the statistical analyses. However, it was decided that they should still be partly included in order to give an approximation about the arsenic levels during the Proterozoic, since this is the time period in which the arsenic levels are expected to have peaked. Note though that the margin of error for these samples is quite high due to their different lithology and/or having been exposed to weathering.

Methods

Whole rock digestion and analysis

In total, 73 samples were analysed, of which 36 derive from three formations in Gabon (BA37, Comilog and FD), 30 from seven formations in South Africa (TF 1/72), five from Canada and two from India. As controls, also two blank samples as well as two BCR-II geologic standards were prepared. The individual samples had already been powdered before they were received. Sample preparations (removing the carbon content and dissolving the samples) were carried out at the Swedish Museum of Natural History, while the chemical analyses were performed at the Department of Geological Sciences, Stockholm University, Sweden, with an ICP-OES (ICAP 6500 from Thermo with dual view). The detection limit was 1 ppb and the precision had a margin of error of 3-5%. All samples were measured three times and an average was calculated. NIST 1643e was used as the certified geologic standard to check the calibration of the ICP-OES.

In order to analyse a sample with an ICP-OES, the material has to be fully dissolved. To dissolve the material, including removing the carbon content, the following protocol was applied. About 0.2 g (mean weight 0.2014 ± 0.002 g, standard deviation 0.0007478) of each sample was transferred into a Teflon capsule specifically designed for rock dissolution. After weighing the

empty capsule as well as the capsule with the sample, a few drops of pure water were added to prevent escape of rock particles, although many appeared to be hydrophobic rather than hydrophilic. 20 drops of concentrated Seastar nitric acid (HNO_3) and 2 mL HF 40% were added to each capsule and placed on a heating plate at 150°C until dried. 1 mL concentrated Seastar HNO_3 was then added and the containers returned to the heating plate to dry once more, at 170°C. However, in those cases where there had been a visible reaction between the sample and the first 20 drops of nitric acid, no HF was added and the sample was instead put on the heating plate to evaporate with nitric acid only. When dry, 20 more drops of HNO_3 were added and first when no visible reaction could be detected, the 2 mL HF was added and the original protocol was resumed. In some cases, the additional nitric acid step had to be repeated up to four times.

Another 20 drops concentrated Seastar HNO_3 and 2 mL HF were added after the 1 mL HNO_3 had evaporated. Each capsule was tightly sealed with a lid and Teflon ring to prevent leakage. They were then placed inside steel canisters and left inside an oven at 205°C.

After three days, the steel canisters were taken out of the oven and left for a few hours to cool. The capsules were then removed from the steel canisters, had their Teflon rings and lids taken off, and left to dry for about two to three hours on a heating plate at 150°C. Once dry, 15 drops of concentrated Seastar HNO_3 and a small amount of ultrapure water were added to each capsule, which then was returned to the heating plate at the same temperature. When dry, 15 new drops of the nitric acid were added, and since all samples were fairly small, no additional water was needed in order to moisten the entire material. The samples were dried again at the same temperature, and again the same amount of nitric acid was added. In total, this was repeated four times, five if including the first round when also water was used. After the last repetition, 5 mL 6M ultrapure HCl was added, and the capsules were once more sealed with both lids and Teflon rings. They were then returned to the steel canisters and left in the oven over night at 205°C. The following morning the canisters were taken out from the oven. After they had cooled down, the capsules were opened and checked for undissolved material before placing them on the heating plate at 80°C underneath an IR-lamp until dry. Those that had been completely dissolved had 3.5 mL 6M Seastar HCl pipetted into them and were left with lids over night in room temperature. The following day, the solution was tipped into acid washed plastic containers. Another 0.5 mL 6 M Seastar HCl was pipetted into the Teflon-capsule to capture any potential leftovers, before it too was poured into the plastic container.

For samples still not being completely dissolved, some additional treatment was necessary. In these cases, 1 mL ultrapure water and 1 mL concentrated Seastar HNO_3 were added, after which

the samples were either left in the oven over night at 205°C, or left at room temperature for five days before placed in the oven over night. One sample received both treatments. Those that had dissolved could thereafter be pipetted with HCl according to the original procedure. However, despite these additional steps, there were still some samples with undissolved material. These were therefore treated with ten drops of perchloric acid (HClO_4), sealed with the lid and left over night on a heating plate at 100°C. The lids were then removed while the capsules remained on the heating plate with unchanged temperature until the material had dried. 15 new drops of the same acid were added and placed to evaporate on the heating plate at 165°C. When dry, 10 more drops were added and the temperature of the heating plate was raised to 190°C. Once no more smoke could be detected from the samples, 3.5 mL 6M Seastar HCl were pipetted into the capsules, and they were left over night in room temperature sealed with their lids.

Those samples that by the next morning had dissolved were left for the time being, while the one sample that still contained undissolved material had to be further treated. The capsule was placed on a heating plate for the sample to dry and then received 20 drops concentrated Seastar HNO_3 and 2 mL HF. When sealed and put inside a steel canister, the sample was left for three days in an oven at 205°C. It was thereafter dried again on a heating plate and 0.5 mL 8M Seastar HNO_3 and 2 mL 6 M Seastar HCl were added, forming the highly corrosive aqua regia. The capsule was then resealed and again placed inside a steel canister in the oven at 205°C. After three nights, it was removed but had still not completely dissolved, although the amount of undissolved matter had both decreased and changed colour. Despite the presence of undissolved material, it was dried one last time together with the other perchloric acid treated samples. The original protocol was then resumed for the final steps, where 5 mL HCl was added and the solution poured into the plastic containers (see above). All samples were thereafter diluted with 1% Seastar HNO_3 in series of x10, x100 and x1000. A margin of error of 1.5% was calculated.

The Teflon-capsules were acid washed between each new round of sample dissolving. After rinsing and some gentle scrubbing, they were placed in a beaker in a way that no air would get trapped. They were covered in 10% HCl and the beaker was placed on the heating plate at 90°C. After three days, the HCl was removed and each container individually rinsed. They were again positioned into the beaker, covered with 10% HNO_3 , and returned to the heating plate. After three more days at 90°C, the HNO_3 was removed and the capsules were rinsed once more, put back into the beaker, and immersed with ultrapure water. Every third day, the water was exchanged, and after the third change, the capsules were moved out of the beaker, rinsed and filled with ultrapure water. With the lid on, they were left on the heating plate at 90°C for three

days, and thereafter emptied and moved to an oven to dry at about 60°C. Once dry, the lid was immediately put on to prevent contamination.

The steel canisters were also acid washed between each use. After disassembling them, all parts were placed in a 10 L bucket with water and about two tablespoons of oxalic acid. After one night in the bath, every part was carefully rinsed and left to dry before assembled again.

Between five and twelve samples were dissolved simultaneously, taking between five days and one month per round, depending on the number of extra steps that had to be added.

Table 1 Chemical treatments and observations of the samples.

| Sample | Reaction with HNO₃ | Extra rounds of HNO₃ | Colour after HF evaporation | Extra rounds after HCl | HClO₄ | Aqua regia |
|---------------|--------------------------------------|--|-------------------------------------|-------------------------------|-------------------------|-------------------|
| FD B9 | Yes | 4 | Pale yellow | 2 | Yes | - |
| FD B16 | Yes | 4 | Beige | 2 | Yes | - |
| FD B26 | Yes | 3 | Pale purple-white | 2 | Yes | - |
| FD 1V 44 | Yes | 3 | Pale yellow | 2 | Yes | - |
| FD 1V 64 | Yes | 3 | Pale beige-white | 2 | Yes | - |
| Comilog 40 | No | - | Very pale yellow | - | - | - |
| Comilog 48 | Yes | 1 | Dark purple | - | - | - |
| Comilog 63 | No | | | - | - | - |
| Comilog 85 | No | | | - | - | - |
| Comilog 100 | Yes | 1 | Pale burgundy | - | - | - |
| Comilog 117 | Yes | 1 | Dark purple | - | - | - |
| Comilog 127 | No | | | - | - | - |
| Comilog 134 | No | | | - | - | - |
| Comilog 140 | No | | | - | - | - |
| Comilog 163.1 | Yes | 1 | Yellow-green, with tiny "fibres" | - | - | - |
| Comilog 165.5 | Yes | 1 | Pale pink-red | - | - | - |
| Comilog 173.8 | No | | | - | - | - |
| BA37 29.2 | No | - | White, pale purple around the edges | - | - | - |
| BA37 34.4 | Yes | 1 | Purple | - | - | - |
| BA37 36.6 | No | - | White, purple around the edges | - | - | - |
| BA37 40.1 | Yes | 1 | Dark grey, very much | 1 | - | - |

| Sample | Reaction with HNO ₃ | Extra rounds of HNO ₃ | Colour after HF evaporation | Extra rounds after HCl | HClO ₄ | Aqua regia |
|------------------------|--------------------------------|----------------------------------|---|------------------------|-------------------|------------|
| BA37 44.18 | Yes | 1 | organic matter Ash grey-blue, much | 1 | - | - |
| BA37 51.1 | No | - | organic matter White, pale purple around the edges | - | - | - |
| BA37 55.1 | No | - | White | - | - | - |
| BA37 62.45 | No | - | White | - | - | - |
| BA37 68.55 | Yes | 1 | Ash grey | 1 | - | - |
| BA37 76.75 | No | - | White, pale yellow around edges | - | - | - |
| BA37 80.1 | No | - | White | - | - | - |
| BA37 84.75 | No | - | Pale blue-green | - | - | - |
| BA37 90.2 | No | - | White | - | - | - |
| BA37 93.3 | No | - | White | - | - | - |
| BA37 100.10 | No | - | White | - | - | - |
| BA37 105.1 | No | - | White | - | - | - |
| BA37 114.5 | No | - | White | - | - | - |
| BA37 133.6 | No | - | White | - | - | - |
| BA37 162.8 | No | - | White | - | - | - |
| TF 1/72 214.4 | No | 1 | Pale turquoise-white | - | - | - |
| TF 1/72 370 | No | 1 | White, very pale turquoise | - | - | - |
| TF 1/72 490 | No | 1 | White, very pale turquoise | - | - | - |
| TF 1/72 711.4 | No | 1 | Dark grey-black | 3 | Yes | Yes |
| TF 1/72 1066.95 | No | 1 | White | 2 | Yes | - |
| TF 1/72 1434.5 | No | 1 | Very pale turquoise-white | - | - | - |
| Sc2007-07 (Canada) | No | - | Yellow-green | - | - | - |
| Sc2007-07b (Canada) | Minor | - | Rusty brown | - | - | - |
| Sc2007-08 (Canada) | Yes | - | Yellow-green | - | - | - |
| Sc2007-09 (Canada) | No | - | Pale white-grey | - | - | - |
| Sc2007-10 (Canada) | Minor | - | Rusty brown-red | - | - | - |
| 404111306 | Yes | - | White | - | - | - |

| Sample | Reaction with HNO ₃ | Extra rounds of HNO ₃ | Colour after HF evaporation | Extra rounds after HCl | HClO ₄ | Aqua regia |
|-----------|--------------------------------|----------------------------------|-----------------------------|------------------------|-------------------|------------|
| (India) | | | | | | |
| 06111307 | Yes | - | White | - | - | - |
| (India) | | | | | | |
| Control 1 | No | - | - | - | - | - |
| Control 2 | No | - | - | - | - | - |
| BCR-2 (1) | No | - | Pale green | - | - | - |
| BCR-2 (2) | No | - | Very pale green | - | - | - |

Carbon isotope analysis

For this analysis, 6 M HCl was added to about 0.2 g of pulverised shale and left at room temperature over night. The solution was centrifuged and washed thoroughly with ultrapure MQ-H₂O. 45% HF was then added to the samples, and left to dissolve at room temperature. The samples were washed ten more times in UP MQ-H₂O. A Finnigan MAT Delta V mass spectrometer was used together with a Carlo Erba NC2500 analyser to perform the stable carbon isotope analysis. The relative error was determined to be <1%. Analysis for total inorganic carbon (TIC) and total organic carbon (TOC) was conducted according to established procedures used for shales (Eigenbrode et al., 2008). The samples from Gabon were analysed at the Southern University of Denmark in collaboration with Dr. Emma Hammarlund, while the samples from the other localities were analysed in collaboration with Dr. Ernest Chi Fru at the Natural History Museum of Stockholm, Sweden. Carbon isotope data was used as a proxy for biological activity.

Iron and sulphur speciation analysis

Iron and sulphur speciation analysis was conducted according to an established protocol (Poulton & Canfield, 2005) at the University of Southern Denmark in collaboration with Dr. Emma Hammarlund. The results were used to determine under which redox conditions the analysed material had been deposited.

Statistical analysis

The geochemical data was statistically tested in several ways in order to determine the type of distribution, identify outliers, and verify correlations between arsenic and other elements.

The Shapiro-Wilk test is commonly used to test whether the distribution is normal if the number of samples is less than 50, although it can be used for sample sizes as big as 2000. The Kolmogorov-Smirnov test can be used as an alternative in those cases where there are a larger number of samples available. Many statistical tests require a normal distribution, but for geochemical data, the distribution is often heavily skewed and even logarithmic rather than normal (McKillup & Dyar, 2010). Therefore, it is necessary to know whether the distribution is normal or not before proceeding with further testing.

The redox conditions in the palaeo-oceans are usually determined either by using redox sensitive elements (e.g. molybdenum, vanadium or uranium) (Och & Shields-Zhou, 2012) or by looking at iron minerals (Poulton & Canfield, 2011). Herein, the latter method was chosen, where reactive iron and total iron (FeHR/FeTOT) and pyrite and reactive iron (FePy/FeHR) data kindly provided by Dr. E. Hammarlund were used to define whether the water was oxygenated, euxinic or ferruginous. A FeHR/FeTOT ratio below 0.38 indicates oxygenated bottom conditions, while anoxic conditions are characterised by higher ratios. If the conditions were anoxic, also the ratio of FePy/FeHR was applied to determine whether the bottom waters were ferruginous or sulphidic. A ratio above 0.8 is indicative of euxinia (Reuschel et al., 2012). FeHR/FeTOT and FePy/FeHR data was only available for samples from the Francevillian Group.

Data normalisation

Arsenic was normalised against a number of elements in order to find correlations and relationships that might help describe its behaviour and possible sources through time. A simple scatter plot of arsenic levels against the arsenic to element ratios (both in molar) was applied and the R^2 -value, which is a measure of how the points are distributed around the linear trend line, was used as an estimation of how much an element was able to influence the variations of arsenic.

Correlation analysis

The correlation between elements was tested using Spearman's rank correlation. Advantages with Spearman's rank correlation are that it does not require a normal distribution, can detect correlations other than linearity, and is able to handle outliers (McKillup & Dyar, 2010). A two-tailed test was selected over a one-tailed test, as the direction of any correlation was unknown. All samples were included in the first analysis, except the Canadian and Indian samples for reasons already mentioned.

Results

General behaviour of arsenic

Plots of arsenic molar concentrations over time suggest that arsenic levels increased by several factors between 2.7 and 2.0 Ga (Fig. 3A). This pattern is highlighted even further when condensing age equivalent samples into distinct groups and plotting them against their average molar concentration (Fig. 3B). Samples from Canada and India were excluded from the analysis because of unclear depositional environment and extensive weathering, respectively.

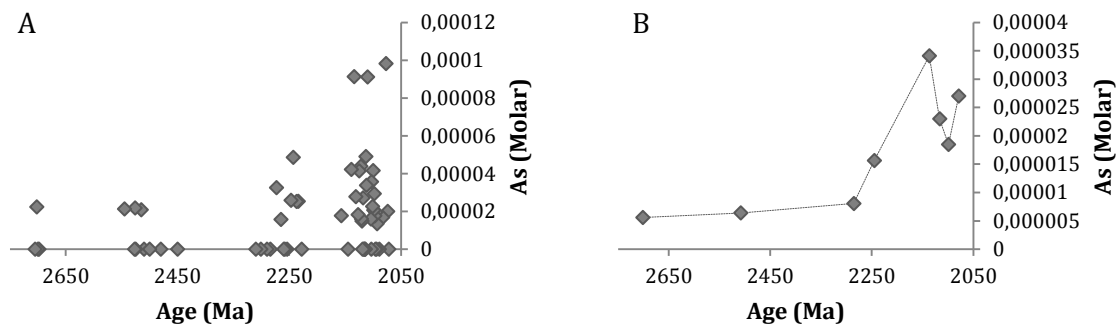


Figure 3 Variation of arsenic through time. Samples with arsenic below detection limit (DL) were set as 0. **A.** Variations of arsenic (in molar) over time for samples from South Africa and Gabon with ages between 2.7 and 2.05 Ga. **B.** Same data as in (A) but divided into age-based groups for which the average arsenic content has been calculated in order to give a better overview.

A summary of the concentration of a few key elements as well as their abundance in various types of sedimentary rocks according to literature data is presented in Table 2.

Table 2 Ranges and means of key elements in the three Gabon formations (FD, Comilog and BA37) as well as in the samples from South Africa (TF 1/72). For comparison, also literature data with concentrations from various mainly sedimentary rocks are presented (from Mason & Moore, 1982). min = lowest amount detected, max = highest amount detected, <DL = below detection limit, N = number of samples.

| | | Al | | As | | Ca | | Fe | | S | |
|--------------------------|------|--------|------|-------------|---------|--------|------|-------|-------|-------|------|
| | | Molar | mg/k | Molar | mg/kg | Molar | mg/k | Molar | mg/kg | Molar | mg/k |
| | | | g | | | | g | | | | g |
| FD (N=5) | min | 0.0394 | 2648 | <DL | <DL | 0.0030 | 3070 | 0.010 | 14470 | 0.002 | 1956 |
| | | 3 | 0 | (1.683E-05) | (31.38) | 91 | | 41 | | 80 | |
| | max | 0.2300 | 1542 | 9.824E-05 | 182.4 | 0.0676 | 6747 | 0.051 | 71580 | 0.002 | 3071 |
| | | | 00 | 05 | | 4 | 0 | 73 | | 614 | |
| | Mean | 0.1550 | 1039 | 2.701E-05 | 50.20 | 0.0207 | 2071 | 0.027 | 38800 | 0.002 | 2271 |
| | | | 00 | 05 | (83.67) | 8 | 0 | 99 | | 851 | |
| | | | | (4.502E-05) | | | | | | | |
| | <DL | - | | 2 | | - | | - | | - | |
| Comilog (N=11) | min | 0.0009 | 641. | <DL | <DL | 0.0053 | 5370 | 0.007 | 10930 | 0.003 | 2404 |
| | | 581 | 8 | (1.598E-05) | (29.72) | 94 | | 883 | | 020 | |
| | max | 0.1522 | 1020 | 4.160E-05 | 76.66 | 0.2642 | 2629 | 0.122 | 16770 | 0.143 | 1129 |
| | | | 00 | 05 | | | 00 | 8 | 0 | 9 | 00 |

| | | | | | | | | | | | |
|-------------------------------------|-----------------------|--------------|------------|----------------------------------|------------------|---------------|------------|--------------|------------|---------------|------------|
| | Mean | 0.0673 5 | 4505 0 | 1.848E- 05 (2.541E- 05) | 34.25 (47.10) | 0.0780 | 7763 0 | 0.033 77 | 46540 | 0.044 40 | 3514 0 |
| BA37 (N=19) | <DL | - | | 3 | | - | | - | | - | |
| | min | 0.0534 4 | 3578 0 | <DL (1.490E- 05) | <DL (27.82) | 0.0009 474 | 942. 2 | 0.002 369 | 3301 | 0.001 686 | 1344 |
| | max | 0.2964 | 1984 00 | 9.132E- 05 | 170.0 | 0.0477 1 | 4749 0 | 0.062 78 | 87170 | 0.067 61 | 5374 0 |
| | Mean | 0.1698 | 1137 00 | 2.709E- 05 (3.960E- 05) | 50.4 (73.64) | 0.0092 39 | 9190 | 0.028 02 | 38900 | 0.013 93 | 1110 0 |
| TF 1/72 (N=28) | <DL | - | | 6 | | - | | - | | - | |
| | min | 0.0053 59 | 441. 5 | <DL (1.574E- 05) | <DL (29.48) | 0.0010 53 | 1056 | 0.001 466 | 395 | 0.001 181 | 946. 5 |
| | max | 0.3153 | 2127 00 | 6.287E- 05 | 117.8 | 0.2567 | 1296 00 | 0.111 0 | 15490 0 | 0.155 0 | 1242 00 |
| | Mean | 0.1519 | 1016 00 | 1.152E- 05 (2.933E- 05) | 21.56 (54.87) | 0.0249 2 | 1589 0 | 0.038 92 | 54060 | 0.011 47 | 9127 |
| TF 1/72 >2.3 Ga (N=16) | <DL | - | | 17 | | - | | - | | - | |
| | Min | 0.0053 59 | 441. 5 | <DL (2.086E- 05) | <DL (38.86) | 0.0016 95 | 1698 | 0.001 466 | 395 | 0.001 823 | 1053 |
| | Max | 0.3153 | 2127 00 | 6.287E- 05 | 117.8 | 0.2567 | 1296 00 | 0.111 0 | 15490 0 | 0.155 0 | 1242 00 |
| | Mean | 0.1425 | 9474 0 | 9.326E- 06 (2.985E- 05) | 17.46 (55.86) | 0.0439 5 | 2812 0 | 0.038 15 | 52790 | 0.013 92 | 1107 0 |
| | <DL | - | | 11 | | - | | - | | - | |
| Lit. data | Shales | 0.1186 | 8000 0 | 6,9406E- 06 | 13 | 0,0220 6 | 2210 0 | 0.033 81 | 47200 | 0.002 994 | 2400 |
| | Sand- stones | 0.0370 6 | 2500 0 | 5.339E- 07 | 1 | 0.0390 2 | 3910 0 | 0.007 019 | 9800 | 2,994 E-04 | 240 |
| | Carbo- nates | 0.0062 27 | 4200 | 5.339E- 07 | 1 | 0.3017 | 3023 00 | 0.002 721 | 3800 | 0.001 497 | 1200 |
| | Igneo- us rocks | 0.1205 | 8130 0 | 9.623E- 07 | 1.8 | 0.0362 3 | 3630 0 | 0,035 81 | 50000 | 3.244 E-04 | 260 |
| | | | | | | | | | | | |

Arsenic plotted against arsenic/iron ratios revealed two distinct correlations, possibly due to two different pools of either arsenic or iron (Fig. 4A). The expected correlation between arsenic and sulphur could, however, not be confirmed (Fig. 4B). Higher R²-values were observed between arsenic and several other elements of which aluminium and silicon were the most interesting (Fig. 4C-D).

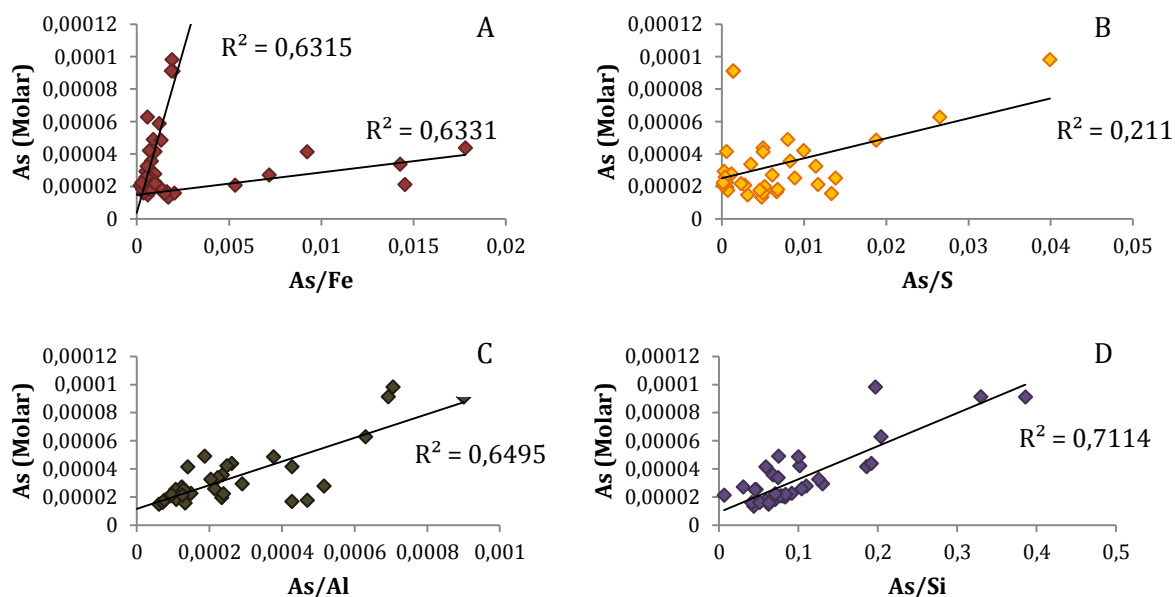


Figure 4 Arsenic normalised to iron (A), sulphur (B), aluminium (C), and silicon (D). Two outliers have been removed in D. The closer to the trendline that the points are distributed, the higher influence does the element exert over arsenic.

Both the Shapiro-Wilk test and the Kolmogorov-Smirnov test frequently gave a p-value below 0.05 (Table 3), meaning the distribution of elemental concentrations deviated significantly from a normal distribution with only a few exceptions; aluminium (according to both tests), and zirconium (according to the Kolmogorov-Smirnov test). The Kolmogorov-Smirnov test suggested that also lead was normally distributed, but as there were only 33 samples containing lead, it was instead the result from the Shapiro-Wilk test that was relevant. Hence, neither lead had a normal distribution. Indeed, if plotting the distribution of all elements, the recurring pattern was not a symmetrical bell curve, but rather a strongly positively skewed curve, often reminding more of a logarithmic distribution than a normal one.

Table 3 Normality tests (Kolmogorov-Smirnov and Shapiro-Wilk) of the elements in all analysed samples. Here, the normality of molar concentrations of the elements is shown. * = Lower bound of the true significance.

| | Kolmogorov-Smirnov | | | Shapiro-Wilk | | |
|-----------|--------------------|----|-------|--------------|----|------|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Al | .051 | 70 | .200* | .980 | 70 | .337 |
| As | .177 | 40 | .003 | .798 | 40 | .000 |
| Ba | .261 | 67 | .000 | .553 | 67 | .000 |
| Ca | .300 | 70 | .000 | .561 | 70 | .000 |
| Co | .283 | 46 | .000 | .619 | 46 | .000 |
| Cr | .137 | 66 | .004 | .921 | 66 | .000 |
| Cu | .319 | 40 | .000 | .706 | 40 | .000 |
| Fe | .166 | 70 | .000 | .892 | 70 | .000 |
| K | .202 | 69 | .000 | .883 | 69 | .000 |

| | | | | | | |
|-----------|------|----|-------|------|----|------|
| Mg | .246 | 70 | .000 | .669 | 70 | .000 |
| Mn | .450 | 63 | .000 | .325 | 63 | .000 |
| Mo | .370 | 14 | .000 | .581 | 14 | .000 |
| Na | .163 | 69 | .000 | .912 | 69 | .000 |
| P | .294 | 69 | .000 | .509 | 69 | .000 |
| Pb | .107 | 33 | .200* | .925 | 33 | .025 |
| S | .328 | 70 | .000 | .551 | 70 | .000 |
| Si | .275 | 70 | .000 | .493 | 70 | .000 |
| Sr | .121 | 60 | .029 | .917 | 60 | .001 |
| Ti | .161 | 67 | .000 | .886 | 67 | .000 |
| V | .139 | 61 | .005 | .885 | 61 | .000 |
| Zn | .303 | 64 | .000 | .533 | 64 | .000 |
| Zr | .085 | 63 | .200* | .881 | 63 | .000 |

Using Spearman's rank correlation, significant correlations could be identified between several elements (see Table 4 for summary or Appendix 2 for details). For arsenic, strong positive correlations ($p < 0.01$) were found when tested against chromium, titanium, vanadium and zirconium, while iron and potassium yielded less strong but still significant correlations ($p < 0.05$), both positive. Arsenic did not correlate with sulphur as had been predicted, but this might be a consequence from running the test on all samples together, regardless of their depositional environment. Therefore, the data set needed to be further divided into groups allowing the environment specific behaviour of arsenic to be detected.

Table 4 Summary of the Spearman correlation analysis for As, Fe and S. N = number of samples. For the complete test result, see Appendix 2.

| | | | As | Fe | S |
|----------------------|--------|------|---------------|-----------|------------|
| All (N=65) | p<0.01 | pos. | Cr, Ti, V, Zr | P, Zn | Ca, Mg, Mn |
| | | neg. | - | - | K |
| | p<0.05 | pos. | Fe, K | As, Na | P, Zn |
| | | neg. | - | - | Al, Zr |

As described in previous chapters (see *Arsenic*), arsenic is known to respond differently to different redox conditions. Mainly determining the behaviour of arsenic is the presence of free oxygen, iron and/or sulphur, meaning that there in total would be three different redox settings; ferruginous, euxinic, and oxygenated. Therefore, by dividing the samples into groups based on which of these settings the material of a specific sample would have been deposited under, it is possible to reveal correlations that were masked in the initial Spearman's rank correlation.

Arsenic changes in response to redox conditions

The FD samples all have FeHR/FeTOT ratios above 0.38, and can thereby be considered as having been deposited under anoxic conditions. The ratios of FePy/FeHR are higher than 0.8 for four of the five samples (80%), showing that the bottom waters were mainly sulphidic.

The Comilog samples display, with only one exception, high ratios of FeHR/FeTOT suggesting constant anoxic conditions. Also the FePy/FeHR ratios are generally high, but with several exceptions. About half of the Comilog samples have a FePy/FeHR ratio >0.77 , with a mean of 0.83. The mean for all Comilog samples, however, is 0.67. For these reasons, the bottom waters are interpreted as to have fluctuated between euxinic and ferruginous.

Of the BA37 samples, 33.3% have FeHR/FeTOT ratios above 0.38, while the remaining 66.7% falls below this limit. The bottom waters thereby appear to have been occasionally oxygenated. The ratios of FePy/FeHR for those samples representing anoxic conditions are all significantly lower than 0.8, leading to the conclusion that the waters were ferruginous instead of sulphidic.

FeHR/FeTOT and FePy/FeHR data were not available for the TF 1/72 samples from South Africa. Considering their age, spanning from approximately 2.7 to 2.2 Ga, they were likely deposited during anoxic and ferruginous conditions. However, as this cannot be confirmed, only samples older than 2.4 Ga (i.e. deposited before GOE) are included in the correlation analysis of anoxic ferruginous samples. Younger samples from this locality are thus temporarily excluded.

In total, of the 65 samples, 36.9% were sorted as definitely ferruginous, 23.0% as coming from oxygenated waters, and at least 7.7% (possibly 13.8%) as being euxinic. 23.0%, all of South African origin, could not be confidently placed in any group, thus only included when running analysis on South African samples alone. Two samples, representing 3%, were excluded.

A Spearman correlation analysis of the elements from the samples representing anoxic ferruginous conditions (24 in total, of which 12 from Gabon and 12 from South Africa) (Table 5, Appendix 3) showed a strong positive correlation (significant at the 0.01 level in a 2-tailed analysis) between arsenic and zirconium. Positive correlations with arsenic (significant at the 0.05 level in a 2-tailed analysis) were found also for aluminium, barium and titanium. The expected correlation between arsenic and iron could not be confirmed; the only element correlating with iron was phosphorous.

Worth noting is the difference in the number of samples containing detectable amounts of arsenic – of the 24 ferruginous samples, eleven contained the metalloid (equal to 45.8%). Of these eleven, seven came from the younger Gabon section, resulting in an arsenic prevalence of 58.3%. This contrasts to what is observed in the older South Africa shales, where arsenic is detected in 33.3% of the samples. Therefore, a Spearman correlation analysis was run again including only the ferruginous Gabon samples (Table 5, Appendix 5). Arsenic and aluminium were now found to have a strong positive correlation, while the positive correlation with titanium remained unchanged. Also a positive correlation with potassium was found. The previously seen correlations with zirconium and barium had, however, disappeared. Due to these changes, another Spearman correlation analysis was run, this time on all of the samples from South Africa (Table 4, Appendix 4). Of the 28 samples, ten (35.7%) had detectable levels of arsenic. The elements now correlating with arsenic were, interestingly, not the same as in the Gabon samples. Instead, arsenic showed a strong positive correlation with cobalt (significant at the 0.01 level in a 2-tailed analysis), while positive correlations were recognised between arsenic and iron, vanadium, and zirconium (significant at the 0.05 level in a 2-tailed analysis).

When running a Spearman's rank correlation on samples identified as coming from oxygenated waters (15 in total, all from Gabon), arsenic correlates (on the 0.05 level) with sulphur, copper and vanadium, also here constantly positively (Table 5, Appendix 6). Likely, copper and vanadium are giving spurious correlations, while sulphur is the element controlling both these and arsenic. Arsenic was detected in 13 of the 15 samples, equalling 86.7%.

Nine samples were interpreted as having been deposited under euxinic conditions, however, only five of which are unambiguously euxinic. As it is not meaningful to run a correlation test on only five samples, the decision was made to also include the four more questionable samples. Still, also nine is a very low number and the results from the Spearman correlation analysis should be treated with caution, especially since only five of these nine samples included detectable concentrations of arsenic. Strong positive correlations (significant at the 0.01 level) were found between arsenic and chromium and vanadium. Positive correlations (significant at the 0.05 level) were identified between arsenic and iron, potassium, phosphorous and strontium. Also a negative correlation was seen between arsenic and calcium (Table 5, Appendix 7). Noteworthy is that iron correlates with all elements also correlating with arsenic, with chromium as the only exception.

Table 5 Summary of the Spearman correlation analysis of As, Al, Fe and S under ferruginous, oxygenated and euxinic conditions, respectively. The samples representing ferruginous conditions have been tested all together as well as divided up in separate groups depending on their origin. Ferr. = ferruginous conditions, Ox. = oxygenated conditions, Eux = euxinic conditions, TF 1/72 = South African samples, N = number of samples. For the complete result, see Appendix 3-7.

| | | | As | Al | Fe | S |
|------------------------------|--------|------|--------------|---------------|----------------------|---------------|
| Ferr. (N=24) | p<0.01 | pos. | Zr | Ba, K, Ti | P | C, Mn, P |
| | | neg. | - | - | - | Cr, Si |
| | p<0.05 | pos. | Al, Ba, Ti | As, Cr, Zr | - | Ca |
| | | neg. | - | Ca | - | K, Ti |
| TF 1/72 (N=28) | p<0.01 | pos. | Co | Ba, Ti | P, Zn | - |
| | | neg. | - | Ca | - | - |
| | p<0.05 | pos. | Fe, V, Zr | K, V, Zr | As, Co, Na | Ca, Cu |
| | | neg. | - | Mn | - | K |
| Ferr. Gabon (N=12) | p<0.01 | pos. | Al | As, Ba, K, Ti | - | Mn, P |
| | | neg. | - | - | - | - |
| | p<0.05 | pos. | K, Ti | - | P | - |
| | | neg. | - | - | - | - |
| Ox. (N=15) | p<0.01 | pos. | - | - | P | Co, Cu |
| | | neg. | - | Co, Zn | - | - |
| | p<0.05 | pos. | Cu, S, V | Ba | Ca, Mn, Zn | As, V |
| | | neg. | - | Mn, S | Cr | Al, Ba, P, Sr |
| Eux. (N=9) | p<0.01 | pos. | Cr, V | K, Na, Ti | - | - |
| | | neg. | - | - | - | - |
| | p<0.05 | pos. | Fe, K, P, Sr | Fe | Al, As, Ba, K, Ti, V | Mg |
| | | neg. | Ca | - | - | - |

Normalisation of arsenic

Based on the Spearman's rank correlation together with the known chemical behaviour of arsenic, it is concluded that aluminium, sulphur and iron have the strongest effects upon the metalloid, depending on the redox state of the ocean. Despite the comparably high R²-value seen when arsenic was plotted against the As/Si ratio (Fig. 4D), a correlation between these two elements could not be confirmed. Therefore, only aluminium, sulphur and iron were chosen for normalisation.

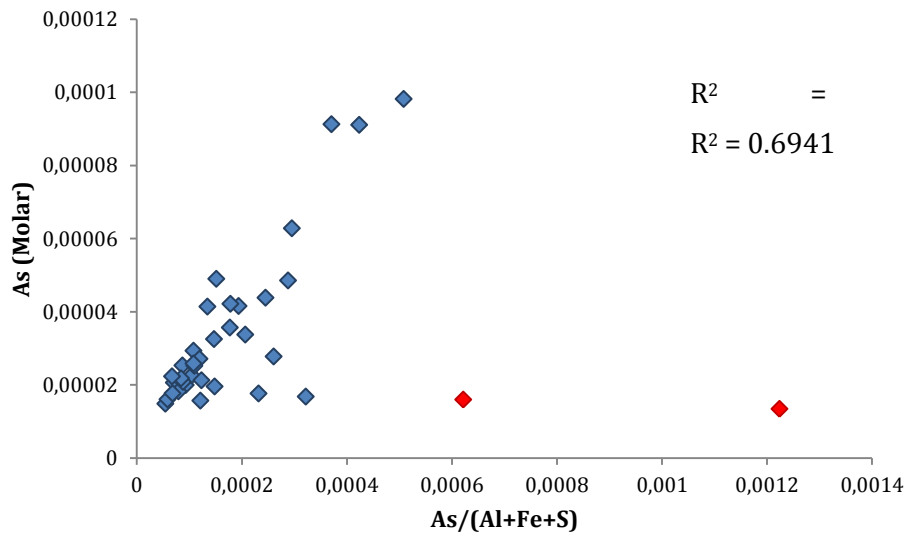


Figure 5 As (in molar) versus As normalised against Al, Fe and S. Outliers are marked in red and the two R^2 -values represent the correlation with (0.0551) and without (0.6941) the outliers included.

Despite the normalisation of arsenic against aluminium, iron and sulphur (Fig. 5), the R^2 -value is only 0.0551 due to two outliers. One of them belongs to Comilog 40, which might be a problematic sample since ten out of 22 elements (45%) failed to reach the detection limit. Thus, Comilog 40 is removed from the data set. The other outlier is traced back to BA37 173.8, which is one of the samples identified as coming from a dolomite rich sequence. If also calcium is included as a normalising element, the distribution improves significantly, having an increased R^2 -value of 0.8518 (including BA37 173.8, excluding Comilog 40) (Fig. 6).

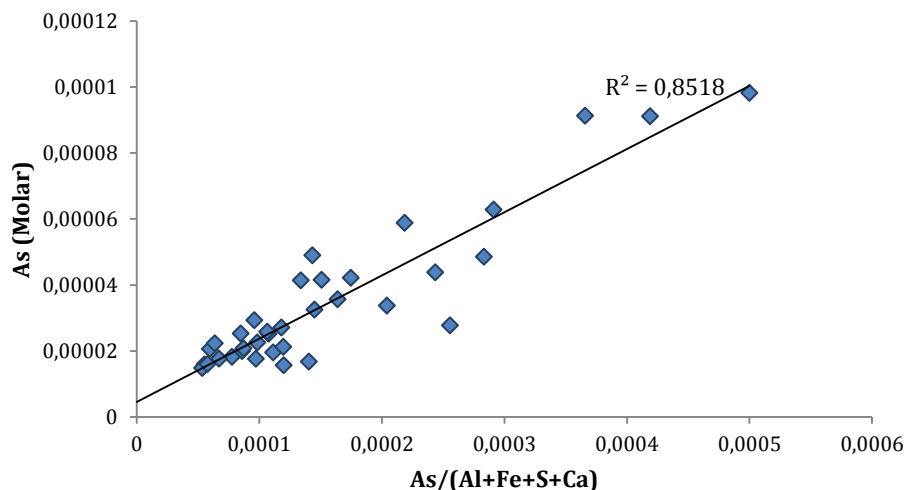


Figure 6 As (in molar) normalised against Al, Fe, S and Ca. One outlier (Comilog 40) removed.

There are reasons to suspect that also organic carbon might influence the arsenic levels, but since not all samples were analysed for carbon content, it could not be used for normalisation.

As shown, changes in the redox conditions affected which elements would have had the strongest effects upon arsenic, partly due to how the availability of these elements were changed (Fig. 7A-D). The iron levels generally decreased over time, whereas sulphur levels instead increased. Aluminium, on the other hand, appears to have remained on rather constant levels, while calcium decreases at about 2.3 Ga before suddenly peaking at about 2.1 Ga. Likely, this is reflecting the solubility and precipitation rates of calcium (carbonates), which depends on the water temperature, rather than the redox conditions.

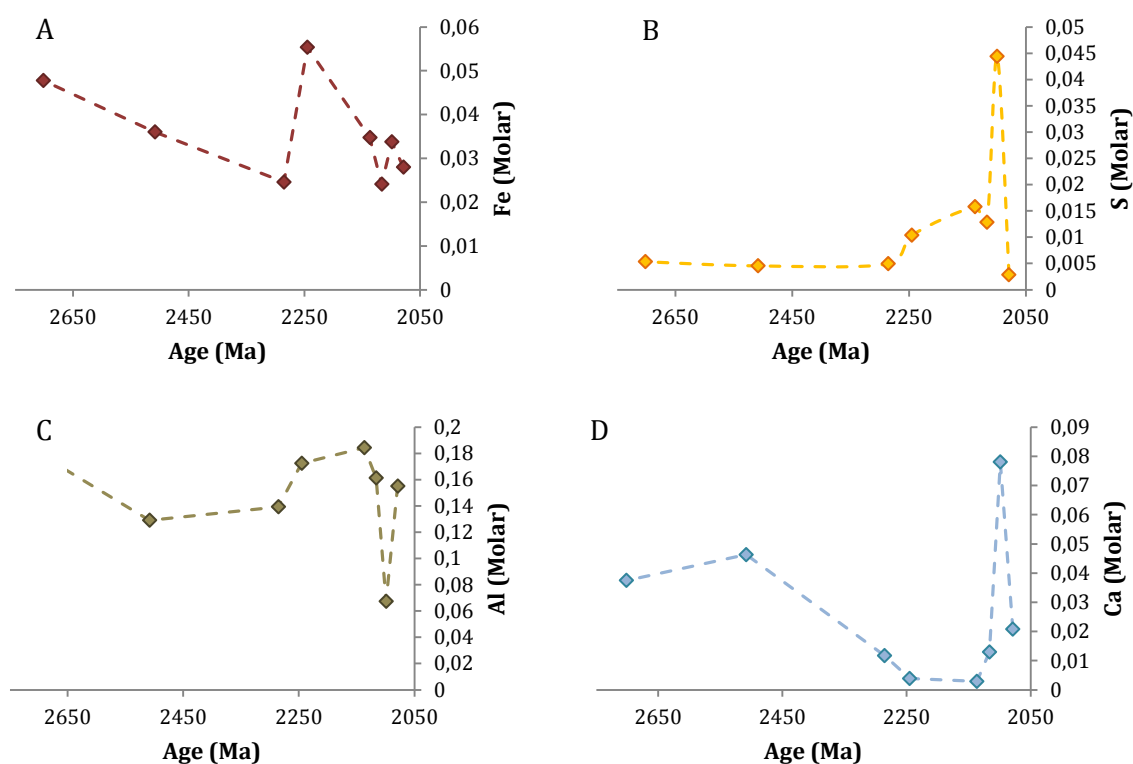


Figure 7 Dynamics of key elements with a strong impact on arsenic through time. Variations of (A) iron, (B) sulphur, (C) aluminium, and (D) calcium.

Considering the changes in the abundance of these elements, it seems fair to expect their influence upon arsenic would change accordingly. Indeed, this change in dependency becomes clear when normalising arsenic to one element at the time and plotting it against age (Fig. 8A-D, to be compared with Fig. 7A-D). Over all, the plots confirm the results of the Spearman's rank correlation. Iron appears to have continuously exerted a strong influence on arsenic through time, although as it got less abundant in the oceans, it also lost in importance (Fig. 8A). The

opposite trend is seen for sulphur, whose influence instead increased with time (Fig. 8B). The plot for arsenic normalised against aluminium over time (Fig. 8C) could be interpreted as aluminium gradually losing in importance, but considering how the aluminium levels were fairly stable throughout this time period, the trend might more reflect the change in weathering patterns rather than aluminium as a controlling element. Which influence calcium had upon arsenic (Fig. 8D) is as unclear as the changes in calcium levels over time.

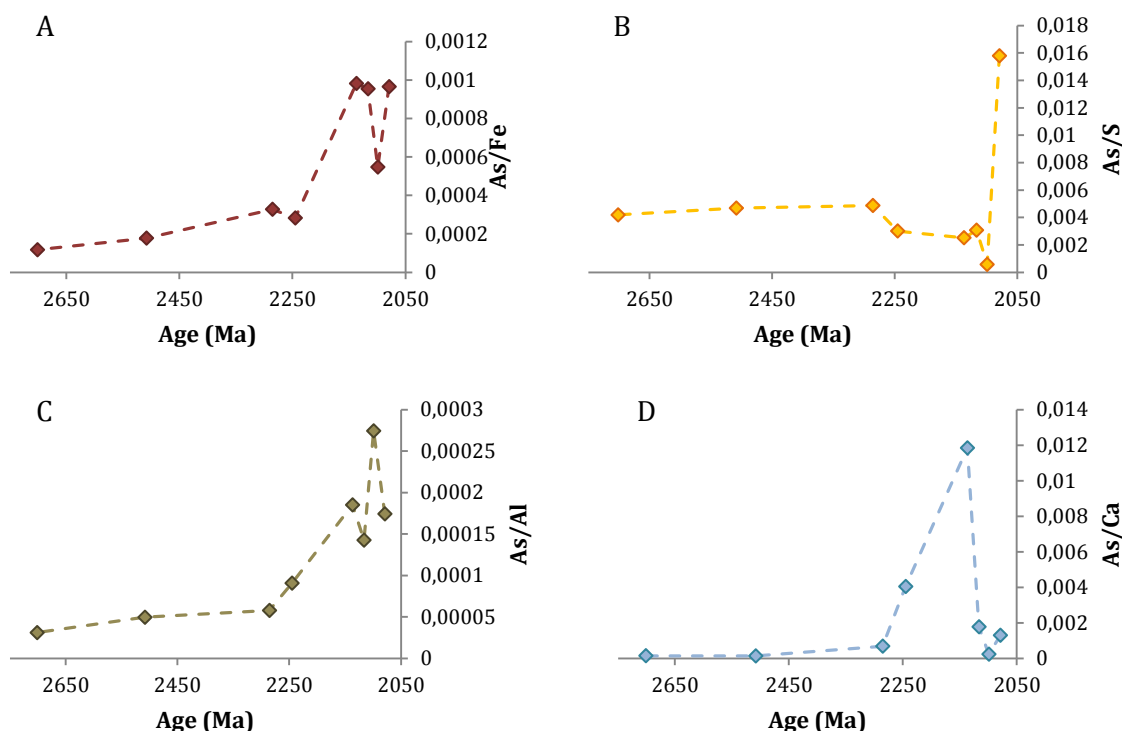


Figure 8 Arsenic normalised to (A) iron, (B) sulphur, (C) aluminium, and (D) calcium. All plotted as the time specific average content against time. <DL not included.

It should be noted, however, that the correlation between arsenic and iron, sulphur, aluminium, and calcium is not always straightforward, not even if taking the redox conditions under consideration. Iron clearly has a strong influence upon arsenic for most of the time, but as was shown already at an early stage (Fig. 4A), their relationship appears to be quite complex. The two separate trends formed by plotting As against As/Fe might suggest the presence of two different pools, but if this would have been the case, it should be possible to verify these pools by looking at the origins of the samples involved in each of the trends (Fig. 9A). One other possibility would be that the two pools would represent anoxic and oxic conditions. However, when plotting As against As/Fe once more, this time with their redox environment identified (Fig. 9B), neither this hypothesis was supported. The cause behind the two trends thereby remains unknown.

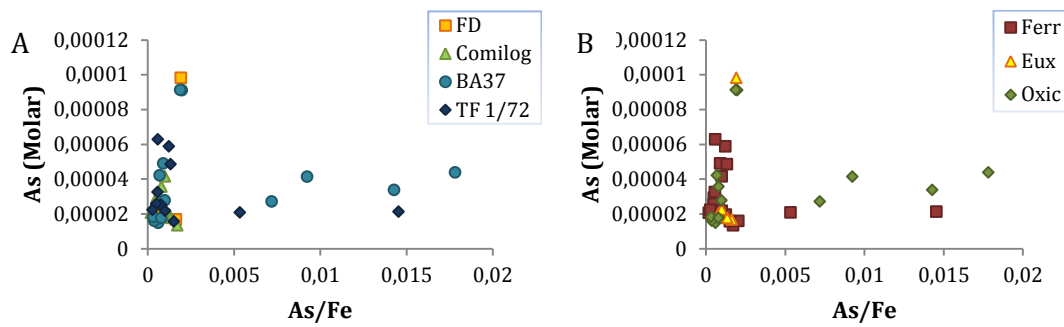


Figure 9 Arsenic versus the ratio of arsenic/iron, showing that its two distinctive behaviours do not depend on either (A) locality or (B) redox conditions.

Arsenic changes in relation to biological activity

63 samples were analysed by Dr. E. Hammarlund and Dr. E. Chi Fru for $\delta^{13}\text{C}$ versus PDB (Fig. 10). The measured $\delta^{13}\text{C}$ values range between -46.22 and -15.85 (mean = -29.92) and generally decrease over time with a prominent negative excursion at about 2.1 Ga. Interestingly, this excursion corresponds with the highest measured levels of arsenic.

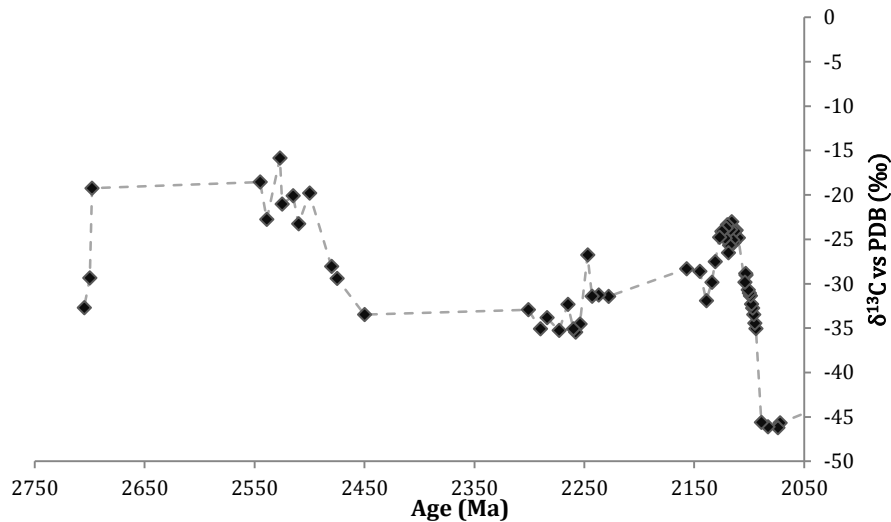


Figure 10 Variation of $\delta^{13}\text{C}$ during the transition from the Archaean to the Proterozoic. Note distinct negative excursion at about 2.1 Ga.

The organic content within many samples was rather high, ranging from 0.53% up to 66.5%, with a mean of 13.0%. A regression analysis of $\delta^{13}\text{C}$ and the organic content (Fig. 11) did not show any correlation between the two variables for most of the samples, except for samples coming from the FD Formation in Gabon. These not only had very low $\delta^{13}\text{C}$ values, but were also

very rich in organic carbon, and it is therefore likely that at least for these samples, the variations in $\delta^{13}\text{C}$ might not reflect changes in biological activity accurately, but rather the deposited amount of organic matter.

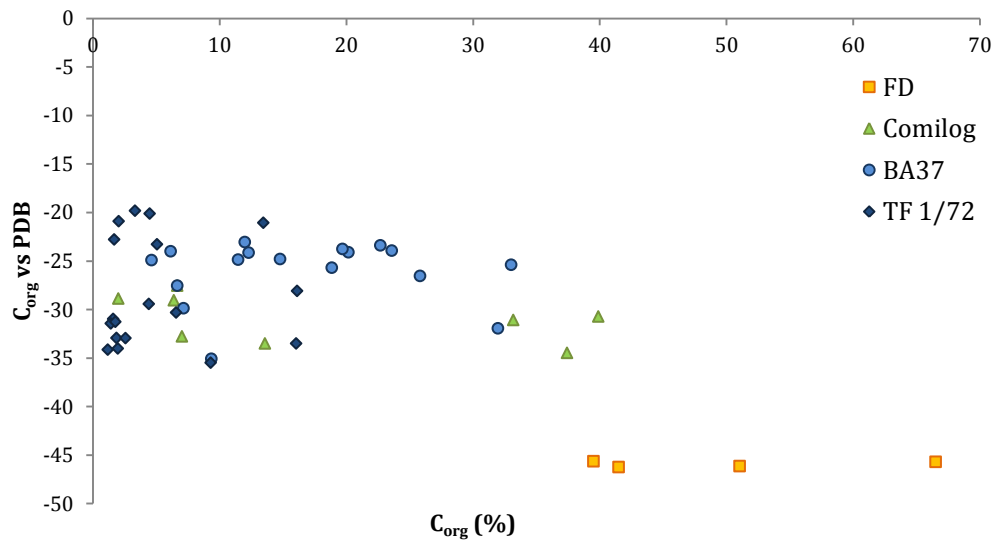


Figure 11 Regression analysis of $\delta^{13}\text{C}$ against organic carbon content. Apart from the FD-samples, there is no significant correlation between the amount of carbon and its isotope composition.

A correlation between arsenic and carbon could not be verified, neither with the total carbon content (Appendix 2-7), nor with $\delta^{13}\text{C}$ (Fig. 12A-B). This means that the arsenic levels appear not to have had any impact upon the biological activity between 2.7 and 2.05 Ga.

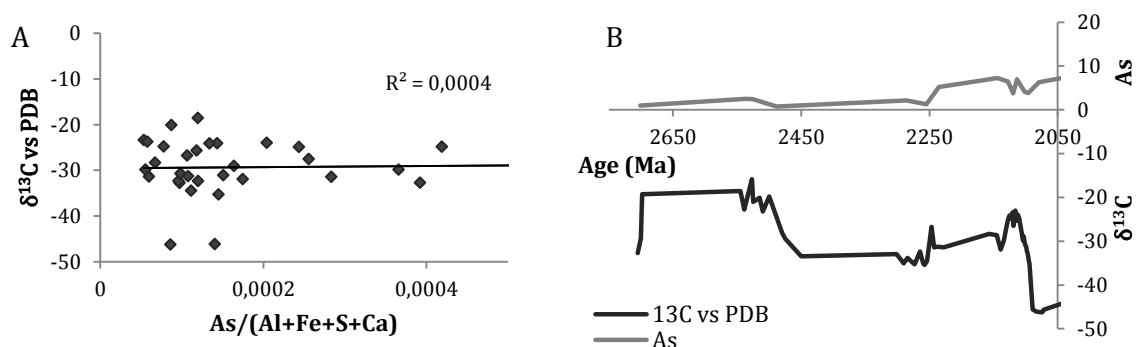


Figure 12 A. Relationships between $\delta^{13}\text{C}$ and As. As has been normalised against Al, Fe, S and Ca. B. Changes in $\delta^{13}\text{C}$ compared to changes in arsenic. For a better overview of the changes of arsenic, the samples have been divided into groups and had their mean values calculated. They are then plotted as relative changes compared to the oldest group of samples. Arsenic has been normalised against Al, Fe, S and Ca.

Changes in arsenic levels over time

When the normalised arsenic values are plotted against time, the relative changes of arsenic concentration are seen (Fig. 13A). However, to achieve a better overview over the large amount of samples and to emphasise the overall trend mean values of the samples were grouped by age and formation (Fig. 13B).

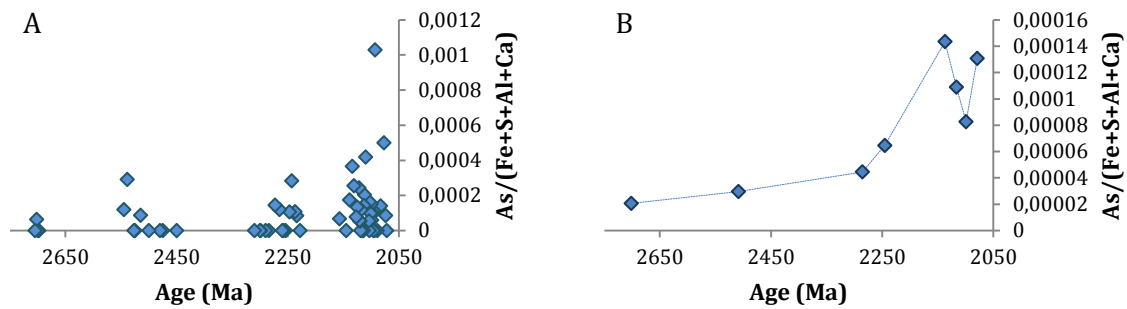


Figure 13 Relative changes of arsenic concentrations over time, A, all samples; B, mean values of samples divided into eight groups. Samples with arsenic levels below detection limit included as 0. Arsenic has been normalised against iron, sulphur, aluminium and calcium. Note that in shales older than 2250 Ma, arsenic is below detection limit in 16 of 23 samples (69.6%). In shales younger than 2200 Ma, arsenic is below detection limit in 12 of 41 samples (29.3%).

The arsenic levels clearly increased during the studied time interval. When calculating the ratio between the mean values of arsenic (molar and normalised concentrations) and the average arsenic concentration of shale (Mason & Moore, 1982), this trend persists. Thus, arsenic levels were up to five times higher than the shale average (Fig. 14) and concentrations of arsenic were significantly elevated between 2.25 Ga and 2.05 Ga.

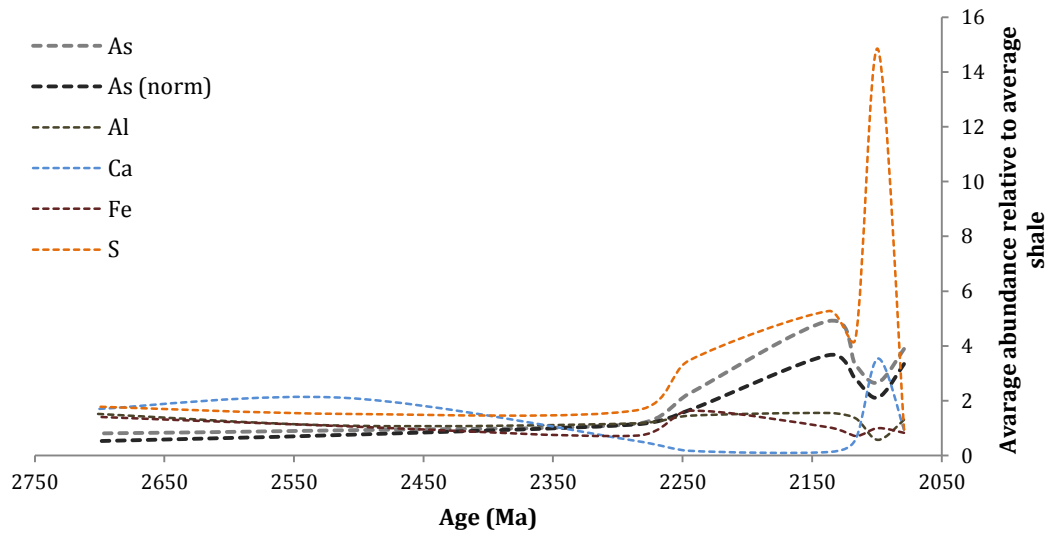


Figure 14 Changes in arsenic, aluminium, calcium, iron and sulphur concentrations through time relative to the shale average (Mason & Moore, 1982). As (norm) represents arsenic normalised against iron, sulphur, aluminium and calcium. Arsenic values of the shale average were recalculated likewise.

Arsenic levels up to five times higher than the shale average is indeed a significant difference. However, as this average is based on considerably younger shales (Cambrian to Tertiary, see Onishi & Sandell, 1955), using it as a reference will reflect variations relative to modern conditions rather than the actual changes in arsenic concentration during the investigated time period. Thus, to provide a fairer picture of arsenic variations, the oldest group of samples was chosen as a reference point instead (Fig. 15). As a result, stronger increases of arsenic are revealed, which are as high as 610% before normalisation and 695% afterwards. The onset of this increase occurred around 2.25 Ga, with a spike at 2.15 and 2.05 Ga.

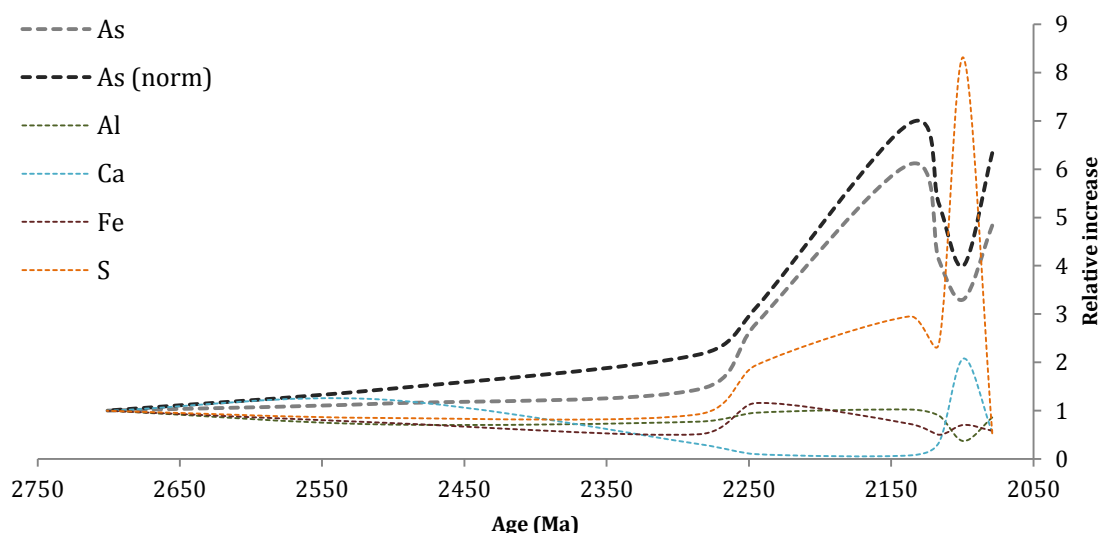


Figure 15 Increase of arsenic, aluminium, calcium, iron and sulphur relative to the oldest group of samples. Arsenic presented both before and after normalisation against iron, sulphur, aluminium and calcium.

So far, the samples from India and Canada have been excluded, as they either had been subjected to recent weathering or differed in sedimentary rock type. Although the accuracy of the data from these samples cannot be verified and despite that they are not directly comparable with the other samples, they might still be able to give some information about how the changes in arsenic levels progressed (Fig. 16, note that silicon has been added to the elements used for normalisation to compensate for the chert content in the Canadian samples).

According to the Canadian samples, the arsenic levels between 2.0 and 1.8 Ga remained on similar levels as they had around 2.15 Ga. After 1.8 Ga, however, it seems as arsenic might have increased considerably, reaching a maximum of close to 1900% higher levels than during the Archaean. As this is based on only one of the two samples from India (the other sample showed severe signs of recent weathering), this result might change significantly if more samples from the same age and formation would be analysed. For now though, it seems as the major increase of arsenic might have taken place during the Mesoproterozoic.

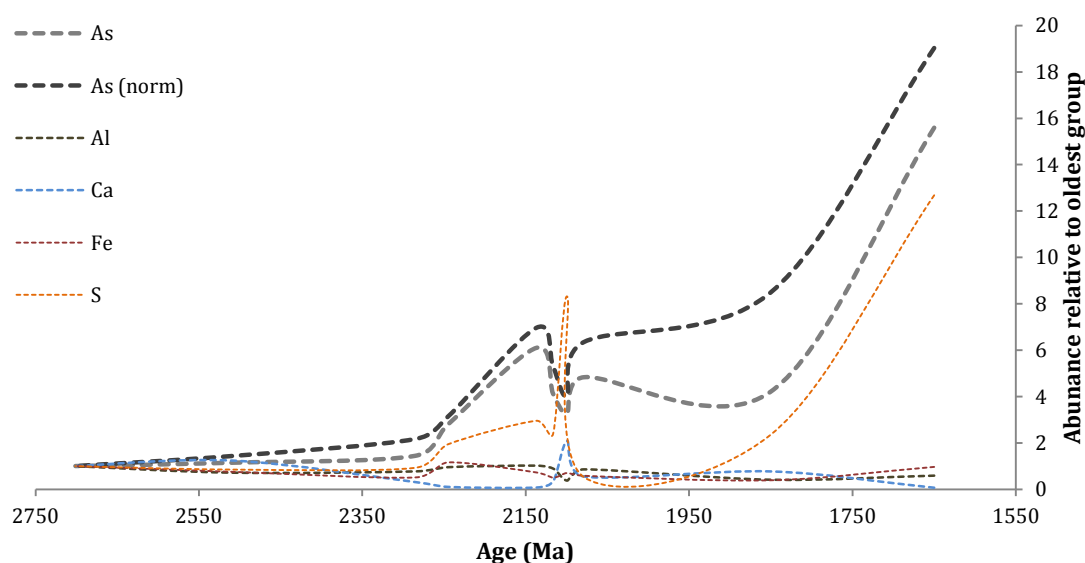


Figure 16 Changes in abundance of arsenic, aluminium, calcium, iron and sulphur relative to the oldest group of samples. Similar to graph in Fig. 15, but with the younger Canadian and Indian samples included to show the potential continuation after 2.05 Ga. Note that the Canadian samples (~1.8 Ga) differ in depositional environment, while the sample from India (~1.6 Ga, note that only one of the two analysed samples were included, meaning that the margin of error ought to be quite high) might have been exposed to weathering. Arsenic normalised to iron, sulphur, aluminium, calcium and silicon.

An interesting pattern appeared when the samples from different redox conditions were separated (but still grouped together based on age and formation). By doing so, both changes in arsenic levels over time as well as how the various redox states of the ocean affected the concentrations of the metalloid were revealed (Fig. 17). The highest levels were found during oxygenated conditions, while arsenic in the sediments from ferruginous waters appears to have been lower. Sulphidic waters generated arsenic levels between oxygenated and ferruginous conditions, while waters which could not clearly be identified as either ferruginous or euxinic were found to have the lowest concentrations of arsenic.

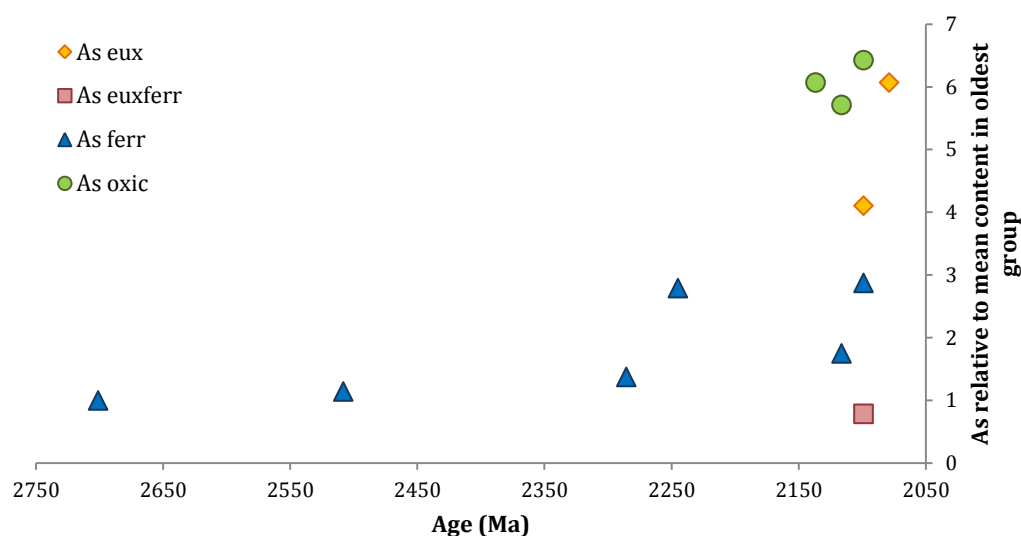


Figure 17 Changes in arsenic levels through time relative to the mean arsenic concentration found within the oldest group. Samples are grouped together based on age and formation. Eux = Euxinic conditions; Euxferr = Euxinic conditions but close to ferruginous; Ferr = Ferruginous conditions; oxic = Oxygenated conditions.

Discussion¹

Evaluating the quality of the samples

The samples are likely to provide a good picture of the Precambrian ocean composition, but there are a few things that need to be considered. The first thing would be any possible mistake or contamination during either the rock digestion or the ICP-OES analysis. The blank controls imply only a low degree of contamination, thus suggesting that the digestion process was correctly executed. The BCR-II controls are, however, of more concern since the obtained elemental composition deviated significantly from the recommended values. The same was true for different dilutions. Several reruns were made, but the reading difficulties persisted. Because of these difficulties, 20 samples (from India, Canada, and South Africa) were reanalysed as well and unlike for the BCR-II, the analysis was able to detect somewhat similar amounts of the elements repeatedly, although still with a larger deviation than what normally would be tolerated. Despite these problems, the decision was made to proceed with the data, mainly because of time restrictions, and by grouping samples and only presenting their average element content instead of the content of each sample individually, any possible deviations should be equalled out somewhat. For future analysis, it might be advisable to use a different, more accurate analysis technique and to test the samples analysed herein once more.

¹ References are virtually absent throughout the discussion apart from in *Evaluating the quality of the samples*. All references have already been mentioned in the introductory chapters.

A second thing to consider is whether the concentration of elements in the sediments reflects the concentration within the water column. To some degree it must always do, as clearly, an element not found in the water would not suddenly appear in the sedimentary deposits. In the ideal situation, there would be a constant balance between the chemical composition of the ocean and the elements deposited at the bottom although this is rarely, if ever, the case. Depending on the lithology, elements can be either enriched or depleted. Indeed, the amount of trace elements in sandstone can be very different from what is found in shale or carbonate of the same age. Thus, it is of critical importance to have such variables under control, i.e. by only include samples of similar lithologies. If not, it cannot be excluded that any change through time is reflecting a change in lithology rather than in water concentration. There are concerns that there might be such a bias in this study, since the majority of the older South African samples come from carbonates, while the younger Gabon samples originate from shales, in which trace elements such as arsenic tends to be enriched. Normalisation of arsenic against iron and sulphur will correct for the ability of these elements to adsorb arsenic in a specific sample (otherwise, the amount of arsenic might only be reflecting the amount of iron and/or sulphur instead of the water concentrations), but it is not enough to account for the different properties between different rock types. By normalising arsenic to also aluminium (representing the clay component) and calcium (representing the precipitation of calcium carbonate), the influence of variations in deposition or precipitation rates will be limited to a certain extent, but again, it is not enough to account for all the lithological differences in composition and chemical properties by which arsenic can be affected. For future studies of the arsenic variations over time, it is necessary to understand the limitations of the normalisation methods and to keep the maximum amount of variables under control by including samples from only the same type of lithology.

A third thing capable of greatly influencing the behaviour of many elements, arsenic included, are the redox conditions. The redox conditions are important not only during but also after deposition since they determine the mobility of elements between sediment and solution. Such post-depositional alterations can be problematic since they can lead to a depletion (or enrichment) of elements in the sediments, which thereby will no longer reflect the original depositional conditions. There are ways to detect these kinds of changes, but unfortunately, the methods are only applicable to carbonates (e.g. Veizer, 1983). However, as other studies have suggested both the sampled South African sequences (Eriksson & Clendenin, 1990) and the formations in Gabon (Gauthier-Lafaye, 2006) to have been preserved largely unchanged, the risk of post-depositional as well as diagenetic alteration should not be of any major concern.

Interpretation of arsenic variations

The predicted increase in arsenic levels between 2.7 and 2.05 Ga has been confirmed, and also established by now are the elements to which arsenic correlates under ferruginous, euxinic, and oxygenated settings, respectively. However, what is causing the correlation is not yet clear, but likely there are several factors that should be considered here. The two most important ones are the source of the material and the redox conditions.

The major source of arsenic in Precambrian sediments can be hydrothermal or continental depending on the time of deposition. During the Archaean, arsenic was delivered to the oceans from hydrothermal vents, which is supported by the Spearman correlation analysis of the South African samples (Appendix 4). In these samples, arsenic correlates with iron, whose main source is commonly considered to be hydrothermal. With elements usually regarded as continental, no correlation could be observed. The younger Gabon samples, on the other hand, tell a different story. When the sulphur concentration started to increase after the GOE, it was accompanied by a simultaneous increase in arsenic. The Spearman correlation analysis confirmed a correlation between arsenic and sulphur during oxygenated conditions, in it also suggested a connection between arsenic and typical continental elements such as aluminium, titanium and potassium, which are all common components of clay. The correlation between arsenic and these (and other) elements persisted for the remainder of the investigated interval, suggesting that the main flux of arsenic to the ocean after the rise of atmospheric oxygen was from continental sources following the weathering of landmasses. Furthermore, the large increase of sulphur in comparison to aluminium supports a shift in weathering patterns, with the CO₂-driven granite-weathering remaining on fairly constant levels, while the O₂-driven sulphide-weathering is not initiated until after the GOE.

The highest levels of arsenic are found in samples recognised as coming from oxygenated conditions, with sulphur as the main controlling element. This appears reasonable due to how the iron levels within the oxygenated zone should be very low, while arsenic and sulphur are assumed to have had a common source. During sulphidic conditions however, iron has the largest control over the arsenic levels. Again, this seems valid, since within this anoxic layer, iron could be present and able to remove the arsenic. It also shows that iron has a stronger controlling effect than sulphur upon arsenic. The lowest arsenic levels are found in the ferruginous waters, regardless of the time period. This could be the case if most of the arsenic had already been removed from the water column in the overlaying oxygenic and sulphidic parts of the ocean, but it might also reflect the dominance of As³⁺ over As⁵⁺. As described earlier (see chapter *Arsenic*), As³⁺ is more soluble than As⁵⁺ and thereby not only less likely to be removed,

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but also more likely to be released from the sediments, allowing it to exist in considerably higher concentrations in the water. This would be the case within the sulphidic layer as well, since also here would As^{3+} be the prevalent form. Thus, the arsenic concentrations within the sulphidic and ferruginous water layers might have been higher than the sediments suggest.

When a sulphidic layer was present, separating the oxygenated waters from the ferruginous deeper waters, these latter two would not have been in contact. Consequently, the removal of arsenic could have been greatly impaired as As^{5+} would have been kept from intermixing directly with iron-rich waters. Hence, the absence of a sulphidic layer would have allowed oxygenated waters with As^{5+} to overlay the ferruginous waters, possibly enhancing the removal rates considerably.

Arsenic started increasing already shortly after the GOE, but a more dramatic increase did not occur until after 1.8 Ga. This is the accepted date for the more permanent sulphidic conditions, which lasted throughout the Mesoproterozoic. It has been suggested that the formation of the supercontinent Nuna would have been responsible for the increase in sulphide flux, something that is also in agreement with the results herein. Indeed, arsenic and sulphur increased simultaneously, which supports a common source for these two elements once the oxidative weathering had started. The formation of the sulphide-rich Nuna could explain why the major increase of arsenic occurred after 1.8 Ga and not immediately after the GOE, despite similar levels of atmospheric oxygen.

Did the increased arsenic levels affect life?

In modern environments, arsenic is more than capable of affecting life, with animals as well as plants suffering already at low concentrations despite their rather sophisticated arsenic protection mechanisms. As a comparison, the World Health Organisation has set 10 $\mu\text{g/L}$ of arsenic in drinking water as a guideline of what to consider as tolerable levels, which is only about four times the arsenic concentration found in sea water. The fact that arsenic protection mechanisms are found in all branches of life suggests that the threat of arsenic has been omnipresent through all times. Indeed, the ancient origin of the bacterial ArsB and ArsC systems, both involved in arsenic detoxification, indicates that arsenic would have challenged already the very first life in the Archaean. However, considering how the more advanced arsenic detoxification systems are distributed between different groups and that these systems are formed through convergent evolution, it appears as the organisms were forced to respond to an increasing arsenic threat at some point later in Earth history. Previous to this event, the lack of

more intricate arsenic detoxification systems would have left the prevailing organisms sensitive to low amounts of the metalloid. During the Archaean, this would not have been an issue since arsenic remained on persistently low levels, but once the flux of arsenic increased due to the oxidative weathering of continental sulphides that followed the GOE, the organisms would soon have found themselves exposed to what could have been critical levels of the element. The increase of arsenic by 600% to 1900% relative to Archaean levels between 2.05-1.6 Ga ago might have been devastating for the still primitive life if it had not yet acquired the necessary genes providing resistance. Indeed, even when these genes had evolved, such high levels might still have contributed significantly to the already harsh environment where the oxygen levels were low, H₂S concentrations high, and the nutrient availability limited.

The idea that elevated arsenic concentrations were responsible for the sudden dip in oxygen production between 2.0 and 1.8 Ga cannot be confirmed at this point. As the cause behind this decreased oxygen levels is unknown, it did appear possible that the oxygen-producing cyanobacteria were among the victims for the increase in arsenic. Once they had evolved more intricate detoxification mechanisms, they could have resumed the oxygenation of the Earth, and although cyanobacteria seem to have gained and lost some traits during their evolution depending on what was required (Miller & Bebout, 2004), they have kept their capability to tolerate comparatively high levels of arsenic until today (Nagy et al., 2005; Bhattacharya & Pal, 2011). However, although the arsenic levels within this time period were about 600% higher than during the Archaean, they had reached this order of magnitude well before the cyanobacteria crisis, making it unlikely that arsenic would have been what caused the oxygen production collapse. The general lack of correlation between arsenic and $\delta^{13}\text{C}$ adds further support to that arsenic was not able to influence the activity of primary producers, at least not between 2.7 and 2.05. However, there are three reasons for why it is still too early to reject the hypothesis. Firstly, there are only four samples from the age of interest, which is much too low for any conclusions to be drawn. Secondly, the samples come from a section consisting mainly of chert, which makes them not directly comparable to the other samples due to differences in both chemical composition and depositional/environmental setting.

Something that does correlate is the increase of arsenic at about 2.1 Ga and the disappearance of the macroscopic colonial or multicellular fossil described from the FC Formation in Gabon. This early organism, if accepting it as such, is surrounded by uncertainties, but it might still be tempting to associate its apparent extinction with the elevated arsenic levels. There are, however, many circumstances that should be considered before coming to this conclusion, including the presence of arsenic-enriched structures on at least one of the Gabon specimens

(Hammarlund, 2012). If this would be a post-mortem enrichment, it would support the organism's sensitiveness for arsenic; if not, it would imply the opposite. Thus for now, it can only be noted that the Gabon organism disappears from the fossil record at the same time as the arsenic levels increased, but whether it was as a direct consequence or purely coincidental remains unknown.

So - is it possible that arsenic contributed to delay the evolution of complex multicellular life? Considering how it has been shown to increase after the initiation of oxidative weathering, it appears possible that arsenic was involved in restraining the evolution, although it should be noted that the water levels of arsenic have not been calculated. However, the exact levels of arsenic might not be as important as the general trend since, as already stated, without the necessary arsenic detoxification mechanisms, even extremely low amounts of arsenic will be fatal. Whether the observed increase is enough to have had any profound impact of such great magnitude is at this point not clear; more data of the arsenic levels is needed from the time between 2.0 and 0.5 Ga. Also important would be to investigate in detail the distribution of the various arsenic resistance systems between different groups in order to put it into a phylogenetic context and reveal the time of the origin of these systems. By doing so, it would be possible to correlate their upcome with the increase in arsenic levels, and where any potential time gap in between would represent a period where arsenic could have had serious effects upon the sensitive organism. The only way to get physical evidence whether arsenic was able to influence the evolution of life is, however, to compare the changes in arsenic levels with changes seen in the fossil record. If arsenic was able to affect life, it must have left its imprint on the life of the time, thus should an increase arsenic levels have been accompanied by a decrease in diversity and vice versa. Before the Precambrian arsenic levels have been compared to what can be seen in the fossil record of that time, it remains unknown whether the evolution of life was affected by the presence of arsenic.

Modelling of arsenic variations in the Precambrian oceans

After having confirmed the predicted increase of arsenic levels following the GOE and seen how it responded to redox changes, the behaviour of arsenic in the Precambrian oceans can be modelled. As the oceans can be divided into three stages, ferruginous, euxinic, and oxygenated, three corresponding scenarios for arsenic have been created. It should be noted, however, that although the behaviour of arsenic during the conditions typical for these stages has been described, most of the samples are not from these specific time periods. Especially the third

stage, representing the Neoproterozoic oxygenated conditions, is at this point mainly speculative and remains to be tested.

Stage 1: Arsenic in ferruginous oceans of the Archaean

Before 2.4 Ga and the rise of free oxygen, the reducing atmosphere would have had a stronger weathering impact upon silicates and carbonates rather than on sulphides. The main source of arsenic at this time would have been hydrothermal vents, from which most of it would have spread throughout the oceans. As^{3+} would have been the most common form since it is the most stable oxidation state in a reducing environment such as the Archaean oceans. If also As^{5+} was present, it would only have been in small amounts.

During most of the Archaean, photoferrotrophic bacteria were producing iron formations to whose iron minerals arsenic could adsorb, thus being removed from the water column. As^{3+} is, however, not as readily removed as As^{5+} , but despite this, the removal rate was likely high enough to balance out the relatively low input. The vast quantities of BIFs that were formed at the time could have kept the arsenic also from accumulating in the water. Thus, prior to the GOE, the oceanic arsenic concentrations would have remained on a low and fairly constantly level (Fig. 18).

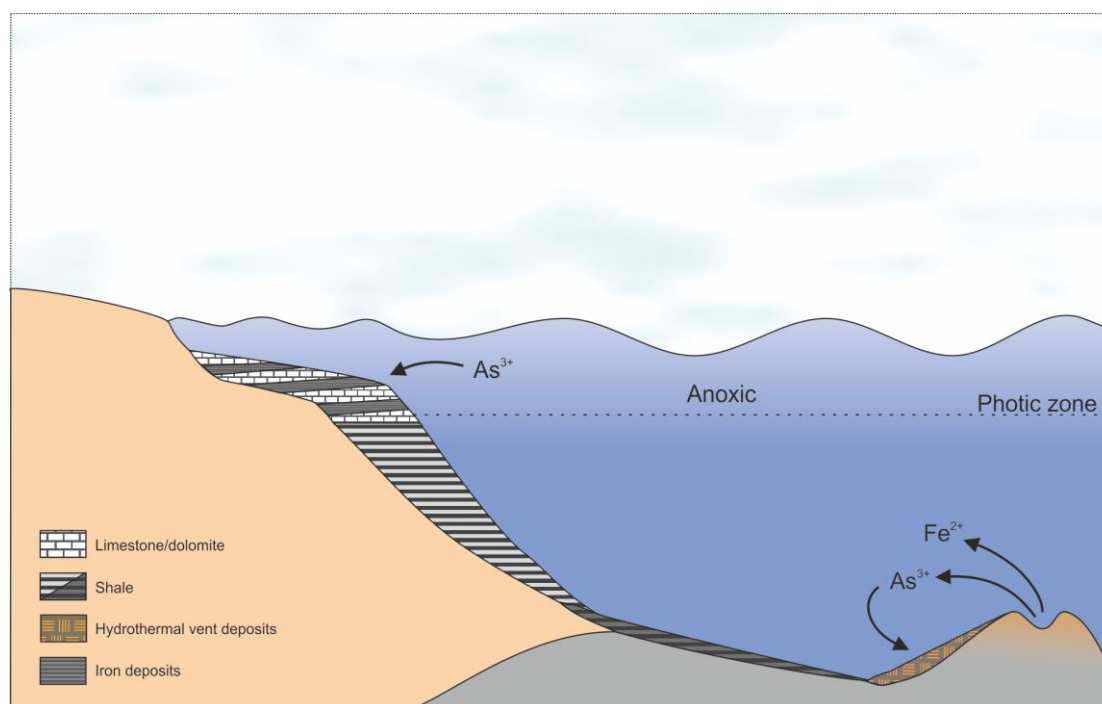


Figure 18 2.4 Ga and before: Input \leq removal. Iron oxides to which the arsenic could adhere were deposited within the photic zone in an anoxic ocean. In combination with a limited arsenic input, the pre-GOE levels of arsenic in the oceans were likely low.

Stage 2: Arsenic in the sulphidic oceans of the mid-Proterozoic

After the GOE, once the continental weathering of sulphide minerals started, the flux of arsenic from land exceeded that from hydrothermal vents. At the same time, an oxygenated upper layer developed in the now stratified oceans, and the redox cycling of arsenic could begin. As^{5+} could now form abiotically in the oxic layer above the redox boundary whereas As^{3+} continued to dominate in the deeper, still anoxic waters. With time, also an intermediate euxinic layer started to spread, reaching from the continental slopes into the open ocean. Within this layer, (oxy)thioarsenates were likely to be formed, although this cannot be verified due to the instability of the compound. Thus, unlike the Archaean oceans, there were now three different redox layers, allowing arsenic to exist in two different oxidation states and as thioarsenates, and overall in considerably higher concentrations (Fig. 19).

During the Archaean, the removal rates of arsenic were likely to have matched or exceeded the supply (see Stage 1). However, this might have changed due to the spreading of sulphidic waters, a transition that was initiated at about 2.1 Ga and increased in magnitude after the formation of the supercontinent Nuna at about 1.9 Ga. Although arsenic can be incorporated into pyrite, a mineral known from sediments within the sulphidic layer, it might also be excluded if the pyrite precipitation rate is high. If this was the case, arsenic could have accumulated to very high levels within the euxinic part of the water column. The accumulation of arsenic would have been further enhanced by the formation of thioarsenates, as these compounds tend to remain in solution. Another factor strongly affecting the arsenic levels would have been the absence of iron oxide precipitation. The photic zone was now oxygenated, which both lowers the solubility of iron as well as prevents the activity of the anaerobic photoferrotrophic bacteria. Ferruginous conditions could have persisted in the deeper parts of the oceans, but without either biotic or abiotic deposition of BIFs. Thus, the main removal mechanism for arsenic would have been eliminated for the time being, allowing the metalloid to accumulate also within the oxic and anoxic water layers.

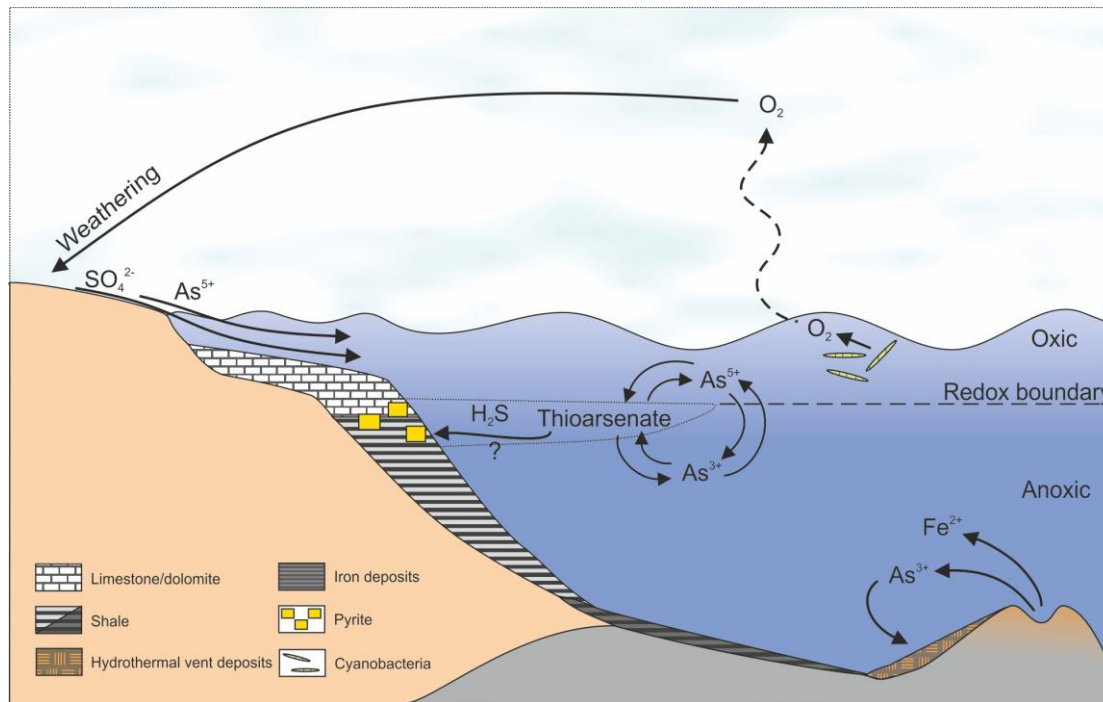


Figure 19 2.4-0.7 Ga: Input > removal. The removal rate of arsenic would have been slower than during the Archaean due to the terminated deposition of BIFs. At the same time, the arsenic input had increased as a consequence of oxidative weathering of the continents. Arsenic could thereby have accumulated within the water column.

Stage 3: Arsenic in the oxygenated oceans of the Neoproterozoic

Towards the end of the Proterozoic, oxygen levels started to increase again, reaching concentrations close to those of today. In the oceans, enough oxygen was produced to not only end the euxinic conditions, but also to oxygenate the bottom waters, at least occasionally. For the most part, however, the deeper waters returned to being ferruginous and either sub- or anoxic. Also the deposition of BIFs was resumed, this time abiotically formed at the boundary between the iron-rich deep waters and the oxygen-rich upper layer.

Without a sulphidic layer, arsenic would now cycle only between As^{3+} and As^{5+} . With BIFs being precipitated at the redox boundary, both of these oxidation states would be present and available for removal by adsorption. However, As^{5+} would be removed at a much higher rate in the oxygenated zone than As^{3+} in the anoxic deeper waters. The input of arsenic from hydrothermal vents was likely still on a similar level as before, but the continental arsenic supply might have started to fluctuate because of several potential reasons. The extensive glaciations of the late Neoproterozoic as well as the potential colonisation of land might have acted as a protective layer limiting or at least slowing down oxidative weathering. However, the most important reason might have been how the mineral composition of the continents gradually had changed during the Mesoproterozoic. The sulphide-rich landmasses that had

formed during the accretion of the supercontinent Nuna had been progressively eroded down until the crystalline bedrock was exposed and the continental flux of arsenic (as well as sulphate) could thereby have decreased accordingly.

The resumed deposition of BIFs in the late Neoproterozoic would have re-established an efficient arsenic removal mechanism. At the same time, As^{5+} would have increased in prevalence on behalf of As^{3+} , while the arsenic flux would have decreased due to the change in weathering patterns. Thus, during the Neoproterozoic the removal rate of arsenic would have exceeded the rates of delivery (Fig. 20). Furthermore, the excessive amounts of arsenic that might have been accumulating during the Early and Middle Proterozoic could now have been swept away, creating a detoxified, nutrient-rich and well oxygenated ocean capable of welcoming the first eukaryotic multicellular life as we know it.

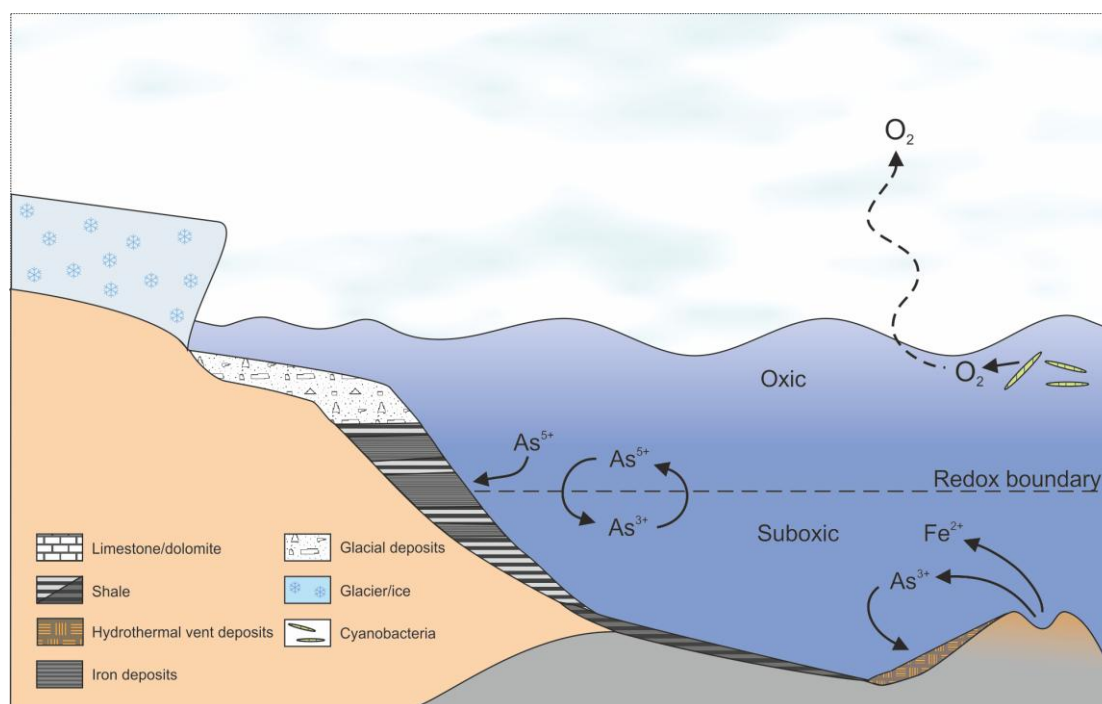


Figure 20 0.7 Ga and onwards: Removal > input. The return of ferruginous conditions may have stripped the oceans from the excessive amounts of arsenic. At the same time, the arsenic input might have decreased due to the previously extensive sulphide deposits having been eroded away.

It should again be pointed out that this large stage is purely speculative, but based on the known behaviour of arsenic as well as the environmental conditions at the time it does appear as a plausible explanation for how any excessive amounts of arsenic could have been removed from the water column. By analysing more samples between 1.9 and 0.5 Ga, the model could be tested in its entirety, thus verifying the predicted changes, sources and removal pathways of arsenic throughout the Proterozoic.

Conclusions

The transition from ferruginous to sulphidic conditions was initiated by the GOE and appears to have accompanied by a simultaneous increase of arsenic levels in the oceans. Before the GOE, the main source of arsenic had been hydrothermal, but once free atmospheric oxygen had emerged, the source changed to being mainly continental. In the anoxic oceans, iron had the strongest effect upon the arsenic levels by removing it through adhesion, while the influence of sulphur increased in importance in oxygenated waters where the amount of iron was limited. It is also hypothesised that arsenic levels were affected by the oxygenation state of the element, with As^{5+} in the oxygenated waters being more readily removed than As^{3+} , which could have remained dissolved in the deeper anoxic parts of the ocean.

Arsenic does not show any correlation with $\delta^{13}\text{C}$ between 2.7 and 2.05, and is therefore believed to not have affected the primary production within this time period. Whether arsenic was one of the factors contributing to the late evolution of complex, multicellular life is still too early to say, but it does appear possible considering how the genes coding for arsenic detoxification mechanisms are distributed within the various branches of life. If so, it should have been during the Mesoproterozoic, which is the time when the sulphidic waters are considered to have had their widest distribution. However, since the focus herein has been on the transition from the anoxic Archaean to the oxygenated Palaeoproterozoic, the concentrations of arsenic during the Mesoproterozoic are still rather speculative. Furthermore, it cannot be excluded that there might be a sample bias in this study, considering the differences in lithology between the Archaean and Proterozoic samples. Finally, before the levels of arsenic have been compared with the evolutionary changes seen in the fossil record, such as changes in taxonomical diversity and abundance, its effects upon life cannot be verified. To conclude: there are reasons to suspect that arsenic could have affected life, but before this can be confidently claimed, more research is needed.

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References

- Alonso, A., Sasin, J., Bottini, N., Friedberg, I., Osterman, A., Godzik, A., Hunter, T., Dixon, J. & Mustelin, T. 2004. Protein tyrosine phosphatases in the human genome. *Cell*, vol. 117: 699-711.
- Allwood, A. C., Grotzinger, J. P., Knoll, A. H., Burch, I. W., Anderson, M. S., Coleman, M. L. & Kanik, I. 2009. Controls on development and diversity of Early Archean stromatolites. *PNAS*, vol. 106: 9548-9555.
- Anbar, A. D. & Knoll, A. H. 2002. Proterozoic ocean chemistry and evolution: A bioinorganic bridge? *Science*, vol. 297: 1137-1142.
- Awramik, S. M., Schopf, J. W. & Walter, M. R. 1983. Filamentous fossil bacteria from the Archean of Western Australia. *Precambrian Research*, vol. 20: 357-374.
- Barley, M. E., Bekker, A. & Krapež, B. 2005. Late Archean to Early Paleoproterozoic global tectonics, environmental change and the rise of atmospheric oxygen. *Earth and Planetary Science Letters*, vol. 238: 156-171.
- Beard, B. L., Johnson, C. M., Cox, L., Sun, H., Nealson, K. H. & Aguilar, C. 1999. Iron isotope biosignatures. *Science*, vol. 285: 1889-1892.
- Bekker, A., Holland, H. D., Wang, P.-L., Rumble III, D., Stein, H. J., Coetzee, L. L. & Beukes, N. J. 2004. Dating the rise of atmospheric oxygen. *Nature*, vol. 427: 117-120.
- Bekker, A., Holmden, C., Beukes, N. J., Kenig, F., Eglinton, B. & Patterson, W. P. 2008. Fractionation between inorganic and organic carbon during the Lomagundi (2.22-2.1 Ga) carbon isotope excursion. *Earth and Planetary Science Letters*, vol. 271: 278-291.
- Bengtson, S., Belinova, V., Rasmussen, B. & Whitehouse, M. 2009. The controversial “Cambrian” fossils of the Vindhyan are real but more than a billion years older. *PNAS*, vol. 106: 7729-7734.
- Benner, S. A. 2011. Comment on “A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus”. *Science*, vol. 332: 1149-c.
- Bertrand-Sarfati, J. & Potin, B. 1994. Microfossiliferous cherty stromatolites in the 2000 Ma Franceville Group, Gabon. *Precambrian Research*, vol. 65: 341-356.
- Bhattacharjee, H., Ho, Y. S. & Rosen, B. P. 2001. Genomic organization and chromosomal localization of the *AsnA1* gene, a mouse homologue of a bacterial arsenic-translocating ATPase gene. *Gene*, vol. 272: 291-299.
- Bhattacharjee, H. & Rosen, B. 2007. Arsenic metabolism in prokaryotic and eukaryotic microbes. In: Nies, D. H. & Silver, S. (eds.), *Molecular microbiology of heavy metals*, 371-406. Springer-Verlag, Berlin, Germany.

- Bhattacharya, P. & Pal, R. 2011. Response of cyanobacteria to arsenic toxicity. *Journal of Applied Phycology*, vol. 23: 293-299.
- Bjerrum, C. J. & Canfield D. E. 2002. Ocean productivity before about 1.9 Gyr ago limited by phosphorus adsorption onto iron oxides. *Nature*, vol. 417: 159-162.
- Borch, T., Kretzschmar, R., Kappler, A., Van Cappellen, P., Ginder-Vogel, M., Voegelin, A. & Campbell, K. 2010. Biogeochemical Redox Processes and their Impact on Contaminant Dynamics. *Environmental Science and Technology*, vol. 44: 15-23.
- Brasier, M. D., Green, O. R., Jephcoat, A. P., Kleppe, A. K., Van Kranendonk, M. J., Lindsay, J. F., Steele, A. & Grassineau, N. V. 2002. Questioning the evidence for Earth's oldest fossils. *Nature*, vol. 416: 76-81.
- Braterman, P. S., Cairns-Smith, A. G. & Sloper, R. W. 1983. Photo-oxidation of hydrated Fe^{2+} — significance for banded iron formations. *Nature*, vol. 303: 163–164.
- Buck, S. G. 1980. Stromatolite and ooid deposits within the fluvial and lacustrine sediments of the Precambrian Ventersdorp Supergroup of South Africa. *Precambrian Research*, vol. 12: 311-330.
- Budd, G. E. 2008. The earliest fossil record of the animals and its significance. *Phil. Trans. R. Soc. B.*, vol. 363: 1425-1434.
- Buick, R. 2008. When did oxygenic photosynthesis evolve? *Phil. Trans. R. Soc. B*, vol. 363: 2731-2743.
- Canfield, D. E. 1998. A New model for Proterozoic ocean chemistry. *Nature*, vol. 396: 450-453.
- Canfield, D. E. 2005. The early history of atmospheric oxygen: homage to Robert M. Garrels. *Annu. Rev. Earth Planet. Sci.*, vol. 33: 1–36.
- Canfield, D. E., Habicht, K. S. & Thamdrup, B. 2000. The Archean Sulfur Cycle and the Early History of Atmospheric Oxygen. *Science*, vol. 288: 658-661.
- Canfield, D. E., Poulton, S. W. & Narbonne, G. M. 2007. Late-Neoproterozoic Deep-Ocean Oxygenation and the Rise of Animal Life. *Science*, vol. 315: 92-95.
- Caravati, E. M. 2004. Arsenic and Arsine Gas. In Dart, R. C. (ed.), *Medical Toxicology*, 1393-1401. Lippincott Williams and Wilkins, Philadelphia, USA.
- Catuneanu, O. & Eriksson, P. G. 1999. The sequence stratigraphic concept and the Precambrian rock record: an example from the 2.7–2.1 Ga Transvaal Supergroup, Kaapvaal craton. *Precambrian Research*, vol. 97: 215-251.

- Catuneanu, O. & Eriksson, P. G. 2002. Sequence stratigraphy of the Precambrian Rooihogte – Timeball Hill rift succession, Transvaal Basin, South Africa. *Sedimentary Geology*, vol. 147: 71-88.
- Chakraborty, C. 2006. Proterozoic intracontinental basin: The Vindhyan example. *Journal of Earth Sciences*, vol. 115: 3-22.
- Chi Fru, E., Hammarlund, E., Pemba, L. N., Arvestål, E. H. M., Bengtson, S., El Albani, A., Andersson, P. & Mörtz, M. Submitted. Microaerophilic biological methane cycling ~2.6-1.6 billion years ago inferred from iron, copper and carbon systematics. *Earth and Planetary Science Letters*.
- Chi Fru, E., Ivarsson, M., Kilias, S. P., Bengtson, S., Belivanova, V., Marone, F., Fortin, D., Broman, C. & Stampanoni, M. 2013. Fossilized iron bacteria reveal pathway to biological origin of banded iron formation. *Nature Communications*, vol. 4: 1-7.
- Clapham, M. E. & Narbonne, G. M. 2002. Ediacaran epifaunal tiering. *Geology*, vol. 30: 627-630.
- Cloud, P. 1965. Significance of the Gunflint (Precambrian) microflora. *Science*, vol. 148: 27-35.
- Cloud, P. 1973. Paleocological significance of the banded iron-formation. *Econ. Geol.*, vol. 68: 1135-1143.
- Cohen, Y., Jørgensen, B. B., Revsbech, N. P. & Poplawski, R. 1986. Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. *Applied and Environmental Microbiology*, vol. 51: 398-407.
- Couture, R-M. & Van Cappellen, P. 2011. Reassessing the role of sulfur geochemistry on arsenic speciation in reducing environments. *Journal of Hazardous Materials*, vol. 189: 647-652.
- Craddock, P. R. & Dauphas, N. 2011. Iron and carbon isotope evidence for microbial iron respiration throughout the Archean. *Earth Planet. Sci. Lett.*, vol. 303: 121-132.
- Croal, L. R., Johnson, C. M., Beard, B. L. & Newman, D. K. 2004. Iron isotope fractionation by Fe(II)-oxidizing photoautotrophic bacteria. *Geochimica et Cosmochimica Acta*, vol. 68: 1227-1242.
- Crow, C. & Condie, K. C. 1988. Geochemistry and origin of Late Archean volcanics from the Ventersdorp Supergroup, South Africa. *Precambrian Research*, vol. 42: 19-37.
- Crowe, S. A., Jones, C., Katsev, S., Magen, C., O'Neill, A. H., Sturm, A., Canfield, D. E., Haffner, G. D., Mucci, A., Sundby, B. & Fowle, D. A. 2008. Photoferrotrophs thrive in an Archean Ocean analogue. *PNAS*, vol. 105: 15938-15943.
- Czaja, A. D., Johnson, C. M., Beard, B. L., Roden, E. E., Li, W. & Moorbath, S. 2013. Biological Fe oxidation controlled deposition of banded iron formation in the ca. 3770 Ma Isua

- Supracrustal Belt (West Greenland). *Earth and Planetary Science Letters*, vol. 363: 192–203.
- Dauphas, N., van Zuilen, M., Wadhwa, M., Davis, A. M., Marty, B. & Janney, P. E. 2004. Clues from Fe Isotope Variations on the Origin of Early Archean BIFs from Greenland. *Science*, vol. 306: 2077–2080.
- Davidov, Y. & Jurkevitch, E. 2009. Predation between prokaryotes and the origin of eukaryotes. *Bioessays*, vol. 31: 748-757.
- Decker, H. & van Holde, K. E. 2011. *Oxygen and the Evolution of Life*. 1-172. Springer-Verlag Berlin Heidelberg.
- Dixit, S. & Hering, J. G. 2003. Comparison of Arsenic(V) and Arsenic(III) Sorption into Iron Oxide Minerals: Implications for Arsenic Mobility. *Environmental Science & Technology*, vol. 37: 4182-4189.
- Dutkiewicz, A., George, S. C., Mossman, D. J., Ridley, J. & Volk, H. 2007. Oil and its biomarkers associated with the Palaeoproterozoic Oklo natural fission reactors, Gabon. *Chemical Geology*, vol. 244: 130-154.
- Eigenbrode, J. L., Freeman, K. H. & Summons, R. E. 2008. Methylhopane biomarker hydrocarbons in Hamersley Province sediments provide evidence for Neoproterozoic aerobiosis. *Earth and Planetary Science Letters*, vol. 273: 323-331.
- El Albani, A., Bengtson, S., Canfield, D. E., Bekker, A., Macchiarelli, E., Mazurier, A., Hammarlund, E. U., Boulvais, P., Dupuy, J.-J., Fintaine, C., Fürsich, F. T., Gauthier-Lafaye, F., Janvier, P., Javaux, E., Ossa, F. O., Pierson-Wickmann, A.-C., Riboulleau, A., Sardini, P., Vachard, D., Whitehouse, M. & Meunier, D. 2010. Large colonial organisms with coordinated growth in oxygenated environments 2.1 Gyr ago. *Nature*, vol. 466: 100-104.
- Eriksson, P. G. & Clendenin, C. W. 1990. A review of the Transvaal Sequence, South Africa. *Journal of African Earth Sciences*, vol. 10: 101-116.
- Eriksson, K. A., McCarthy, T. S. & Truswell, J. F. 1975. Limestone Formation and Dolomitization in a Lower Proterozoic Succession from South Africa. *Journal of Sedimentary Research*, vol. 45: 604-614.
- Fekry, M. I., Tipton, P. A. & Gates, K. S. 2011. Kinetic Consequences of Replacing the Internucleotide Phosphorus Atoms in DNA with Arsenic. *ACS Chem Biol*, vol. 6: 127-130.
- Flora, S. J. S. 2011. Arsenic-induced oxidative stress and its reversibility. *Free Radical Biology and Medicine*, vol. 51: 257-281.
- Frausto da Silva, F. I. J. R. & Williams, R. S. P. 2001. *The biological chemistry of the elements*, 2nd edn. Oxford University Press, Oxford.

- Frei, R., Gaucher, C., Poulton, S. W. & Canfield, D. E. 2009. Fluctuations in Precambrian atmospheric oxygenation recorded by chromium isotopes. *Nature*, vol. 461(7261): 250–253.
- Gauthier-Lafaye, F. 2006. Time constraint for the occurrence of uranium deposits and natural fission reactors in the Paleoproterozoic Franceville Basin (Gabon). In: Kesler, S. E. & Ohmoto (eds.), *Evolution of Early Earth's Atmosphere, Hydrosphere, and Biosphere: Constraints from Ore Deposits*, 157-167. The Geological Society of America, Inc., Boulder, USA.
- German, C. R., Campbell, A. C. & Edmond, J. M. 1991. Hydrothermal scavenging at the Mid-Atlantic Ridge: Modification of trace element dissolved fluxes. *Earth and Planetary Science Letters*, vol. 107: 101-114.
- Glass, J. B., Wolfe-Simon, F. & Anbar, A. D. 2009. Coevolution of metal availability and nitrogen assimilation in cyanobacteria and algae. *Geobiology*, vol. 7: 100-123.
- Gruhl, J. W. 2005. Evolution of photosynthesis and biospheric oxygenation contingent upon nitrogen fixation? *International Journal of Astrobiology*, vol. 4: 251-257.
- Halverson, G. P., Wade, B. P., Hurtgen, M. T. & Barovich, K. M. 2010. Neoproterozoic chemostratigraphy. *Precambrian Research*, vol. 182: 337-350.
- Hammarlund, E. U. 2012. Ocean chemistry and the evolution of multicellularity. *Meddelanden från Stockholms universitets institution för geologiska vetenskaper*, vol. 350: 1-51.
- Han, T.-M. & Runnegar, B. 1992. Megascopic eukaryotic algae from the 2.1-billion-year-old Negaunee Iron-Formation, Michigan. *Science*, vol. 257: 232-235.
- Handley, K. M., Héry, M. & Lloyd, J. R. 2009. Redox cycling of arsenic by the hydrothermal marine bacterium *Marinobacter santoriniensis*. *Environmental Microbiology*, vol. 11: 1601-1611.
- Heinrich-Salmeron, A., Corid, A., Brochier-Armanet, C., Halter, D., Pagnout, C., Abbaszadeh-fard, E., Montaut, D., Seby, F., Bertin, P. N., Bauda, P. & Ploetze-Arsène, F. 2011. Unsuspected Diversity of Arsenite-Oxidizing Bacteria as Revealed by Widespread Distribution of the *aoxB* Gene in Prokaryotes. *Appl Environ Microb*, vol. 77: 4685-4692.
- Hofman, H. J., Grey, K., Hickman, A. H. & Thorpe, R. I. 1999. Origin of 3.45 Ga coniform stromatolites in Warrawoona Group, Western Australia. *Geol Soc Am Bull* vol. 111: 1256-1262.
- Holland, H. D. 1984. *The Chemical Evolution of the Atmosphere and Oceans*. Princeton University Press, Princeton.
- Holland, H. D. 2006. The oxygenation of the atmosphere and oceans. *Philos Trans R Soc Lond B Biol Sci*, vol. 361: 903-915.

- Hu, Q. H., Sun, G. X., Gao, X. B. & Zhu, Y. G. 2012. Conversation, sorption, and transport of arsenic species in geological media. *Applied Geochemistry*; doi:10.1016/j.apgeochem.2012.01.012.
- James, H. L. 1954. Sedimentary facies of iron formation. *Economic Geology*, vol. 49: 235-293.
- Javaux, E. 2011. Early eukaryotes in Precambrian oceans. In: Gargaud, M., López-García, P. & Martin, H. (eds.), *Origins and Evolution of life: An Astrobiological Perspective*, 414-449. Cambridge University Press, Cambridge, UK.
- Johnston, R. B. & Singer, P. C. 2007. Redox reactions in the Fe-As-O₂ system. *Chemosphere*, vol. 69: 517-525.
- Johnston, D. T., Wolfe-Simon, F., Pearson, A. & Knoll, A. H. 2009. Anoxygenic photosynthesis modulated Proterozoic oxygen and sustained Earth's middle age. *PNAS*, vol. 106: 16925-16929.
- Kappler, A., Pasquero, C., Konhauser, K. O. & Newman, D. K. 2005. Deposition of banded iron formations by anoxygenic phototrophic Fe(II)-oxidizing bacteria. *Geology*, vol. 33: 865-868.
- Kasting, J. F. 2005. Methane and climate during the Precambrian era. *Precambrian Research*, vol. 137: 119-129.
- Kasting, J. F. & Ono, S. 2006. Palaeoclimates: the first two billion years. *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 361: 917-929.
- Kaur, S., Kamli, M. R. & Ali, A. 2011. Role of arsenic and its resistance in nature. *Canadian Journal of Microbiology*, vol. 57: 769-774.
- Kendall, B., Gordon, G. W., Poulton, S. W. & Anbar, A. D. 2011. Molybdenum isotope constraints on the extent of late Paleoproterozoic ocean euxinia. *Earth and Planetary Science Letters*, vol. 307: 450-460.
- Kendall, B., Reinhard, C. T., Lyons, T., Kaufman, A. J., Poulton, S. W. & Anbar, A. D. 2010. Pervasive oxygenation along late Archaean ocean margins. *Nature Geoscience*, vol. 3: 647-652.
- Klein, C. & Ladeira, E. A. 2004. Geochemistry and mineralogy of Neoproterozoic banded Iron-Formations and some selected, siliceous manganese formations from the Urucum district, Mato Grosso do Sul, Brazil. *Economic Geology*, vol. 99:1233-1244.
- Klein, C. 2005. Some Precambrian banded iron-formations (BIFs) from around the world: Their age, geologic setting, mineralogy, metamorphism, geochemistry, and origins. *American Mineralogist*, vol. 90: 1473-1499.
- Knauth, L. P. 2005. Temperature and salinity history of the Precambrian ocean: implications for the course of microbial evolution. *Palaeogeography, Palaeoclimatology, Palaeoecology*, vol. 219: 53-69.

- Knauth, L. P. & Kennedy, M. J. 2009. The late Precambrian greening of the Earth. *Nature*, vol. 460: 728-732.
- Knoll, A. 2008. Cyanobacteria and Earth History. In: Herrero, A. & Flores, E. (eds.), *The Cyanobacteria – Molecular Biology, Genetics and Evolution*, 1-20. Caister Academic Press, Norfolk, UK.
- Knoll, A. 2011. The Multiple Origins of Complex Multicellularity. *Annu. Rev. Earth Planet. Sci.*, vol. 39: 217-239.
- Konhauser, K. O., Hamade, T., Raiswell, R., Morris, R. C., Ferris, F. G., Southam, G. & Canfield, D. E. 2002. Could bacteria have formed the Precambrian banded iron formations? *Geology*, vol. 30: 1079-1082.
- Konhauser, K. O. & Riding, R. 2012. Bacterial Biomineralisation. In: Knoll, A. H., Canfield, D. E. & Konhauser, K. O., *Fundamentals of Geobiology*, 105-130. John Wiley & Sons, Ltd, Chichester, UK.
- Kopp, R. E., Kirschvink, J. L., Hilburn, I. A. & Nash, C. Z. 2005. The Paleoproterozoic snowball Earth: A climatic disaster triggered by the evolution of oxygenic photosynthesis. *PNAS*, vol. 102: 11131-11136.
- Kuhn, W. R. & Atreya, S. K. 1979. Ammonia photolysis and the greenhouse effect in the primordial atmosphere of the Earth. *Icarus*, vol. 37: 207- 213.
- Kump, L. R., Pavlov, A. & Arthur, M. A. 2005. Massive release of hydrogen sulfide to the surface ocean and atmosphere during intervals of oceanic anoxia. *Geology*, vol. 33: 397-400.
- Lasaga, M. A., Holland, H. D. & Dwyer, M. J. 1971. Primordial Oil Slick. *Science*, vol. 174: 53-55.
- Ledbetter, R. N. & Magnuson, T. S. 2010. The Geomicrobiology of Arsenic. In: Barton, L. L., Mandl, M. & Lay, A. (eds.), *Geomicrobiology: Molecular and Environmental Perspective*, 147-168. Springer, Netherlands.
- Li, C., Love, G. D., Lyons, T. W., Fike, D. A., Sessions, A. L. & Chu, X. 2010. A Stratified Redox Model for the Ediacaran Ocean. *Science*, vol. 328: 80-83.
- Lièvremon, D., Bertin, P. N. & Lett, M-C. 2009. Arsenic in contaminated waters: Biogeochemical cycle, microbial metabolism and biotreatment processes. *Biochimie*, vol. 91: 1229-1237.
- Liu, S. X., Athar, M., Lippai, I., Waldren, C. & Hei, T. K. 2001. Induction of oxyradicals by arsenic: Implications for mechanism of genotoxicity. *PNAS*, vol. 98: 1643-1648.
- Lloyd, D. 2006. Hydrogen sulfide: clandestine microbial messenger? *Trends in Microbiology*, vol. 14: 456-462.

- Lunine, J. I. 2006. Physical conditions on the early Earth. *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 361: 1721-1731.
- Lyons, T. W. & Gill, B. C. 2010. Ancient Sulphur Cycling and Oxygenation of the Early Biosphere. *Elements*, vol. 6: 93-99.
- Lyons, T. W. & Reinhard, C. T. 2009. Oxygen for heavy-metal fans. *Nature*, vol. 461: 179 -181.
- Lyons, T. W. & Reinhard, C. T. 2011. Sea change for the rise in oxygen. *Nature*, vol. 478: 194 -195.
- Maciaszczyk-Dziubinska, E., Wawrzycka, D. & Wysocki, R. 2012. Arsenic and Antimony Transporters in Eukaryotes. *International Journal of Molecular Sciences*, vol. 13: 3527-3548.
- Mandal, B. K. & Suzuki, K. T. 2002. Arsenic round the world: a review. *Talanta*, vol. 58: 201-235.
- Mason, B. & Moore, C. B. 1982. *Principles of geochemistry*. Fourth edition. John Wiley & Sons, Inc, USA.
- McCollom, T. M. & Seewald, J. S. 2006. Carbon isotope composition of organic compounds produced by abiotic synthesis under hydrothermal conditions. *Earth and Planetary Science Letters*, vol. 243: 74-84.
- McKillup, S. & Dyar, M. D. 2012. *Geostatistics Explained – An Introductory Guide for Earth Scientists*. Cambridge University Press, Cambridge, UK.
- Melezhik, V. A. 2006. Multiple causes of Earth's earliest global glaciation. *Terra Nova*, vol. 18: 130-137.
- Miller, S. L. 1953. A Production of Amino Acids under Possible Primitive Earth Conditions. *Science*, vol. 117: 528–529.
- Miller, S. R. & Bebout, B. M. 2004. Variation in sulfide tolerance of photosystem II in phylogenetically diverse cyanobacteria from sulphidic habitats. *Applied and Environmental Microbiology*, vol. 70: 736-744.
- Moorhead, G. B. G., De Wever, V., Templeton, G. & Kerk, D. 2009. Evolution of protein phosphatases in plants and animals. *Biochem J*, vol. 417: 401-409.
- Mukhopadhyay, R., Rosen, B. P., Phung, L. T. & Silver, S. 2006. Microbial arsenic: from geocycles to genes and anzymes. *FEMS microbiology reviews*, vol. 26: 311-325.
- Nagy, M. L., Johansen, J. R., St. Clair, L. L. & Webb, B. L. 2005. Recovery patterns of microbial soil crusts 70 years after arsenic contamination. *Journal of Arid Environments*, vol. 63: 304-323.

- Nascimento, V. A., Melnikov, P. & Consolo, L. Z. Z. 2012. Computerized Modeling of Adenosine Triphosphate, Adenosine Triarsenate and Adenosine Trivanadate. *Molecules*, vol. 17: 9489-9495.
- Och, L. & Shields-Zhou, G. 2012. The Neoproterozoic oxygenation event: Environmental perturbations and biogeochemical cycling. *Earth Science Reviews*, vol. 110: 26-57.
- O'Day, P. A., Vlassopoulos, D., Root, R. & Rivera, N. 2004. The influence from sulphur and iron on dissolved arsenic concentrations in the shallow subsurface under changing redox conditions. *Proc. Natl. Acad. Sci. USA*, vol. 101: 13703-13708.
- O'Day, P. 2006. Chemistry and mineralogy of arsenic. *Elements*, vol. 2: 77-83.
- Onishi, H. & Sandell, E. B. 1955. Geochemistry of arsenic. *Geochimica et Cosmochimica Acta*, vol. 7: 1-33.
- Oremland, R. S., Saltikov, C. W., Wolfe-Simon, F. & Stolz, J. F. 2009. Arsenic in the Evolution of Earth and Extraterrestrial Ecosystems. *Geomicrobiology Journal*, vol. 26: 522-536.
- Oremland, R. S. & Stoltz, J. F. 2003. The Ecology of Arsenic. *Science*, vol. 300: 939-944.
- Papineau, D. 2010. Mineral Environments on the Earliest Earth. *Elements*, vol. 6: 25-30.
- Parnell, J., Hole, M., Boyce, A. J., Spinks, S. & Bowden, S. 2012. Heavy metal, sex and granites: Crustal differentiation and bioavailability in the mid-Proterozoic. *Geology*, vol. 40: 751-754.
- Peretó, J. 2011. Origin and evolution of metabolisms. In: Gargaud, M., López-García, P. & Martin, H. (eds.), *Origins and Evolution of life: An Astrobiological Perspective*, 270-287. Cambridge University Press, Cambridge, UK.
- Peters, S. E. & Gaines, R. R. 2012. Formation of the 'Great Unconformity' as a trigger for the Cambrian explosion. *Nature*, vol. 484: 363-366.
- Petsch, S. T. 2004. The Global Oxygen Cycle. In: Holland, H. D. & Turekian, K. K. (eds.), *Treatise on Geochemistry*, vol. 8: 515-555. Elsevier, Amsterdam, Netherlands.
- Pierrehumbert, R. T., Abbot, D. S., Voigt, A. & Koll, D. 2011. Climate of the Neoproterozoic. *Annual Review of Earth and Planetary Sciences*, vol. 39: 417-460.
- Planavsky, N., Rouxel, O., Bekker, A., Shapiro, R., Fralick, P. & Knudsen, A. 2009. Iron oxidizing microbial ecosystems thrived in late Paleoproterozoic redox-stratified oceans. *Earth and Planetary Science Letters*, vol. 286: 230-242.
- Planer-Friedrich, B., Franke, D., Merkel, B. & Wallschläger, D. 2008. Acute toxicity of thioarsenates to *Vibrio fischeri*. *Environmental Toxicology and Chemistry*, vol. 10: 2027-2035.

- Posth, N. R., Hegler, F., Konhauser, K. O. & Kappler, A. 2008. Alternating Si and Fe deposition caused by temperature fluctuations in Precambrian oceans. *Nature Geoscience*, vol. 1: 703-708.
- Poulton, S. W. & Canfield, D. E. 2005. Development of a sequential extraction procedure for iron: implications for iron partitioning in continentally derived particulates. *Chemical Geology*, vol. 214: 209-221.
- Poulton, S. W. & Canfield, D. E. 2011. Ferruginous Conditions: A Dominant Feature of the Ocean through Earth's History. *Elements*, vol. 7: 107-112.
- Poulton, S. W., Fralick, P. W. & Canfield, D. E. 2004. The transition to a sulphidic ocean ~1.84 billion years ago. *Nature*, vol. 431: 173-177.
- Poulton, S. W., Fralick, P. W. & Canfield, D. E. 2010. Spatial variability in oceanic redox structure 1.8 billion years ago. *Nature Geoscience*, vol. 3: 486-490.
- Reaves, M. L., Sinha, S., Rabinowitz, J. D., Kruglyak, L. & Redfield, R. J. 2012. Absence of Detectable Arsenate in DNA from Arsenate-Grown GFAJ-1 Cells. *Science*, vol. 337: 470-473.
- Reiffenstein, R. J., Hulbert, W. C. & Roth, S. H. 1992. Toxicology of Hydrogen-Sulfide. *Annual Review of Pharmacology and Toxicology*, vol. 32: 109-134.
- Reuschel, S., Mehezhik, V. A. Strauss, H. 2012. Sulfur isotopic trends and iron speciation from the c. 2.0 Ga Pilgūjärvi Sedimentary Formation, NW Russia. *Precambrian Research*, vol. 196-197: 193-203.
- Roberts, N. M. W. 2013. The boring billion? – Lid tectonics, continental growth and environmental change associated with the Columbia supercontinent. *Geoscience Frontiers*, in press: 1-11.
- Rollinson, H. 2007. *Early Earth Systems – A Geochemical Approach*. 1-285. Blackwell Publishing Ltd, Singapore.
- Rosen, B. P., Ajees, A. A. & McDermott, T. R. 2011. Life and death with arsenic. *Bioessays*, vol. 33: 350-357.
- Saito, M. A., Sigman, D. M. & Morel, F. M. M. 2003. The bioinorganic chemistry of the ancient ocean: the co-evolution of cyanobacterial metal requirements and biogeochemical cycles at the Archean-Proterozoic boundary? *Inorganica Chimica Acta*, vol. 356: 308-318.
- Saito, M. A. 2009. Less nickel for more oxygen. *Nature*, vol. 458: 714-715.
- Sarkar, A., Chakraborty, P. P., Mishra, B., Bera, M. K., Sanyal, P. & Paul, S. 2010. Mesoproterozoic sulphidic ocean, delayed oxygenation and evolution of early life: sulphur isotope clues from Indian Proterozoic basins. *Geological Magazine*, vol. 147: 206-218.

- Schaefer, L. & Fegley, B. 2007. Outgasing of ordinary chondritic material and some of its implications for the chemistry of asteroids, planets, and satellites. *Icarus*, vol. 186: 462-483.
- Schidlowski, M., Appel, P. W. U., Eichmann, R. & Junge, C. E. 1979. Carbon isotope geochemistry of the 3.7 x 10⁹-yr-old Isua sediments, West Greenland: implications for the Archaean carbon and oxygen cycles. *Geochimica et Cosmochimica Acta*, vol. 43: 189-199.
- Schidlowski, M. 1988. A 3,800-million-year isotopic record of life from carbon in sedimentary rocks. *Nature*, vol. 333: 313-318.
- Schoepp-Cothenet, B., Nitschke, W., Barge, L. M., Ponce, A., Russel, M. J. & Tsapin, A. I. 2011. Comment on "A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus". *Science*, vol. 332: 1149-d.
- Scott, C., Lyons, T. W., Bekker, A., Shen, Y., Poulton, S. W., Chu, X. & Anbar, A. D. 2008. Tracing the stepwise oxygenation of the Proterozoic ocean. *Nature*, vol. 452: 456-459.
- Senn, D. B. & Hemond, H. F. 2002. Nitrate controls on Iron and Arsenic in an Urban Lake. *Science*, vol. 296: 2373-2376.
- Sessions, A. L., Doughty, D. M., Welander, P. V. Summons, R. E. & Newman, D. K. 2009. The Continuing Puzzle of the Great Oxidation Event. *Current Biology*, vol. 19: R567-R574.
- Sharma, V. K. & Sohn, M. 2009. Aquatic arsenic: Toxicity, speciation, transformations, and remediation. *Environment International*, vol. 35: 743-759.
- Shields-Zhou, G. & Och, L. 2011. The case for a Neoproterozoic Oxygenation Event: Geochemical evidence and biological consequences. *GSAT*, vol. 21: 4-11.
- Schopf, J. W. & Packer, B. M. 1987. Early Archean (3.3-billion to 3.5-billion-year-old) microfossils from Warrawoona Group, Australia. *Science*, vol. 237: 70-73.
- Smedley, P. L. & Kinniburgh, D. G. 2002. A review of the source, behaviour and distribution of arsenic in natural waters. *Applied Geochemistry*, vol. 17: 517-568.
- Stolz, J. F., Basu, P. & Oremland, R. S. 2010. Microbial arsenic metabolism: new twists on an old poison. *Microbe*, vol. 5: 53-59.
- Suess, E. & Planer-Friedrich, B. 2012. Thioarsenate formation upon dissolution of orpiment and arsenopyrite. *Chemosphere*, vol. 89: 1390-1398.
- Suess, E., Wallschläger, D. & Planer-Friedrich, B. 2011. Stabilization of thioarsenates in iron-rich waters. *Chemosphere*, vol. 83: 1524-1531.

- Summons, R. E., Jahnke, L. L., Hope, J. M. & Logan, G. A. 1999. 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature*, vol. 400: 554-557.
- Tadanier, C. J., Schreiber, M. E. & Roller, J. W. 2005. Arsenic Mobilization through Microbially Medated Deflocculation of Ferrihydrite. *Environmental Science and Technology*, vol. 39: 3061-3068.
- Tamás, M. J. & Wysocki, R. 2001. Mechanisms involved in metalloid transport and tolerance acquisition. *Current genetics*, vol. 40: 2-12.
- Tyler, R. & Tyler, N. 1996. Stratigraphic and structural controls on gold mineralization in the Pilgrim's Rest goldfield, eastern Transvaal, South Africa. *Precambrian Research*, vol. 79: 141-169.
- van Zuilen, M. A., Lepland, A. & Arrhenius, G. 2002. Reassessing the evidence for the earliest traces of life. *Nature*, vol. 418: 627-630.
- Vaughan, D. J. 2006. Arsenic. *Elements*, vol. 2: 71-75.
- Veizer, J. 1983. Geologic Evolution of the Archean – Early Proterozoic Earth. In: Schopf, J. W. (ed.), *Earth's Earliest Biosphere, its Origin and Evolution*, 240-259. Princeton University Press, New Jersey, USA.
- Wacey, D., Kilburn, M. R., Saunders, M., Cliff, J. & Brasier, M. D. 2011. Microfossils of sulphur-metabolizing cells in 3.4-billion-year-old rocks of Western Australia. *Nature Geoscience*, vol. 4: 698-702.
- Walker, J. C. G., Klein, C., Schidlowski, M., Schopf, J. W., Stevenson, D. J., & Walter, M. R. 1983. Environmental evolution of the Archean-Early Proterozoic Earth. In: Schopf, J. W. (ed.), *Earth's Earliest Biosphere, its Origin and Evolution*, 260-290. Princeton University Press, New Jersey, USA.
- Wallmann, K. & Aloisi, G. 2012. The Global Carbon Cycle: Geological Processes. In: Knoll, A. H., Canfield, D. E. & Konhauser, K. O. (eds.), *Fundamentals of Geobiology*, 20-35. John Wiley & Sons Ltd, Chichester, UK.
- Wang, S. & Mulligan, C. N. 2006. Occurrence of arsenic contamination in Canada: Sources, behavior and distribution. *Science of the Total Environment*, vol. 366: 701-721.
- Watanabe, Y., Naraoka, H., Wronkiewicz, D. J., Condie, K. C. & Ohmoto, H. 1997. Carbon, nitrogen, and sulfur geochemistry of Archean and Proterozoic shales from the Kaapvaal Craton, South Africa. *Geochimica et Cosmochimica Acta*, vol. 61: 3441-3459.
- WHO. 2010. Exposure to arsenic: a major public health concern. Geneva: World Health Organization (WHO): 2013-10-03.
- Williams, R. J. P. 2012. Zinc in evolution. *Journal of Inorganic Biochemistry*, vol. 111: 104-109.

- Wilde, S. A., Valley, J. W., Peck, W. H. & Graham, C. M. 2001. Evidence from detrital zircons for the existence of continental crust and oceans on the Earth 4.4 Gyr ago. *Nature*, vol. 409: 175-178.
- Wilkin, R. T., Wallschläger, D. & Ford, R. G. 2003. Speciation of arsenic in sulfidic waters. *Geochem. Trans.*, vol. 4(1): 1-7.
- Wolfe-Simon, F., Blum, J. S., Kulp, T. R., Gordon, G. W., Hoefft, S. E., Pett-Ridge, J., Stolz, J. F., Webb, D. M., Weber, P. K. & Davies, P. C. W. 2011. A bacterium that can grow by using arsenic instead of phosphorus. *Science*, vol. 332(6034): 1163-1166.
- Wronkiewicz, D. J. & Condie, K. J. 1990. Geochemistry and mineralogy of sediments from the Ventersdorp and Transvaal Supergroups, South Africa: Cratonic evolution during the early Proterozoic. *Geochimica et Cosmochimica Acta*, vol. 54: 343-354.
- Zahnle, K., Schaefer, L. & Fegley, B. 2010. Earth's Earliest Atmosphere. *Cold Spring Harb Perspect Biol* 2010;2:a004895.
- Zalasiewicz, J. & Williams, M. 2012. The goldilocks planet – the four billion year story of earth's climate. 1-303. Oxford University Press, New York, USA.

Appendix

Appendix 1 Sample information including age, formation, lithology and other details.

| Sample | Approx. age | Formation | Lithology | Details |
|---------------|-------------|---------------------------------|------------------|---|
| FD 9 | 2077 | FD, Francevillian Group, Gabon | | |
| FD 16 | 2079 | FD, Francevillian Group, Gabon | | |
| FD 26 | 2081 | FD, Francevillian Group, Gabon | | |
| FD 1V 44 | 2085 | FD, Francevillian Group, Gabon | | |
| FD 1V 64 | 2089 | FD, Francevillian Group, Gabon | | |
| Comilog 40 | 2093 | FB2, Francevillian Group, Gabon | Black shale | |
| Comilog 48 | 2094 | FB2, Francevillian Group, Gabon | Black shale | Some laminations |
| Comilog 63 | 2095 | FB2, Francevillian Group, Gabon | Black shale | Possibly a slump |
| Comilog 85 | 2096 | FB2, Francevillian Group, Gabon | Black shale | 1 laminae of coarser (a bit weathered) |
| Comilog 100 | 2097 | FB2, Francevillian Group, Gabon | Black shale | |
| Comilog 117 | 2098 | FB2, Francevillian Group, Gabon | Black shale | White of gypsum (weathered) |
| Comilog 127 | 2099 | FB2, Francevillian Group, Gabon | Black shale | A few silt laminae |
| Comilog 134 | 2100 | FB2, Francevillian Group, Gabon | Black shale | White of gypsum |
| Comilog 140 | 2101 | FB2, Francevillian Group, Gabon | Black shale | |
| Comilog 163.1 | 2103 | FB2, Francevillian Group, Gabon | Black shale | With 0,5 cm pyritelaminae |
| Comilog 165.5 | 2103.3 | FB2, Francevillian Group, Gabon | Black shale | Crack of Si |
| Comilog 173.8 | 2104 | FB2, Francevillian Group, Gabon | Black shale | Slightly coarser |
| BA37 29.2 | 2110 | FB1, Francevillian Group, Gabon | Black shale | With siltstone laminations |
| BA37 34.4 | 2112 | FB1, Francevillian Group, Gabon | Black shale | With thin laminations |
| BA37 36.6 | 2113 | FB1, Francevillian Group, Gabon | Black shale | Above 2-3 layers of 0.5-1 cm thick siltstone layers |
| BA37 40.1 | 2114 | FB1, Francevillian Group, Gabon | Black shale | Ampelit |
| BA37 44.18 | 2115 | FB1, Francevillian Group, Gabon | Black shale, | Weak laminations, 1 event with paler shale |
| BA37 51.1 | 2116 | FB1, Francevillian Group, Gabon | Black shale | Dense |
| BA37 55.1 | 2117 | FB1, Francevillian Group, Gabon | Very black shale | Dense |
| BA37 62.45 | 2118 | FB1, Francevillian Group, Gabon | Very black shale | Chips |

| Sample | Approx. age | Formation | Lithology | Details |
|----------------|-------------|---|---|---|
| BA37 68.55 | 2119 | Group, Gabon FB1, Francevillian | Black shale | Stromatolites, pyritebands |
| BA37 76.75 | 2120 | Group, Gabon FB1, Francevillian | Very black shale | Chips |
| BA37 80.1 | 2121 | Group, Gabon FB1, Francevillian | Very black shale | Chips |
| BA37 84.75 | 2122 | Group, Gabon FB1, Francevillian | Black shale | Thick siltevent (1 cm) on top |
| BA37 90.2 | 2125 | Group, Gabon FB1, Francevillian | Very black shale | Chips |
| BA37 93.3 | 2127 | Group, Gabon FB1, Francevillian | Black shale | Dense/close to siltbeds |
| BA37 100.10 | 2131 | Group, Gabon FB1, Francevillian | Black shale | Dense/chips |
| BA37 105.1 | 2134 | Group, Gabon FB1, Francevillian | Black shale | Dense |
| BA37 114.5 | 2139 | Group, Gabon FB1, Francevillian | Strange shale | Possibly bioturbated |
| BA37 133.6 | 2145 | Group, Gabon FB1, Francevillian | Black shale | Some silt, dense |
| BA37 162.8 | 2155 | Group, Gabon FB1, Francevillian | Green/grey shale | Thin laminations |
| TF 1/72 49.75 | 2230 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | Black shale | Visible bedding and cleavage, pyrite in either lens bedding or in secondary fractures |
| TF 1/72 85 | 2234 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | Black shale | Visible bedding and cleavage |
| TF 1/72 112 | 2237 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | Black shale | Visible bedding and cleavage |
| TF 1/72 180.4 | 2243 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | Homogenous shale | |
| TF 1/72 214.4 | 2247 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | Quartzite with a few levels of decimetre thick shale | |
| TF 1/72 279.8 | 2254 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | Alternating shales | |
| TF 1/72 300.1 | 2258 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | Alternating shales | |
| TF 1/72 310.05 | 2260 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | Alternating thin shales (1-2 cm) with folds, quartz-breccia | |

| Sample | Approx. age | Formation | Lithology | Details |
|-----------------|----------------|---|--|---------|
| TF 1/72 320.3 | 2263 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | veins, and faults with quartz and pyrite Dominantly black shales, alternated with some very fine material | |
| TF 1/72 329.9 | 2265 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | Dominantly black shales, alternated with some very fine material | |
| TF 1/72 370 | 2273 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | Dominantly black shales, alternated with some very fine material | |
| TF 1/72 460 | 2284 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | Dominantly black shales, alternated with some very fine material | |
| TF 1/72 490 | 2290 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | Diabase? | |
| TF 1/72 568 | 2230 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | Black shale | |
| TF 1/72 570 | 2301 | Timeball Hill, Pretoria group, Transvaal supergroup, South Africa | Black shale | |
| TF 1/72 594.15 | 2510 | Lyttleton formation, Chuniespoort Group, Transvaal supergroup, South Africa | Black quartzite with interbedded shales | |
| TF 1/72 711.4 | 2450 | Frisco formation, Chunispoort group, Transvaal supergroup, South Africa | Dolomite | |
| TF 1/72 1066.95 | 2475 | Eccles formation, Chunispoort group, Transvaal supergroup, South Africa | Dolomite | |
| TF 1/72 1068.75 | 2480 | Eccles formation, Chunispoort group, Transvaal supergroup, South Africa | Dolomite | |
| TF 1/72 1213.7 | 2500 | Eccles formation, Chunispoort group, Transvaal supergroup, South Africa | Dolomite | |
| TF 1/72 1434.5 | 2515 | Lyttleton formation, Chunispoort group, | Dolomite | |

| Sample | Approx. age | Formation | Lithology | Details |
|-----------------|----------------|---|------------------------|---------|
| TF 1/72 1843.3 | 2525 | Transvaal supergroup, South Africa Monte Christo formation, Chunispoort group, Transvaal supergroup, South Africa | Dolomite | |
| TF 1/72 1852.7 | 2526 | Monte Christo formation, Chunispoort group, Transvaal supergroup, South Africa | Dolomite | |
| TF 1/72 2048 | 2527 | Monte Christo formation, Chunispoort group, Transvaal supergroup, South Africa | Dolomite | |
| TF 1/72 2138.2 | 2539 | Oaktree formation, Chunispoort group, Transvaal supergroup, South Africa | Dolomite | |
| TF 1/72 2239.21 | 2545 | Oaktree formation, Chunispoort group, Transvaal supergroup, South Africa | Dolomite | |
| TF 1/72 2391.8 | 2698 | Bothaville formation, Ventersdorp supergroup, South Africa | Dolomite and shales | |
| TF 1/72 2430 | 2700 | Bothaville formation, Ventersdorp supergroup, South Africa | Dolomite and shales | |
| TF 1/72 2440 | 2702 | Bothaville formation, Ventersdorp supergroup, South Africa | Dolomite and shales | |
| TF 1/72 2452.2 | 2705 | Bothaville formation, Ventersdorp supergroup, South Africa | Dolomite and shales | |
| Sc2007-07 | 1840 | Gunflint Formation, Animikie Group, Canada | | |
| Sc2007-07b | 1845 | Gunflint Formation, Animikie Group, Canada | | |
| Sc2007-08 | 1850 | Gunflint Formation, Animikie Group, Canada | | |
| Sc2007-09 | 1855 | Gunflint Formation, Animikie Group, Canada | | |
| Sc2007-10 | 1860 | Gunflint Formation, Animikie Group, Canada | | |
| 404111306 | 1590 | Vindhyan Supergroup, | | |

| Sample | Approx. age | Formation | Lithology | Details |
|----------|----------------|--|-----------|---------|
| 06111307 | 1600 | India Vindhyan Supergroup, India | | |

Appendix 2 Complete results from the Spearman correlation analysis, including all 65 samples from South Africa and Gabon.

| | | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|----|------------|---------|--------|--------|---------|---------|--------|---------|-------|-------|---------|---------|---------|--------|---------|-------|---------|---------|---------|---------|---------|--------|---------|
| Al | Corr Coeff | 1,000 | ,133 | ,563** | -,081 | -,513** | -,318* | ,293* | -,259 | ,177 | ,653** | -,195 | -,421** | ,373** | ,018 | -,035 | -,309* | ,135 | ,087 | ,658** | ,298* | -,088 | ,435** |
| | Sig. | . | ,439 | ,000 | ,548 | ,000 | ,033 | ,022 | ,111 | ,158 | ,000 | ,120 | ,001 | ,002 | ,889 | ,848 | ,012 | ,287 | ,516 | ,000 | ,023 | ,504 | ,001 |
| | N | 65 | 36 | 62 | 57 | 64 | 45 | 61 | 39 | 65 | 65 | 65 | 59 | 64 | 63 | 32 | 65 | 64 | 58 | 64 | 58 | 60 | 58 |
| As | Corr Coeff | ,133 | 1,000 | ,285 | -,140 | -,224 | ,238 | ,475** | ,085 | ,373* | ,368* | -,260 | ,232 | ,176 | ,132 | -,298 | ,092 | ,067 | -,090 | ,529** | ,604** | ,221 | ,600** |
| | Sig. | ,439 | . | ,097 | ,461 | ,189 | ,232 | ,004 | ,706 | ,025 | ,027 | ,125 | ,209 | ,305 | ,444 | ,202 | ,593 | ,696 | ,629 | ,001 | ,000 | ,217 | ,000 |
| | N | 36 | 36 | 35 | 30 | 36 | 27 | 35 | 22 | 36 | 36 | 36 | 31 | 36 | 36 | 20 | 36 | 36 | 31 | 35 | 32 | 33 | 33 |
| Ba | Corr Coeff | ,563** | ,285 | 1,000 | ,138 | -,210 | -,006 | ,015 | -,216 | ,162 | ,378** | -,363** | -,226 | ,356** | ,212 | -,108 | -,224 | -,022 | ,387** | ,343** | ,123 | ,011 | ,249 |
| | Sig. | ,000 | ,097 | . | ,317 | ,101 | ,970 | ,913 | ,198 | ,210 | ,002 | ,004 | ,094 | ,005 | ,100 | ,561 | ,080 | ,863 | ,003 | ,006 | ,360 | ,932 | ,059 |
| | N | 62 | 35 | 62 | 55 | 62 | 45 | 58 | 37 | 62 | 62 | 62 | 56 | 61 | 61 | 31 | 62 | 62 | 57 | 62 | 57 | 60 | 58 |
| C | Corr Coeff | -,081 | -,140 | ,138 | 1,000 | ,230 | -,082 | -,442** | ,100 | -,095 | -,315* | ,020 | ,086 | ,198 | ,258 | ,160 | ,256 | -,354** | ,286* | -,318* | -,286* | ,107 | -,132 |
| | Sig. | ,548 | ,461 | ,317 | . | ,089 | ,613 | ,001 | ,580 | ,481 | ,017 | ,882 | ,548 | ,143 | ,057 | ,425 | ,054 | ,007 | ,042 | ,016 | ,040 | ,448 | ,356 |
| | N | 57 | 30 | 55 | 57 | 56 | 40 | 53 | 33 | 57 | 57 | 57 | 51 | 56 | 55 | 27 | 57 | 56 | 51 | 57 | 52 | 53 | 51 |
| Ca | Corr Coeff | -,513** | -,224 | -,210 | ,230 | 1,000 | ,094 | -,598** | -,199 | -,061 | -,570** | ,599** | ,439** | -,009 | ,414** | -,073 | ,432** | ,124 | ,061 | -,585** | -,714** | ,017 | -,565** |
| | Sig. | ,000 | ,189 | ,101 | ,089 | . | ,541 | ,000 | ,230 | ,632 | ,000 | ,000 | ,001 | ,943 | ,001 | ,698 | ,000 | ,328 | ,654 | ,000 | ,000 | ,899 | ,000 |
| | N | 64 | 36 | 62 | 56 | 64 | 45 | 60 | 38 | 64 | 64 | 64 | 58 | 63 | 63 | 31 | 64 | 64 | 57 | 63 | 57 | 60 | 58 |
| Co | Corr Coeff | -,318* | ,238 | -,006 | -,082 | ,094 | 1,000 | -,142 | ,204 | ,283 | -,223 | -,016 | ,200 | -,193 | ,192 | ,318 | ,075 | ,013 | ,069 | -,039 | ,254 | ,204 | -,153 |
| | Sig. | ,033 | ,232 | ,970 | ,613 | ,541 | . | ,359 | ,289 | ,060 | ,141 | ,918 | ,223 | ,205 | ,212 | ,099 | ,622 | ,933 | ,666 | ,799 | ,100 | ,190 | ,321 |
| | N | 45 | 27 | 45 | 40 | 45 | 45 | 44 | 29 | 45 | 45 | 45 | 39 | 45 | 44 | 28 | 45 | 45 | 42 | 45 | 43 | 43 | 44 |
| Cr | Corr Coeff | ,293* | ,475** | ,015 | -,442** | -,598** | -,142 | 1,000 | ,031 | -,084 | ,700** | -,249 | -,335* | -,136 | -,466** | -,155 | -,211 | ,278* | -,434** | ,655** | ,707** | -,212 | ,584** |
| | Sig. | ,022 | ,004 | ,913 | ,001 | ,000 | ,359 | . | ,856 | ,519 | ,000 | ,053 | ,012 | ,298 | ,000 | ,406 | ,102 | ,031 | ,001 | ,000 | ,000 | ,117 | ,000 |
| | N | 61 | 35 | 58 | 53 | 60 | 44 | 61 | 37 | 61 | 61 | 61 | 55 | 60 | 60 | 31 | 61 | 60 | 55 | 60 | 57 | 56 | 57 |
| Cu | Corr Coeff | -,259 | ,085 | -,216 | ,100 | -,199 | ,204 | ,031 | 1,000 | -,161 | -,007 | -,130 | ,477** | -,105 | -,183 | ,332 | ,269 | -,194 | ,042 | -,200 | ,233 | ,386* | ,149 |
| | Sig. | ,111 | ,706 | ,198 | ,580 | ,230 | ,289 | ,856 | . | ,326 | ,965 | ,430 | ,003 | ,523 | ,277 | ,091 | ,098 | ,244 | ,807 | ,223 | ,178 | ,018 | ,394 |
| | N | 39 | 22 | 37 | 33 | 38 | 29 | 37 | 39 | 39 | 39 | 39 | 37 | 39 | 37 | 27 | 39 | 38 | 36 | 39 | 35 | 37 | 35 |
| Fe | Corr Coeff | ,177 | ,373* | ,162 | -,095 | -,061 | ,283 | -,084 | -,161 | 1,000 | -,087 | ,051 | ,065 | ,301* | ,477** | -,142 | ,014 | -,077 | ,042 | ,205 | ,201 | ,434** | ,196 |
| | Sig. | ,158 | ,025 | ,210 | ,481 | ,632 | ,060 | ,519 | ,326 | . | ,492 | ,685 | ,626 | ,016 | ,000 | ,437 | ,911 | ,546 | ,757 | ,105 | ,131 | ,001 | ,141 |
| | N | 65 | 36 | 62 | 57 | 64 | 45 | 61 | 39 | 65 | 65 | 65 | 59 | 64 | 63 | 32 | 65 | 64 | 58 | 64 | 58 | 60 | 58 |
| K | Corr Coeff | ,653** | ,368* | ,378** | -,315* | -,570** | -,223 | ,700** | -,007 | -,087 | 1,000 | -,264* | -,279* | ,057 | -,161 | -,071 | -,388** | ,277* | -,217 | ,731** | ,612** | -,266* | ,573** |
| | Sig. | ,000 | ,027 | ,002 | ,017 | ,000 | ,141 | ,000 | ,965 | ,492 | . | ,033 | ,032 | ,652 | ,206 | ,697 | ,001 | ,027 | ,101 | ,000 | ,000 | ,040 | ,000 |
| | N | 65 | 36 | 62 | 57 | 64 | 45 | 61 | 39 | 65 | 65 | 65 | 59 | 64 | 63 | 32 | 65 | 64 | 58 | 64 | 58 | 60 | 58 |

| | | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|----|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Mg | Corr Coeff | -,195 | -,260 | -,363** | ,020 | ,599** | -,016 | -,249 | -,130 | ,051 | -,264* | 1,000 | ,413** | ,005 | ,145 | -,319 | ,483** | ,188 | -,252 | -,259* | -,434** | ,066 | -,505** |
| | Sig. N | ,120 65 | ,125 36 | ,004 62 | ,882 57 | ,000 64 | ,918 45 | ,053 61 | ,430 39 | ,685 65 | ,033 65 | . | ,001 59 | ,967 64 | ,258 63 | ,075 32 | ,000 65 | ,136 64 | ,056 58 | ,039 64 | ,001 58 | ,615 60 | ,000 58 |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Mn | Corr Coeff | -,421** | ,232 | -,226 | ,086 | ,439** | ,200 | -,335* | ,477** | ,065 | -,279* | ,413** | 1,000 | -,347** | ,243 | ,253 | ,367** | -,012 | -,032 | -,410** | -,117 | ,316* | -,055 |
| | Sig. N | ,001 59 | ,209 31 | ,094 56 | ,548 51 | ,001 58 | ,223 39 | ,012 55 | ,003 37 | ,626 59 | ,032 59 | ,001 59 | . | ,008 58 | ,069 57 | ,162 32 | ,004 59 | ,928 58 | ,818 53 | ,001 58 | ,410 52 | ,017 56 | ,700 52 |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Na | Corr Coeff | ,373** | ,176 | ,356** | ,198 | -,009 | -,193 | -,136 | -,105 | ,301* | ,057 | ,005 | -,347** | 1,000 | ,264* | -,329 | ,154 | -,164 | ,231 | ,217 | -,073 | ,159 | -,058 |
| | Sig. N | ,002 64 | ,305 36 | ,005 61 | ,143 56 | ,943 63 | ,205 45 | ,298 60 | ,523 39 | ,016 64 | ,652 64 | ,967 64 | ,008 58 | . | ,038 62 | ,066 32 | ,224 64 | ,200 63 | ,084 57 | ,088 63 | ,588 57 | ,228 59 | ,666 57 |
| | | | | | | | | | | | | | | | | | | | | | | | |
| P | Corr Coeff | ,018 | ,132 | ,212 | ,258 | ,414** | ,192 | -,466** | -,183 | ,477** | -,161 | ,145 | ,243 | ,264* | 1,000 | ,224 | ,258* | -,043 | ,354** | -,003 | -,264* | ,320* | -,033 |
| | Sig. N | ,889 63 | ,444 36 | ,100 61 | ,057 55 | ,001 63 | ,212 44 | ,000 60 | ,277 37 | ,000 63 | ,206 63 | ,258 63 | ,069 57 | ,038 62 | . | ,235 30 | ,041 63 | ,736 63 | ,007 56 | ,982 62 | ,050 56 | ,014 59 | ,807 57 |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Pb | Corr Coeff | -,035 | -,298 | -,108 | ,160 | -,073 | ,318 | -,155 | ,332 | -,142 | -,071 | -,319 | ,253 | -,329 | ,224 | 1,000 | -,328 | ,151 | ,345 | -,122 | -,002 | ,152 | ,243 |
| | Sig. N | ,848 32 | ,202 20 | ,561 31 | ,425 27 | ,698 31 | ,099 28 | ,406 31 | ,091 27 | ,437 32 | ,697 32 | ,075 32 | ,162 32 | ,066 32 | ,235 30 | . | ,066 32 | ,417 31 | ,053 32 | ,504 32 | ,993 30 | ,416 31 | ,196 30 |
| | | | | | | | | | | | | | | | | | | | | | | | |
| S | Corr Coeff | -,309* | ,092 | -,224 | ,256 | ,432** | ,075 | -,211 | ,269 | ,014 | -,388** | ,483** | ,367** | ,154 | ,258* | -,328 | 1,000 | -,222 | -,150 | -,200 | -,024 | ,318* | -,305* |
| | Sig. N | ,012 65 | ,593 36 | ,080 62 | ,054 57 | ,000 64 | ,622 45 | ,102 61 | ,098 39 | ,911 65 | ,001 65 | ,000 65 | ,004 59 | ,224 64 | ,041 63 | ,066 32 | . | ,078 64 | ,260 58 | ,113 64 | ,856 58 | ,013 60 | ,020 58 |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Si | Corr Coeff | ,135 | ,067 | -,022 | -,354** | ,124 | ,013 | ,278* | -,194 | -,077 | ,277* | ,188 | -,012 | -,164 | -,043 | ,151 | -,222 | 1,000 | -,226 | ,354** | ,044 | -,079 | ,190 |
| | Sig. N | ,287 64 | ,696 36 | ,863 62 | ,007 56 | ,328 64 | ,933 45 | ,031 60 | ,244 38 | ,546 64 | ,027 64 | ,136 64 | ,928 58 | ,200 63 | ,736 63 | ,417 31 | ,078 64 | . | ,091 57 | ,004 63 | ,744 57 | ,551 60 | ,153 58 |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Sr | Corr Coeff | ,087 | -,090 | ,387** | ,286* | ,061 | ,069 | -,434** | ,042 | ,042 | -,217 | -,252 | -,032 | ,231 | ,354** | ,345 | -,150 | -,226 | 1,000 | -,301* | -,206 | -,146 | -,035 |
| | Sig. N | ,516 58 | ,629 31 | ,003 57 | ,042 51 | ,654 57 | ,666 42 | ,001 55 | ,807 36 | ,757 58 | ,101 58 | ,056 58 | ,818 53 | ,084 57 | ,007 56 | ,053 32 | ,260 58 | ,091 57 | . | ,021 58 | ,136 54 | ,289 55 | ,802 54 |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Ti | Corr Coeff | ,658** | ,529** | ,343** | -,318* | -,585** | -,039 | ,655** | -,200 | ,205 | ,731** | -,259* | -,410** | ,217 | -,003 | -,122 | -,200 | ,354** | -,301* | 1,000 | ,741** | -,024 | ,726** |
| | Sig. N | ,000 64 | ,001 35 | ,006 62 | ,016 57 | ,000 63 | ,799 45 | ,000 60 | ,223 39 | ,105 64 | ,000 64 | ,039 64 | ,001 58 | ,088 63 | ,982 62 | ,504 32 | ,113 64 | ,004 63 | ,021 58 | . | ,000 58 | ,854 60 | ,000 58 |
| | | | | | | | | | | | | | | | | | | | | | | | |
| V | Corr Coeff | ,298* | ,604** | ,123 | -,286* | -,714** | ,254 | ,707** | ,233 | ,201 | ,612** | -,434** | -,117 | -,073 | -,264* | -,002 | -,024 | ,044 | -,206 | ,741** | 1,000 | ,058 | ,600** |
| | Sig. N | ,023 58 | ,000 32 | ,360 57 | ,040 52 | ,000 57 | ,100 43 | ,000 57 | ,178 35 | ,131 58 | ,000 58 | ,001 58 | ,410 52 | ,588 57 | ,050 56 | ,993 30 | ,856 58 | ,744 57 | ,136 54 | ,000 58 | . | ,674 55 | ,000 56 |
| | | | | | | | | | | | | | | | | | | | | | | | |
| Zn | Corr Coeff | -,088 | ,221 | ,011 | ,107 | ,017 | ,204 | -,212 | ,386* | ,434** | -,266* | ,066 | ,316* | ,159 | ,320* | ,152 | ,318* | -,079 | -,146 | -,024 | ,058 | 1,000 | ,022 |

| | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|-------|-----------|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|----------|-----------|-----------|-----------|----------|-----------|----------|-----------|-----------|-----------|----------|-----------|-----------|
| Sig. | ,504 | ,217 | ,932 | ,448 | ,899 | ,190 | ,117 | ,018 | ,001 | ,040 | ,615 | ,017 | ,228 | ,014 | ,416 | ,013 | ,551 | ,289 | ,854 | ,674 | . | ,874 |
| N | 60 | 33 | 60 | 53 | 60 | 43 | 56 | 37 | 60 | 60 | 60 | 56 | 59 | 59 | 31 | 60 | 60 | 55 | 60 | 55 | 60 | 56 |
| Corr | ,435** | ,600** | ,249 | -,132 | -,565** | -,153 | ,584** | ,149 | ,196 | ,573** | -,505** | -,055 | -,058 | -,033 | ,243 | -,305* | ,190 | -,035 | ,726** | ,600** | ,022 | 1,000 |
| Coeff | | | | | | | | | | | | | | | | | | | | | | |
| Sig. | ,001 | ,000 | ,059 | ,356 | ,000 | ,321 | ,000 | ,394 | ,141 | ,000 | ,000 | ,700 | ,666 | ,807 | ,196 | ,020 | ,153 | ,802 | ,000 | ,000 | ,874 | . |
| N | 58 | 33 | 58 | 51 | 58 | 44 | 57 | 35 | 58 | 58 | 58 | 52 | 57 | 57 | 30 | 58 | 58 | 54 | 58 | 56 | 56 | 58 |

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Appendix 3 Complete result from the Spearman correlation analysis of the 24 samples deposited under ferruginous conditions.

| | | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|----|------------|--------|-------|--------|--------|--------|-------|--------|-------|-------|--------|--------|--------|--------|---------|--------|---------|---------|-------|---------|--------|-------|---------|
| Al | Corr Coeff | 1,000 | ,700* | ,590** | ,083 | -,483* | -,157 | ,523* | -,301 | ,135 | ,774** | -,107 | -,369 | ,260 | ,067 | -,176 | -,333 | ,257 | ,062 | ,855** | ,364 | ,077 | ,545* |
| | Sig. | . | ,016 | ,003 | ,737 | ,017 | ,548 | ,012 | ,342 | ,530 | ,000 | ,619 | ,084 | ,231 | ,756 | ,627 | ,112 | ,226 | ,788 | ,000 | ,115 | ,732 | ,011 |
| | N | 24 | 11 | 23 | 19 | 24 | 17 | 22 | 12 | 24 | 24 | 24 | 23 | 23 | 24 | 10 | 24 | 24 | 21 | 23 | 20 | 22 | 21 |
| As | Corr Coeff | ,700* | 1,000 | ,673* | ,036 | -,082 | ,117 | ,467 | -,300 | ,455 | ,573 | ,164 | -,042 | ,564 | ,364 | -,314 | -,027 | -,100 | ,100 | ,673* | ,357 | ,133 | ,800** |
| | Sig. | ,016 | . | ,033 | ,939 | ,811 | ,765 | ,174 | ,624 | ,160 | ,066 | ,631 | ,907 | ,071 | ,272 | ,544 | ,937 | ,770 | ,798 | ,033 | ,385 | ,732 | ,010 |
| | N | 11 | 11 | 10 | 7 | 11 | 9 | 10 | 5 | 11 | 11 | 11 | 10 | 11 | 11 | 6 | 11 | 11 | 9 | 10 | 8 | 9 | 9 |
| Ba | Corr Coeff | ,590** | ,673* | 1,000 | ,487* | -,347 | -,012 | ,271 | -,098 | ,172 | ,531** | -,458* | -,074 | ,209 | ,211 | -,067 | -,009 | -,036 | ,169 | ,533** | ,430 | ,266 | ,425 |
| | Sig. | ,003 | ,033 | . | ,035 | ,105 | ,963 | ,234 | ,762 | ,433 | ,009 | ,028 | ,744 | ,349 | ,333 | ,855 | ,968 | ,872 | ,464 | ,009 | ,058 | ,232 | ,055 |
| | N | 23 | 10 | 23 | 19 | 23 | 17 | 21 | 12 | 23 | 23 | 23 | 22 | 22 | 23 | 10 | 23 | 23 | 21 | 23 | 20 | 22 | 21 |
| C | Corr Coeff | ,083 | ,036 | ,487* | 1,000 | ,072 | ,058 | -,552* | ,190 | ,280 | -,330 | -,235 | ,103 | ,662** | ,644** | ,314 | ,578** | -,637** | ,589* | -,076 | -,082 | ,547* | -,144 |
| | Sig. | ,737 | ,939 | ,035 | . | ,770 | ,851 | ,022 | ,651 | ,245 | ,167 | ,332 | ,683 | ,003 | ,003 | ,544 | ,010 | ,003 | ,013 | ,759 | ,754 | ,019 | ,583 |
| | N | 19 | 7 | 19 | 19 | 19 | 13 | 17 | 8 | 19 | 19 | 19 | 18 | 18 | 19 | 6 | 19 | 19 | 17 | 19 | 17 | 18 | 17 |
| Ca | Corr Coeff | -,483* | -,082 | -,347 | ,072 | 1,000 | ,355 | -,519* | -,105 | -,145 | -,368 | ,629** | ,715** | -,224 | ,323 | ,721* | ,443* | ,084 | ,083 | -,616** | -,514* | -,220 | -,613** |
| | Sig. | ,017 | ,811 | ,105 | ,770 | . | ,162 | ,013 | ,746 | ,498 | ,077 | ,001 | ,000 | ,304 | ,123 | ,019 | ,030 | ,695 | ,720 | ,002 | ,020 | ,326 | ,003 |
| | N | 24 | 11 | 23 | 19 | 24 | 17 | 22 | 12 | 24 | 24 | 24 | 23 | 23 | 24 | 10 | 24 | 24 | 21 | 23 | 20 | 22 | 21 |
| Co | Corr Coeff | -,157 | ,117 | -,012 | ,058 | ,355 | 1,000 | -,105 | ,033 | ,404 | -,255 | ,191 | ,347 | -,444 | ,319 | ,817** | ,044 | ,250 | -,094 | ,162 | ,059 | ,144 | ,159 |
| | Sig. | ,548 | ,765 | ,963 | ,851 | ,162 | . | ,687 | ,932 | ,107 | ,323 | ,462 | ,188 | ,074 | ,213 | ,007 | ,866 | ,333 | ,729 | ,535 | ,829 | ,594 | ,541 |
| | N | 17 | 9 | 17 | 13 | 17 | 17 | 17 | 9 | 17 | 17 | 17 | 16 | 17 | 17 | 9 | 17 | 17 | 16 | 17 | 16 | 16 | 17 |
| Cr | Corr Coeff | ,523* | ,467 | ,271 | -,552* | -,519* | -,105 | 1,000 | -,164 | -,162 | ,796** | -,316 | -,419 | -,221 | -,557** | -,055 | -,582** | ,251 | -,364 | ,673** | ,764** | -,405 | ,639** |
| | Sig. | ,012 | ,174 | ,234 | ,022 | ,013 | ,687 | . | ,631 | ,471 | ,000 | ,152 | ,058 | ,336 | ,007 | ,881 | ,004 | ,259 | ,115 | ,001 | ,000 | ,077 | ,002 |
| | N | 22 | 10 | 21 | 17 | 22 | 17 | 22 | 11 | 22 | 22 | 22 | 21 | 21 | 22 | 10 | 22 | 22 | 20 | 21 | 20 | 20 | 21 |
| Cu | Corr Coeff | -,301 | -,300 | -,098 | ,190 | -,105 | ,033 | -,164 | 1,000 | ,070 | -,308 | -,224 | ,343 | -,035 | -,238 | ,214 | ,427 | -,091 | -,273 | -,490 | ,212 | ,636* | ,082 |
| | Sig. | ,342 | ,624 | ,762 | ,651 | ,746 | ,932 | ,631 | . | ,829 | ,331 | ,484 | ,276 | ,914 | ,457 | ,610 | ,167 | ,779 | ,417 | ,106 | ,556 | ,026 | ,811 |
| | N | 12 | 5 | 12 | 8 | 12 | 9 | 11 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 8 | 12 | 12 | 11 | 12 | 10 | 12 | 11 |
| Fe | Corr Coeff | ,135 | ,455 | ,172 | ,280 | -,145 | ,404 | -,162 | ,070 | 1,000 | -,114 | ,173 | ,218 | ,292 | ,518** | -,115 | ,274 | -,323 | ,335 | ,158 | ,102 | ,392 | ,355 |
| | Sig. | ,530 | ,160 | ,433 | ,245 | ,498 | ,107 | ,471 | ,829 | . | ,596 | ,419 | ,317 | ,177 | ,009 | ,751 | ,195 | ,124 | ,138 | ,471 | ,668 | ,071 | ,115 |
| | N | 24 | 11 | 23 | 19 | 24 | 17 | 22 | 12 | 24 | 24 | 24 | 23 | 23 | 24 | 10 | 24 | 24 | 21 | 23 | 20 | 22 | 21 |
| K | Corr Coeff | ,774** | ,573 | ,531** | -,330 | -,368 | -,255 | ,796** | -,308 | -,114 | 1,000 | -,132 | -,252 | ,076 | -,182 | -,236 | -,435* | ,344 | -,323 | ,701** | ,624** | -,126 | ,410 |
| | Sig. | ,000 | ,066 | ,009 | ,167 | ,077 | ,323 | ,000 | ,331 | ,596 | . | ,538 | ,246 | ,730 | ,395 | ,511 | ,034 | ,099 | ,153 | ,000 | ,003 | ,577 | ,065 |
| | N | 24 | 11 | 23 | 19 | 24 | 17 | 22 | 12 | 24 | 24 | 24 | 23 | 23 | 24 | 10 | 24 | 24 | 21 | 23 | 20 | 22 | 21 |

| | | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|-----------|------------|--------|-------|--------|---------|---------|--------|---------|-------|--------|--------|---------|--------|-------|--------|-------|---------|---------|--------|--------|---------|-------|--------|
| Mg | Corr Coeff | -,107 | ,164 | -,458* | -,235 | ,629** | ,191 | -,316 | -,224 | ,173 | -,132 | 1,000 | ,411 | -,006 | ,163 | -,127 | ,163 | ,384 | ,006 | -,205 | -,585** | -,112 | -,397 |
| | Sig. | ,619 | ,631 | ,028 | ,332 | ,001 | ,462 | ,152 | ,484 | ,419 | ,538 | . | ,051 | ,979 | ,445 | ,726 | ,445 | ,064 | ,978 | ,349 | ,007 | ,619 | ,074 |
| | N | 24 | 11 | 23 | 19 | 24 | 17 | 22 | 12 | 24 | 24 | 24 | 23 | 23 | 24 | 10 | 24 | 24 | 21 | 23 | 20 | 22 | 21 |
| Mn | Corr Coeff | -,369 | -,042 | -,074 | ,103 | ,715** | ,347 | -,419 | ,343 | ,218 | -,252 | ,411 | 1,000 | -,185 | ,425* | ,733* | ,565** | -,158 | ,068 | -,475* | -,068 | ,137 | -,281 |
| | Sig. | ,084 | ,907 | ,744 | ,683 | ,000 | ,188 | ,058 | ,276 | ,317 | ,246 | ,051 | . | ,411 | ,043 | ,016 | ,005 | ,471 | ,777 | ,026 | ,781 | ,543 | ,230 |
| | N | 23 | 10 | 22 | 18 | 23 | 16 | 21 | 12 | 23 | 23 | 23 | 23 | 22 | 23 | 10 | 23 | 23 | 20 | 22 | 19 | 22 | 20 |
| Na | Corr Coeff | ,260 | ,564 | ,209 | ,662** | -,224 | -,444 | -,221 | -,035 | ,292 | ,076 | -,006 | -,185 | 1,000 | ,169 | -,479 | ,065 | -,340 | ,556* | ,042 | -,284 | ,264 | ,060 |
| | Sig. | ,231 | ,071 | ,349 | ,003 | ,304 | ,074 | ,336 | ,914 | ,177 | ,730 | ,979 | ,411 | . | ,441 | ,162 | ,768 | ,113 | ,011 | ,852 | ,238 | ,248 | ,801 |
| | N | 23 | 11 | 22 | 18 | 23 | 17 | 21 | 12 | 23 | 23 | 23 | 22 | 23 | 23 | 10 | 23 | 23 | 20 | 22 | 19 | 21 | 20 |
| P | Corr Coeff | ,067 | ,364 | ,211 | ,644** | ,323 | ,319 | -,557** | -,238 | ,518** | -,182 | ,163 | ,425* | ,169 | 1,000 | ,358 | ,713** | -,451* | ,582** | -,120 | -,226 | ,313 | -,100 |
| | Sig. | ,756 | ,272 | ,333 | ,003 | ,123 | ,213 | ,007 | ,457 | ,009 | ,395 | ,445 | ,043 | ,441 | . | ,310 | ,000 | ,027 | ,006 | ,587 | ,339 | ,156 | ,666 |
| | N | 24 | 11 | 23 | 19 | 24 | 17 | 22 | 12 | 24 | 24 | 24 | 23 | 23 | 24 | 10 | 24 | 24 | 21 | 23 | 20 | 22 | 21 |
| Pb | Corr Coeff | -,176 | -,314 | -,067 | ,314 | ,721* | ,817** | -,055 | ,214 | -,115 | -,236 | -,127 | ,733* | -,479 | ,358 | 1,000 | ,030 | ,188 | -,164 | -,030 | ,483 | ,261 | -,285 |
| | Sig. | ,627 | ,544 | ,855 | ,544 | ,019 | ,007 | ,881 | ,610 | ,751 | ,511 | ,726 | ,016 | ,162 | ,310 | . | ,934 | ,603 | ,651 | ,934 | ,187 | ,467 | ,425 |
| | N | 10 | 6 | 10 | 6 | 10 | 9 | 10 | 8 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 10 | 10 |
| S | Corr Coeff | -,333 | -,027 | -,009 | ,578** | ,443* | ,044 | -,582** | ,427 | ,274 | -,435* | ,163 | ,565** | ,065 | ,713** | ,030 | 1,000 | -,531** | ,360 | -,472* | -,218 | ,257 | -,192 |
| | Sig. | ,112 | ,937 | ,968 | ,010 | ,030 | ,866 | ,004 | ,167 | ,195 | ,034 | ,445 | ,005 | ,768 | ,000 | ,934 | . | ,008 | ,109 | ,023 | ,356 | ,248 | ,404 |
| | N | 24 | 11 | 23 | 19 | 24 | 17 | 22 | 12 | 24 | 24 | 24 | 23 | 23 | 24 | 10 | 24 | 24 | 21 | 23 | 20 | 22 | 21 |
| Si | Corr Coeff | ,257 | -,100 | -,036 | -,637** | ,084 | ,250 | ,251 | -,091 | -,323 | ,344 | ,384 | -,158 | -,340 | -,451* | ,188 | -,531** | 1,000 | -,445* | ,306 | -,241 | -,154 | -,055 |
| | Sig. | ,226 | ,770 | ,872 | ,003 | ,695 | ,333 | ,259 | ,779 | ,124 | ,099 | ,064 | ,471 | ,113 | ,027 | ,603 | ,008 | . | ,043 | ,155 | ,307 | ,493 | ,814 |
| | N | 24 | 11 | 23 | 19 | 24 | 17 | 22 | 12 | 24 | 24 | 24 | 23 | 23 | 24 | 10 | 24 | 24 | 21 | 23 | 20 | 22 | 21 |
| Sr | Corr Coeff | ,062 | ,100 | ,169 | ,589* | ,083 | -,094 | -,364 | -,273 | ,335 | -,323 | ,006 | ,068 | ,556* | ,582** | -,164 | ,360 | -,445* | 1,000 | -,231 | -,219 | ,060 | ,054 |
| | Sig. | ,788 | ,798 | ,464 | ,013 | ,720 | ,729 | ,115 | ,417 | ,138 | ,153 | ,978 | ,777 | ,011 | ,006 | ,651 | ,109 | ,043 | . | ,313 | ,367 | ,801 | ,821 |
| | N | 21 | 9 | 21 | 17 | 21 | 16 | 20 | 11 | 21 | 21 | 21 | 20 | 20 | 21 | 10 | 21 | 21 | 21 | 21 | 19 | 20 | 20 |
| Ti | Corr Coeff | ,855** | ,673* | ,533** | -,076 | -,616** | ,162 | ,673** | -,490 | ,158 | ,701** | -,205 | -,475* | ,042 | -,120 | -,030 | -,472* | ,306 | -,231 | 1,000 | ,565** | ,073 | ,692** |
| | Sig. | ,000 | ,033 | ,009 | ,759 | ,002 | ,535 | ,001 | ,106 | ,471 | ,000 | ,349 | ,026 | ,852 | ,587 | ,934 | ,023 | ,155 | ,313 | . | ,009 | ,747 | ,001 |
| | N | 23 | 10 | 23 | 19 | 23 | 17 | 21 | 12 | 23 | 23 | 23 | 22 | 22 | 23 | 10 | 23 | 23 | 21 | 23 | 20 | 22 | 21 |
| V | Corr Coeff | ,364 | ,357 | ,430 | -,082 | -,514* | ,059 | ,764** | ,212 | ,102 | ,624** | -,585** | -,068 | -,284 | -,226 | ,483 | -,218 | -,241 | -,219 | ,565** | 1,000 | ,042 | ,659** |
| | Sig. | ,115 | ,385 | ,058 | ,754 | ,020 | ,829 | ,000 | ,556 | ,668 | ,003 | ,007 | ,781 | ,238 | ,339 | ,187 | ,356 | ,307 | ,367 | ,009 | . | ,864 | ,002 |
| | N | 20 | 8 | 20 | 17 | 20 | 16 | 20 | 10 | 20 | 20 | 20 | 19 | 19 | 20 | 9 | 20 | 20 | 19 | 20 | 20 | 19 | 20 |
| Zn | Corr Coeff | ,077 | ,133 | ,266 | ,547* | -,220 | ,144 | -,405 | ,636* | ,392 | -,126 | -,112 | ,137 | ,264 | ,313 | ,261 | ,257 | -,154 | ,060 | ,073 | ,042 | 1,000 | -,069 |

| | | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|-----------|-------|-----------|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|----------|-----------|-----------|-----------|----------|-----------|----------|-----------|-----------|-----------|----------|-----------|-----------|
| | Sig. | ,732 | ,732 | ,232 | ,019 | ,326 | ,594 | ,077 | ,026 | ,071 | ,577 | ,619 | ,543 | ,248 | ,156 | ,467 | ,248 | ,493 | ,801 | ,747 | ,864 | . | ,772 |
| | N | 22 | 9 | 22 | 18 | 22 | 16 | 20 | 12 | 22 | 22 | 22 | 22 | 21 | 22 | 10 | 22 | 22 | 20 | 22 | 19 | 22 | 20 |
| | Corr | | | | | | | | | | | | | | | | | | | | | | |
| | Coeff | ,545* | ,800** | ,425 | -,144 | -,613** | ,159 | ,639** | ,082 | ,355 | ,410 | -,397 | -,281 | ,060 | -,100 | -,285 | -,192 | -,055 | ,054 | ,692** | ,659** | -,069 | 1,000 |
| Zr | Sig. | ,011 | ,010 | ,055 | ,583 | ,003 | ,541 | ,002 | ,811 | ,115 | ,065 | ,074 | ,230 | ,801 | ,666 | ,425 | ,404 | ,814 | ,821 | ,001 | ,002 | ,772 | . |
| | N | 21 | 9 | 21 | 17 | 21 | 17 | 21 | 11 | 21 | 21 | 21 | 20 | 20 | 21 | 10 | 21 | 21 | 20 | 21 | 20 | 20 | 21 |

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 4 Complete result from the Spearman correlation analysis of the 28 samples from South Africa.

| | | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|----|------------|---------|--------|--------|-------|---------|--------|---------|-------|-------|---------|--------|--------|---------|---------|-------|--------|-------|--------|---------|---------|--------|---------|
| Al | Corr Coeff | 1,000 | -,188 | ,538** | -,026 | -,541** | -,242 | ,284 | -,559 | ,043 | ,448* | -,246 | -,456* | ,170 | ,227 | -,126 | -,299 | ,120 | ,332 | ,635** | ,468* | ,199 | ,404* |
| | Sig. | . | ,603 | ,004 | ,901 | ,003 | ,267 | ,160 | ,059 | ,827 | ,017 | ,207 | ,019 | ,396 | ,245 | ,697 | ,122 | ,542 | ,113 | ,000 | ,014 | ,320 | ,040 |
| | N | 28 | 10 | 27 | 25 | 28 | 23 | 26 | 12 | 28 | 28 | 28 | 26 | 27 | 28 | 12 | 28 | 28 | 24 | 28 | 27 | 27 | 26 |
| As | Corr Coeff | -,188 | 1,000 | ,588 | ,405 | ,079 | ,900** | -,370 | ,000 | ,697* | -,430 | -,200 | ,567 | ,430 | ,527 | ,700 | ,164 | -,018 | ,119 | ,503 | ,636* | ,400 | ,697* |
| | Sig. | ,603 | . | ,074 | ,320 | ,829 | ,001 | ,293 | 1,000 | ,025 | ,214 | ,580 | ,112 | ,214 | ,117 | ,188 | ,651 | ,960 | ,779 | ,138 | ,048 | ,286 | ,025 |
| | N | 10 | 10 | 10 | 8 | 10 | 9 | 10 | 4 | 10 | 10 | 10 | 9 | 10 | 10 | 5 | 10 | 10 | 8 | 10 | 10 | 9 | 10 |
| Ba | Corr Coeff | ,538** | ,588 | 1,000 | ,031 | -,212 | ,140 | ,065 | -,264 | ,056 | ,355 | -,421* | -,218 | ,270 | ,426* | -,308 | -,175 | -,035 | ,477* | ,529** | ,458* | ,118 | ,454* |
| | Sig. | ,004 | ,074 | . | ,884 | ,287 | ,523 | ,751 | ,433 | ,783 | ,069 | ,029 | ,296 | ,181 | ,027 | ,331 | ,382 | ,861 | ,019 | ,005 | ,018 | ,566 | ,020 |
| | N | 27 | 10 | 27 | 25 | 27 | 23 | 26 | 11 | 27 | 27 | 27 | 25 | 26 | 27 | 12 | 27 | 27 | 24 | 27 | 26 | 26 | 26 |
| C | Corr Coeff | -,026 | ,405 | ,031 | 1,000 | ,168 | ,011 | -,246 | ,109 | -,031 | -,265 | ,106 | ,001 | ,252 | ,006 | -,176 | ,287 | -,063 | -,056 | ,005 | -,050 | ,226 | -,004 |
| | Sig. | ,901 | ,320 | ,884 | . | ,423 | ,961 | ,247 | ,781 | ,882 | ,200 | ,613 | ,995 | ,235 | ,978 | ,626 | ,164 | ,766 | ,805 | ,980 | ,818 | ,287 | ,985 |
| | N | 25 | 8 | 25 | 25 | 25 | 21 | 24 | 9 | 25 | 25 | 25 | 23 | 24 | 25 | 10 | 25 | 25 | 22 | 25 | 24 | 24 | 24 |
| Ca | Corr Coeff | -,541** | ,079 | -,212 | ,168 | 1,000 | -,163 | -,604** | ,329 | -,193 | -,557** | ,351 | ,344 | ,056 | ,118 | -,273 | ,477* | ,294 | -,066 | -,651** | -,703** | ,016 | -,532** |
| | Sig. | ,003 | ,829 | ,287 | ,423 | . | ,457 | ,001 | ,297 | ,326 | ,002 | ,067 | ,086 | ,781 | ,551 | ,391 | ,010 | ,129 | ,759 | ,000 | ,000 | ,935 | ,005 |
| | N | 28 | 10 | 27 | 25 | 28 | 23 | 26 | 12 | 28 | 28 | 28 | 26 | 27 | 28 | 12 | 28 | 28 | 24 | 28 | 27 | 27 | 26 |
| Co | Corr Coeff | -,242 | ,900** | ,140 | ,011 | -,163 | 1,000 | ,129 | ,000 | ,471* | ,014 | -,251 | ,193 | ,014 | ,058 | ,100 | -,271 | ,101 | ,052 | ,222 | ,388 | ,097 | ,245 |
| | Sig. | ,267 | ,001 | ,523 | ,961 | ,457 | . | ,556 | 1,000 | ,023 | ,950 | ,249 | ,402 | ,948 | ,792 | ,770 | ,210 | ,645 | ,818 | ,309 | ,067 | ,667 | ,261 |
| | N | 23 | 9 | 23 | 21 | 23 | 23 | 23 | 10 | 23 | 23 | 23 | 21 | 23 | 23 | 11 | 23 | 23 | 22 | 23 | 23 | 22 | 23 |
| Cr | Corr Coeff | ,284 | -,370 | ,065 | -,246 | -,604** | ,129 | 1,000 | -,127 | -,255 | ,781** | -,117 | -,186 | -,642** | -,572** | ,252 | -,342 | -,021 | -,471* | ,491* | ,593** | -,345 | ,240 |
| | Sig. | ,160 | ,293 | ,751 | ,247 | ,001 | ,556 | . | ,726 | ,209 | ,000 | ,568 | ,384 | ,001 | ,002 | ,430 | ,088 | ,918 | ,020 | ,011 | ,001 | ,091 | ,237 |
| | N | 26 | 10 | 26 | 24 | 26 | 23 | 26 | 10 | 26 | 26 | 26 | 24 | 25 | 26 | 12 | 26 | 26 | 24 | 26 | 26 | 25 | 26 |
| Cu | Corr Coeff | -,559 | ,000 | -,264 | ,109 | ,329 | ,000 | -,127 | 1,000 | ,063 | -,392 | ,098 | ,615* | ,280 | -,028 | ,150 | ,692* | -,091 | -,176 | -,357 | -,118 | ,126 | -,333 |
| | Sig. | ,059 | 1,000 | ,433 | ,781 | ,297 | 1,000 | ,726 | . | ,846 | ,208 | ,762 | ,033 | ,379 | ,931 | ,700 | ,013 | ,779 | ,627 | ,255 | ,729 | ,697 | ,347 |
| | N | 12 | 4 | 11 | 9 | 12 | 10 | 10 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 9 | 12 | 12 | 10 | 12 | 11 | 12 | 10 |
| Fe | Corr Coeff | ,043 | ,697* | ,056 | -,031 | -,193 | ,471* | -,255 | ,063 | 1,000 | -,328 | ,015 | ,244 | ,428* | ,516** | ,252 | -,080 | -,094 | ,256 | ,158 | ,107 | ,609** | ,314 |
| | Sig. | ,827 | ,025 | ,783 | ,882 | ,326 | ,023 | ,209 | ,846 | . | ,088 | ,941 | ,230 | ,026 | ,005 | ,430 | ,684 | ,633 | ,228 | ,421 | ,596 | ,001 | ,119 |
| | N | 28 | 10 | 27 | 25 | 28 | 23 | 26 | 12 | 28 | 28 | 28 | 26 | 27 | 28 | 12 | 28 | 28 | 24 | 28 | 27 | 27 | 26 |
| K | Corr Coeff | ,448* | -,430 | ,355 | -,265 | -,557** | ,014 | ,781** | -,392 | -,328 | 1,000 | -,025 | -,266 | -,332 | -,247 | ,000 | -,473* | ,234 | -,181 | ,596** | ,611** | -,329 | ,259 |
| | Sig. | ,017 | ,214 | ,069 | ,200 | ,002 | ,950 | ,000 | ,208 | ,088 | . | ,901 | ,190 | ,091 | ,205 | 1,000 | ,011 | ,231 | ,398 | ,001 | ,001 | ,094 | ,202 |
| | N | 28 | 10 | 27 | 25 | 28 | 23 | 26 | 12 | 28 | 28 | 28 | 26 | 27 | 28 | 12 | 28 | 28 | 24 | 28 | 27 | 27 | 26 |

| | | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|----|------------|--------|-------|--------|-------|---------|-------|---------|-------|--------|--------|---------|--------|--------|--------|-------|-------|-------|---------|--------|--------|--------|---------|
| Mg | Corr Coeff | -,246 | -,200 | -,421* | ,106 | ,351 | -,251 | -,117 | ,098 | ,015 | -,025 | 1,000 | ,539** | -,062 | -,028 | -,203 | ,346 | ,317 | -,569** | -,239 | -,433* | ,143 | -,572** |
| | Sig. | ,207 | ,580 | ,029 | ,613 | ,067 | ,249 | ,568 | ,762 | ,941 | ,901 | . | ,004 | ,758 | ,886 | ,527 | ,071 | ,101 | ,004 | ,220 | ,024 | ,477 | ,002 |
| | N | 28 | 10 | 27 | 25 | 28 | 23 | 26 | 12 | 28 | 28 | 28 | 26 | 27 | 28 | 12 | 28 | 28 | 24 | 28 | 27 | 27 | 26 |
| Mn | Corr Coeff | -,456* | ,567 | -,218 | ,001 | ,344 | ,193 | -,186 | ,615* | ,244 | -,266 | ,539** | 1,000 | ,188 | -,035 | ,021 | ,347 | ,156 | -,347 | -,175 | -,182 | ,181 | -,155 |
| | Sig. | ,019 | ,112 | ,296 | ,995 | ,086 | ,402 | ,384 | ,033 | ,230 | ,190 | ,004 | . | ,369 | ,867 | ,948 | ,082 | ,446 | ,113 | ,391 | ,383 | ,377 | ,470 |
| | N | 26 | 9 | 25 | 23 | 26 | 21 | 24 | 12 | 26 | 26 | 26 | 26 | 25 | 26 | 12 | 26 | 26 | 22 | 26 | 25 | 26 | 24 |
| Na | Corr Coeff | ,170 | ,430 | ,270 | ,252 | ,056 | ,014 | -,642** | ,280 | ,428* | -,332 | -,062 | ,188 | 1,000 | ,618** | -,182 | ,300 | -,188 | ,707** | ,071 | ,091 | ,490* | ,057 |
| | Sig. | ,396 | ,214 | ,181 | ,235 | ,781 | ,948 | ,001 | ,379 | ,026 | ,091 | ,758 | ,369 | . | ,001 | ,572 | ,128 | ,348 | ,000 | ,723 | ,657 | ,011 | ,787 |
| | N | 27 | 10 | 26 | 24 | 27 | 23 | 25 | 12 | 27 | 27 | 27 | 25 | 27 | 27 | 12 | 27 | 27 | 23 | 27 | 26 | 26 | 25 |
| P | Corr Coeff | ,227 | ,527 | ,426* | ,006 | ,118 | ,058 | -,572** | -,028 | ,516** | -,247 | -,028 | -,035 | ,618** | 1,000 | ,266 | ,148 | ,022 | ,677** | ,121 | -,034 | ,576** | ,095 |
| | Sig. | ,245 | ,117 | ,027 | ,978 | ,551 | ,792 | ,002 | ,931 | ,005 | ,205 | ,886 | ,867 | ,001 | . | ,404 | ,451 | ,910 | ,000 | ,540 | ,868 | ,002 | ,645 |
| | N | 28 | 10 | 27 | 25 | 28 | 23 | 26 | 12 | 28 | 28 | 28 | 26 | 27 | 28 | 12 | 28 | 28 | 24 | 28 | 27 | 27 | 26 |
| Pb | Corr Coeff | -,126 | ,700 | -,308 | -,176 | -,273 | ,100 | ,252 | ,150 | ,252 | ,000 | -,203 | ,021 | -,182 | ,266 | 1,000 | -,259 | ,357 | -,224 | ,336 | ,266 | ,126 | ,266 |
| | Sig. | ,697 | ,188 | ,331 | ,626 | ,391 | ,770 | ,430 | ,700 | ,430 | 1,000 | ,527 | ,948 | ,572 | ,404 | . | ,417 | ,255 | ,484 | ,286 | ,404 | ,697 | ,404 |
| | N | 12 | 5 | 12 | 10 | 12 | 11 | 12 | 9 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| S | Corr Coeff | -,299 | ,164 | -,175 | ,287 | ,477* | -,271 | -,342 | ,692* | -,080 | -,473* | ,346 | ,347 | ,300 | ,148 | -,259 | 1,000 | -,256 | -,183 | -,224 | -,248 | ,289 | -,158 |
| | Sig. | ,122 | ,651 | ,382 | ,164 | ,010 | ,210 | ,088 | ,013 | ,684 | ,011 | ,071 | ,082 | ,128 | ,451 | ,417 | . | ,188 | ,393 | ,251 | ,211 | ,144 | ,442 |
| | N | 28 | 10 | 27 | 25 | 28 | 23 | 26 | 12 | 28 | 28 | 28 | 26 | 27 | 28 | 12 | 28 | 28 | 24 | 28 | 27 | 27 | 26 |
| Si | Corr Coeff | ,120 | -,018 | -,035 | -,063 | ,294 | ,101 | -,021 | -,091 | -,094 | ,234 | ,317 | ,156 | -,188 | ,022 | ,357 | -,256 | 1,000 | -,194 | ,071 | -,162 | -,008 | -,145 |
| | Sig. | ,542 | ,960 | ,861 | ,766 | ,129 | ,645 | ,918 | ,779 | ,633 | ,231 | ,101 | ,446 | ,348 | ,910 | ,255 | ,188 | . | ,363 | ,718 | ,421 | ,969 | ,480 |
| | N | 28 | 10 | 27 | 25 | 28 | 23 | 26 | 12 | 28 | 28 | 28 | 26 | 27 | 28 | 12 | 28 | 28 | 24 | 28 | 27 | 27 | 26 |
| Sr | Corr Coeff | ,332 | ,119 | ,477* | -,056 | -,066 | ,052 | -,471* | -,176 | ,256 | -,181 | -,569** | -,347 | ,707** | ,677** | -,224 | -,183 | -,194 | 1,000 | ,125 | ,161 | ,094 | ,143 |
| | Sig. | ,113 | ,779 | ,019 | ,805 | ,759 | ,818 | ,020 | ,627 | ,228 | ,398 | ,004 | ,113 | ,000 | ,000 | ,484 | ,393 | ,363 | . | ,550 | ,443 | ,670 | ,506 |
| | N | 24 | 8 | 24 | 22 | 24 | 22 | 24 | 10 | 24 | 24 | 24 | 22 | 23 | 24 | 12 | 24 | 24 | 25 | 25 | 25 | 23 | 24 |
| Ti | Corr Coeff | ,635** | ,503 | ,529** | ,005 | -,651** | ,222 | ,491* | -,357 | ,158 | ,596** | -,239 | -,175 | ,071 | ,121 | ,336 | -,224 | ,071 | ,125 | 1,000 | ,903** | ,201 | ,853** |
| | Sig. | ,000 | ,138 | ,005 | ,980 | ,000 | ,309 | ,011 | ,255 | ,421 | ,001 | ,220 | ,391 | ,723 | ,540 | ,286 | ,251 | ,718 | ,550 | . | ,000 | ,314 | ,000 |
| | N | 28 | 10 | 27 | 25 | 28 | 23 | 26 | 12 | 28 | 28 | 28 | 26 | 27 | 28 | 12 | 28 | 28 | 25 | 29 | 28 | 27 | 26 |
| V | Corr Coeff | ,468* | ,636* | ,458* | -,050 | -,703** | ,388 | ,593** | -,118 | ,107 | ,611** | -,433* | -,182 | ,091 | -,034 | ,266 | -,248 | -,162 | ,161 | ,903** | 1,000 | ,057 | ,699** |
| | Sig. | ,014 | ,048 | ,018 | ,818 | ,000 | ,067 | ,001 | ,729 | ,596 | ,001 | ,024 | ,383 | ,657 | ,868 | ,404 | ,211 | ,421 | ,443 | ,000 | . | ,782 | ,000 |
| | N | 27 | 10 | 26 | 24 | 27 | 23 | 26 | 11 | 27 | 27 | 27 | 25 | 26 | 27 | 12 | 27 | 27 | 25 | 28 | 28 | 26 | 26 |
| Zn | Corr Coeff | ,199 | ,400 | ,118 | ,226 | ,016 | ,097 | -,345 | ,126 | ,609** | -,329 | ,143 | ,181 | ,490* | ,576** | ,126 | ,289 | -,008 | ,094 | ,201 | ,057 | 1,000 | ,275 |

| | | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|-----------|-------|-----------|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|----------|-----------|-----------|-----------|----------|-----------|----------|-----------|-----------|-----------|----------|-----------|-----------|
| | Sig. | ,320 | ,286 | ,566 | ,287 | ,935 | ,667 | ,091 | ,697 | ,001 | ,094 | ,477 | ,377 | ,011 | ,002 | ,697 | ,144 | ,969 | ,670 | ,314 | ,782 | . | ,183 |
| | N | 27 | 9 | 26 | 24 | 27 | 22 | 25 | 12 | 27 | 27 | 27 | 26 | 26 | 27 | 12 | 27 | 27 | 23 | 27 | 26 | 27 | 25 |
| | Corr | | | | | | | | | | | | | | | | | | | | | | |
| | Coeff | ,404* | ,697* | ,454* | -,004 | -,532** | ,245 | ,240 | -,333 | ,314 | ,259 | -,572** | -,155 | ,057 | ,095 | ,266 | -,158 | -,145 | ,143 | ,853** | ,699** | ,275 | 1,000 |
| Zr | Sig. | ,040 | ,025 | ,020 | ,985 | ,005 | ,261 | ,237 | ,347 | ,119 | ,202 | ,002 | ,470 | ,787 | ,645 | ,404 | ,442 | ,480 | ,506 | ,000 | ,000 | ,183 | . |
| | N | 26 | 10 | 26 | 24 | 26 | 23 | 26 | 10 | 26 | 26 | 26 | 24 | 25 | 26 | 12 | 26 | 26 | 24 | 26 | 26 | 25 | 26 |

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Appendix 5 Complete result from the Spearman correlation analysis of the 12 samples deposited under ferruginous samples (Gabon).

| | | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|----|------------|--------|--------|--------|-------|-------|-------|-------|-------|-------|--------|--------|--------|-------|-------|--------|-------|-------|-------|--------|-------|--------|--------|
| Al | Corr Coeff | 1,000 | ,929** | ,736** | ,167 | -,238 | -,048 | ,155 | -,367 | ,392 | ,860** | -,028 | -,371 | ,378 | ,189 | -,190 | -,161 | ,098 | ,527 | ,936** | -,183 | ,127 | ,564 |
| | Sig. | . | ,003 | ,010 | ,693 | ,457 | ,911 | ,650 | ,332 | ,208 | ,000 | ,931 | ,236 | ,226 | ,557 | ,651 | ,618 | ,762 | ,096 | ,000 | ,637 | ,709 | ,090 |
| | N | 12 | 7 | 11 | 8 | 12 | 8 | 11 | 9 | 12 | 12 | 12 | 12 | 12 | 12 | 8 | 12 | 12 | 11 | 11 | 9 | 11 | 10 |
| As | Corr Coeff | ,929** | 1,000 | ,714 | ,200 | ,000 | -,500 | ,486 | -,400 | ,643 | ,821* | ,250 | -,036 | ,571 | ,607 | -,500 | ,321 | -,214 | ,543 | ,886* | ,400 | ,429 | ,800 |
| | Sig. | ,003 | . | ,111 | ,800 | 1,000 | ,391 | ,329 | ,600 | ,119 | ,023 | ,589 | ,939 | ,180 | ,148 | ,391 | ,482 | ,645 | ,266 | ,019 | ,600 | ,397 | ,104 |
| | N | 7 | 7 | 6 | 4 | 7 | 5 | 6 | 4 | 7 | 7 | 7 | 7 | 7 | 7 | 5 | 7 | 7 | 6 | 6 | 4 | 6 | 5 |
| Ba | Corr Coeff | ,736** | ,714 | 1,000 | ,333 | -,591 | -,238 | ,648* | -,033 | ,182 | ,700* | -,545 | -,345 | ,118 | -,209 | -,119 | -,109 | ,109 | ,073 | ,564 | ,517 | ,245 | ,539 |
| | Sig. | ,010 | ,111 | . | ,420 | ,056 | ,570 | ,043 | ,932 | ,593 | ,016 | ,083 | ,298 | ,729 | ,537 | ,779 | ,750 | ,750 | ,832 | ,071 | ,154 | ,467 | ,108 |
| | N | 11 | 6 | 11 | 8 | 11 | 8 | 10 | 9 | 11 | 11 | 11 | 11 | 11 | 11 | 8 | 11 | 11 | 11 | 11 | 9 | 11 | 10 |
| C | Corr Coeff | ,167 | ,200 | ,333 | 1,000 | -,619 | ,000 | ,036 | ,086 | ,238 | ,381 | -,833* | -,190 | ,595 | ,143 | ,400 | ,095 | -,571 | ,071 | ,024 | ,250 | ,286 | -,607 |
| | Sig. | ,693 | ,800 | ,420 | . | ,102 | 1,000 | ,939 | ,872 | ,570 | ,352 | ,010 | ,651 | ,120 | ,736 | ,505 | ,823 | ,139 | ,867 | ,955 | ,589 | ,493 | ,148 |
| | N | 8 | 4 | 8 | 8 | 8 | 5 | 7 | 6 | 8 | 8 | 8 | 8 | 8 | 8 | 5 | 8 | 8 | 8 | 8 | 7 | 8 | 7 |
| Ca | Corr Coeff | -,238 | ,000 | -,591 | -,619 | 1,000 | ,833* | -,373 | ,133 | ,126 | -,448 | ,650* | ,748** | -,455 | ,531 | ,738* | ,510 | ,147 | -,118 | -,427 | -,067 | -,336 | ,030 |
| | Sig. | ,457 | 1,000 | ,056 | ,102 | . | ,010 | ,259 | ,732 | ,697 | ,145 | ,022 | ,005 | ,138 | ,075 | ,037 | ,090 | ,649 | ,729 | ,190 | ,865 | ,312 | ,934 |
| | N | 12 | 7 | 11 | 8 | 12 | 8 | 11 | 9 | 12 | 12 | 12 | 12 | 12 | 12 | 8 | 12 | 12 | 11 | 11 | 9 | 11 | 10 |
| Co | Corr Coeff | -,048 | -,500 | -,238 | ,000 | ,833* | 1,000 | -,310 | ,214 | ,167 | -,643 | ,048 | ,667 | -,643 | ,690 | ,857** | ,405 | ,524 | -,143 | ,190 | ,000 | ,190 | -,119 |
| | Sig. | ,911 | ,391 | ,570 | 1,000 | ,010 | . | ,456 | ,645 | ,693 | ,086 | ,911 | ,071 | ,086 | ,058 | ,007 | ,320 | ,183 | ,736 | ,651 | 1,000 | ,651 | ,779 |
| | N | 8 | 5 | 8 | 5 | 8 | 8 | 8 | 7 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | 8 |
| Cr | Corr Coeff | ,155 | ,486 | ,648* | ,036 | -,373 | -,310 | 1,000 | ,117 | ,091 | ,191 | -,336 | -,273 | ,009 | -,427 | -,214 | -,209 | -,155 | ,236 | ,176 | ,717* | -,127 | ,782** |
| | Sig. | ,650 | ,329 | ,043 | ,939 | ,259 | ,456 | . | ,765 | ,790 | ,574 | ,312 | ,417 | ,979 | ,190 | ,610 | ,537 | ,650 | ,511 | ,627 | ,030 | ,726 | ,008 |
| | N | 11 | 6 | 10 | 7 | 11 | 8 | 11 | 9 | 11 | 11 | 11 | 11 | 11 | 11 | 8 | 11 | 11 | 10 | 10 | 9 | 10 | 10 |
| Cu | Corr Coeff | -,367 | -,400 | -,033 | ,086 | ,133 | ,214 | ,117 | 1,000 | -,317 | -,200 | -,367 | ,550 | -,333 | -,167 | ,607 | ,333 | -,100 | -,500 | -,467 | ,690 | ,833** | -,200 |
| | Sig. | ,332 | ,600 | ,932 | ,872 | ,732 | ,645 | ,765 | . | ,406 | ,606 | ,332 | ,125 | ,381 | ,668 | ,148 | ,381 | ,798 | ,170 | ,205 | ,058 | ,005 | ,606 |
| | N | 9 | 4 | 9 | 6 | 9 | 7 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 7 | 9 | 9 | 9 | 9 | 8 | 9 | 9 |
| Fe | Corr Coeff | ,392 | ,643 | ,182 | ,238 | ,126 | ,167 | ,091 | -,317 | 1,000 | ,273 | ,259 | ,154 | ,126 | ,650* | -,024 | ,469 | -,294 | ,073 | ,345 | ,250 | ,145 | ,297 |
| | Sig. | ,208 | ,119 | ,593 | ,570 | ,697 | ,693 | ,790 | ,406 | . | ,391 | ,417 | ,633 | ,697 | ,022 | ,955 | ,124 | ,354 | ,832 | ,298 | ,516 | ,670 | ,405 |
| | N | 12 | 7 | 11 | 8 | 12 | 8 | 11 | 9 | 12 | 12 | 12 | 12 | 12 | 12 | 8 | 12 | 12 | 11 | 11 | 9 | 11 | 10 |
| K | Corr Coeff | ,860** | ,821* | ,700* | ,381 | -,448 | -,643 | ,191 | -,200 | ,273 | 1,000 | -,042 | -,476 | ,608* | -,091 | -,595 | -,273 | -,084 | ,391 | ,645* | ,050 | ,364 | ,321 |
| | Sig. | ,000 | ,023 | ,016 | ,352 | ,145 | ,086 | ,574 | ,606 | ,391 | . | ,897 | ,118 | ,036 | ,779 | ,120 | ,391 | ,795 | ,235 | ,032 | ,898 | ,272 | ,365 |
| | N | 12 | 7 | 11 | 8 | 12 | 8 | 11 | 9 | 12 | 12 | 12 | 12 | 12 | 12 | 8 | 12 | 12 | 11 | 11 | 9 | 11 | 10 |

| | | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|-----------|------------|--------|-------|-------|--------|--------|--------|-------|--------|-------|-------|-------|--------|--------|--------|-------|--------|-------|--------|-------|-------|-------|-------|
| Mg | Corr Coeff | -,028 | ,250 | -,545 | -,833* | ,650* | ,048 | -,336 | -,367 | ,259 | -,042 | 1,000 | ,168 | -,077 | ,182 | -,143 | ,042 | ,245 | ,282 | -,109 | -,383 | -,218 | ,091 |
| | Sig. | ,931 | ,589 | ,083 | ,010 | ,022 | ,911 | ,312 | ,332 | ,417 | ,897 | . | ,602 | ,812 | ,572 | ,736 | ,897 | ,443 | ,401 | ,750 | ,308 | ,519 | ,803 |
| | N | 12 | 7 | 11 | 8 | 12 | 8 | 11 | 9 | 12 | 12 | 12 | 12 | 12 | 12 | 8 | 12 | 12 | 11 | 11 | 9 | 11 | 10 |
| Mn | Corr Coeff | -,371 | -,036 | -,345 | -,190 | ,748** | ,667 | -,273 | ,550 | ,154 | -,476 | ,168 | 1,000 | -,608* | ,559 | ,714* | ,797** | -,119 | -,727* | -,600 | ,417 | ,045 | -,285 |
| | Sig. | ,236 | ,939 | ,298 | ,651 | ,005 | ,071 | ,417 | ,125 | ,633 | ,118 | ,602 | . | ,036 | ,059 | ,047 | ,002 | ,713 | ,011 | ,051 | ,265 | ,894 | ,425 |
| | N | 12 | 7 | 11 | 8 | 12 | 8 | 11 | 9 | 12 | 12 | 12 | 12 | 12 | 12 | 8 | 12 | 12 | 11 | 11 | 9 | 11 | 10 |
| Na | Corr Coeff | ,378 | ,571 | ,118 | ,595 | -,455 | -,643 | ,009 | -,333 | ,126 | ,608* | -,077 | -,608* | 1,000 | -,077 | -,405 | -,294 | -,413 | ,482 | ,136 | -,383 | ,018 | -,079 |
| | Sig. | ,226 | ,180 | ,729 | ,120 | ,138 | ,086 | ,979 | ,381 | ,697 | ,036 | ,812 | ,036 | . | ,812 | ,320 | ,354 | ,183 | ,133 | ,689 | ,308 | ,958 | ,829 |
| | N | 12 | 7 | 11 | 8 | 12 | 8 | 11 | 9 | 12 | 12 | 12 | 12 | 12 | 12 | 8 | 12 | 12 | 11 | 11 | 9 | 11 | 10 |
| P | Corr Coeff | ,189 | ,607 | -,209 | ,143 | ,531 | ,690 | -,427 | -,167 | ,650* | -,091 | ,182 | ,559 | -,077 | 1,000 | ,452 | ,776** | -,280 | -,155 | ,082 | -,267 | -,036 | -,042 |
| | Sig. | ,557 | ,148 | ,537 | ,736 | ,075 | ,058 | ,190 | ,668 | ,022 | ,779 | ,572 | ,059 | ,812 | . | ,260 | ,003 | ,379 | ,650 | ,811 | ,488 | ,915 | ,907 |
| | N | 12 | 7 | 11 | 8 | 12 | 8 | 11 | 9 | 12 | 12 | 12 | 12 | 12 | 12 | 8 | 12 | 12 | 11 | 11 | 9 | 11 | 10 |
| Pb | Corr Coeff | -,190 | -,500 | -,119 | ,400 | ,738* | ,857** | -,214 | ,607 | -,024 | -,595 | -,143 | ,714* | -,405 | ,452 | 1,000 | ,405 | ,429 | -,167 | -,095 | ,321 | ,333 | -,286 |
| | Sig. | ,651 | ,391 | ,779 | ,505 | ,037 | ,007 | ,610 | ,148 | ,955 | ,120 | ,736 | ,047 | ,320 | ,260 | . | ,320 | ,289 | ,693 | ,823 | ,482 | ,420 | ,493 |
| | N | 8 | 5 | 8 | 5 | 8 | 8 | 8 | 7 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | 8 |
| S | Corr Coeff | -,161 | ,321 | -,109 | ,095 | ,510 | ,405 | -,209 | ,333 | ,469 | -,273 | ,042 | ,797** | -,294 | ,776** | ,405 | 1,000 | -,441 | -,573 | -,382 | ,300 | ,064 | -,188 |
| | Sig. | ,618 | ,482 | ,750 | ,823 | ,090 | ,320 | ,537 | ,381 | ,124 | ,391 | ,897 | ,002 | ,354 | ,003 | ,320 | . | ,152 | ,066 | ,247 | ,433 | ,853 | ,603 |
| | N | 12 | 7 | 11 | 8 | 12 | 8 | 11 | 9 | 12 | 12 | 12 | 12 | 12 | 12 | 8 | 12 | 12 | 11 | 11 | 9 | 11 | 10 |
| Si | Corr Coeff | ,098 | -,214 | ,109 | -,571 | ,147 | ,524 | -,155 | -,100 | -,294 | -,084 | ,245 | -,119 | -,413 | -,280 | ,429 | -,441 | 1,000 | ,127 | ,400 | -,233 | ,145 | ,018 |
| | Sig. | ,762 | ,645 | ,750 | ,139 | ,649 | ,183 | ,650 | ,798 | ,354 | ,795 | ,443 | ,713 | ,183 | ,379 | ,289 | ,152 | . | ,709 | ,223 | ,546 | ,670 | ,960 |
| | N | 12 | 7 | 11 | 8 | 12 | 8 | 11 | 9 | 12 | 12 | 12 | 12 | 12 | 12 | 8 | 12 | 12 | 11 | 11 | 9 | 11 | 10 |
| Sr | Corr Coeff | ,527 | ,543 | ,073 | ,071 | -,118 | -,143 | ,236 | -,500 | ,073 | ,391 | ,282 | -,727* | ,482 | -,155 | -,167 | -,573 | ,127 | 1,000 | ,527 | -,417 | -,336 | ,406 |
| | Sig. | ,096 | ,266 | ,832 | ,867 | ,729 | ,736 | ,511 | ,170 | ,832 | ,235 | ,401 | ,011 | ,133 | ,650 | ,693 | ,066 | ,709 | . | ,096 | ,265 | ,312 | ,244 |
| | N | 11 | 6 | 11 | 8 | 11 | 8 | 10 | 9 | 11 | 11 | 11 | 11 | 11 | 11 | 8 | 11 | 11 | 11 | 11 | 9 | 11 | 10 |
| Ti | Corr Coeff | ,936** | ,886* | ,564 | ,024 | -,427 | ,190 | ,176 | -,467 | ,345 | ,645* | -,109 | -,600 | ,136 | ,082 | -,095 | -,382 | ,400 | ,527 | 1,000 | -,233 | ,082 | ,491 |
| | Sig. | ,000 | ,019 | ,071 | ,955 | ,190 | ,651 | ,627 | ,205 | ,298 | ,032 | ,750 | ,051 | ,689 | ,811 | ,823 | ,247 | ,223 | ,096 | . | ,546 | ,811 | ,150 |
| | N | 11 | 6 | 11 | 8 | 11 | 8 | 10 | 9 | 11 | 11 | 11 | 11 | 11 | 11 | 8 | 11 | 11 | 11 | 11 | 9 | 11 | 10 |
| V | Corr Coeff | -,183 | ,400 | ,517 | ,250 | -,067 | ,000 | ,717* | ,690 | ,250 | ,050 | -,383 | ,417 | -,383 | -,267 | ,321 | ,300 | -,233 | -,417 | -,233 | 1,000 | ,550 | ,250 |
| | Sig. | ,637 | ,600 | ,154 | ,589 | ,865 | 1,000 | ,030 | ,058 | ,516 | ,898 | ,308 | ,265 | ,308 | ,488 | ,482 | ,433 | ,546 | ,265 | ,546 | . | ,125 | ,516 |
| | N | 9 | 4 | 9 | 7 | 9 | 7 | 9 | 8 | 9 | 9 | 9 | 9 | 9 | 9 | 7 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Zn | Corr Coeff | ,127 | ,429 | ,245 | ,286 | -,336 | ,190 | -,127 | ,833** | ,145 | ,364 | -,218 | ,045 | ,018 | -,036 | ,333 | ,064 | ,145 | -,336 | ,082 | ,550 | 1,000 | -,309 |

| | | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|-----------|-------|-----------|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|----------|-----------|-----------|-----------|----------|-----------|----------|-----------|-----------|-----------|----------|-----------|-----------|
| | Sig. | ,709 | ,397 | ,467 | ,493 | ,312 | ,651 | ,726 | ,005 | ,670 | ,272 | ,519 | ,894 | ,958 | ,915 | ,420 | ,853 | ,670 | ,312 | ,811 | ,125 | . | ,385 |
| | N | 11 | 6 | 11 | 8 | 11 | 8 | 10 | 9 | 11 | 11 | 11 | 11 | 11 | 11 | 8 | 11 | 11 | 11 | 11 | 9 | 11 | 10 |
| | Corr | ,564 | ,800 | ,539 | -,607 | ,030 | -,119 | ,782** | -,200 | ,297 | ,321 | ,091 | -,285 | -,079 | -,042 | -,286 | -,188 | ,018 | ,406 | ,491 | ,250 | -,309 | 1,000 |
| Zr | Coeff | | | | | | | | | | | | | | | | | | | | | | |
| | Sig. | ,090 | ,104 | ,108 | ,148 | ,934 | ,779 | ,008 | ,606 | ,405 | ,365 | ,803 | ,425 | ,829 | ,907 | ,493 | ,603 | ,960 | ,244 | ,150 | ,516 | ,385 | . |
| | N | 10 | 5 | 10 | 7 | 10 | 8 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 8 | 10 | 10 | 10 | 10 | 9 | 10 | 10 |

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Appendix 6 Complete result from the Spearman correlation analysis of the 15 samples deposited under oxygenated conditions (Gabon).

| | | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|----|------------|---------|-------|-------|--------|---------|---------|---------|--------|--------|--------|-------|---------|---------|--------|-------|--------|--------|--------|--------|---------|---------|--------|
| Al | Corr Coeff | 1,000 | -,549 | ,521* | ,357 | -,129 | -,903** | -,054 | -,527 | -,236 | ,464 | ,200 | -,700* | ,039 | ,121 | ,314 | -,568* | ,379 | ,110 | ,304 | -,279 | -,714** | ,204 |
| | Sig. | . | ,052 | ,046 | ,231 | ,648 | ,000 | ,850 | ,096 | ,398 | ,081 | ,475 | ,016 | ,889 | ,666 | ,544 | ,027 | ,164 | ,721 | ,271 | ,315 | ,004 | ,467 |
| | N | 15 | 13 | 15 | 13 | 15 | 10 | 15 | 11 | 15 | 15 | 15 | 11 | 15 | 15 | 6 | 15 | 15 | 13 | 15 | 15 | 14 | 15 |
| As | Corr Coeff | -,549 | 1,000 | -,363 | -,497 | -,187 | ,515 | ,544 | ,648* | ,192 | ,154 | -,319 | ,633 | -,104 | -,104 | -,429 | ,637* | -,049 | -,564 | ,231 | ,588* | ,441 | ,357 |
| | Sig. | ,052 | . | ,223 | ,101 | ,541 | ,128 | ,055 | ,043 | ,529 | ,616 | ,289 | ,067 | ,734 | ,734 | ,397 | ,019 | ,873 | ,071 | ,448 | ,035 | ,152 | ,231 |
| | N | 13 | 13 | 13 | 12 | 13 | 10 | 13 | 10 | 13 | 13 | 13 | 9 | 13 | 13 | 6 | 13 | 13 | 11 | 13 | 13 | 12 | 13 |
| Ba | Corr Coeff | ,521* | -,363 | 1,000 | ,396 | ,314 | -,261 | -,396 | -,291 | -,086 | ,054 | ,100 | -,773** | ,589* | ,200 | ,771 | -,518* | ,046 | ,571* | -,157 | -,464 | -,495 | -,307 |
| | Sig. | ,046 | ,223 | . | ,181 | ,254 | ,467 | ,143 | ,385 | ,761 | ,850 | ,723 | ,005 | ,021 | ,475 | ,072 | ,048 | ,869 | ,041 | ,576 | ,081 | ,072 | ,265 |
| | N | 15 | 13 | 15 | 13 | 15 | 10 | 15 | 11 | 15 | 15 | 15 | 11 | 15 | 15 | 6 | 15 | 15 | 13 | 15 | 15 | 14 | 15 |
| C | Corr Coeff | ,357 | -,497 | ,396 | 1,000 | ,434 | -,564 | -,681* | -,576 | ,280 | -,253 | ,346 | -,417 | ,220 | ,077 | -,429 | -,500 | ,005 | ,345 | -,467 | -,725** | -,161 | -,407 |
| | Sig. | ,231 | ,101 | ,181 | . | ,138 | ,090 | ,010 | ,082 | ,354 | ,405 | ,247 | ,265 | ,471 | ,803 | ,397 | ,082 | ,986 | ,298 | ,108 | ,005 | ,618 | ,168 |
| | N | 13 | 12 | 13 | 13 | 13 | 10 | 13 | 10 | 13 | 13 | 13 | 9 | 13 | 13 | 6 | 13 | 13 | 11 | 13 | 13 | 12 | 13 |
| Ca | Corr Coeff | -,129 | -,187 | ,314 | ,434 | 1,000 | ,030 | -,682** | -,545 | ,604* | -,493 | -,161 | ,000 | ,704** | ,693** | ,600 | -,407 | -,100 | ,736** | -,464 | -,693** | ,345 | -,439 |
| | Sig. | ,648 | ,541 | ,254 | ,138 | . | ,934 | ,005 | ,083 | ,017 | ,062 | ,567 | 1,000 | ,003 | ,004 | ,208 | ,132 | ,723 | ,004 | ,081 | ,004 | ,227 | ,101 |
| | N | 15 | 13 | 15 | 13 | 15 | 10 | 15 | 11 | 15 | 15 | 15 | 11 | 15 | 15 | 6 | 15 | 15 | 13 | 15 | 15 | 14 | 15 |
| Co | Corr Coeff | -,903** | ,515 | -,261 | -,564 | ,030 | 1,000 | -,188 | ,952** | ,079 | -,721* | -,079 | ,086 | ,358 | ,006 | ,200 | ,794** | -,648* | -,119 | -,479 | ,539 | ,517 | -,661* |
| | Sig. | ,000 | ,128 | ,467 | ,090 | ,934 | . | ,603 | ,000 | ,829 | ,019 | ,829 | ,872 | ,310 | ,987 | ,747 | ,006 | ,043 | ,779 | ,162 | ,108 | ,154 | ,038 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 8 | 10 | 10 | 10 | 6 | 10 | 10 | 5 | 10 | 10 | 8 | 10 | 10 | 9 | 10 |
| Cr | Corr Coeff | -,054 | ,544 | -,396 | -,681* | -,682** | -,188 | 1,000 | ,582 | -,539* | ,693** | -,207 | ,382 | -,693** | -,379 | ,200 | ,325 | ,396 | -,621* | ,689** | ,600* | -,152 | ,811** |
| | Sig. | ,850 | ,055 | ,143 | ,010 | ,005 | ,603 | . | ,060 | ,038 | ,004 | ,459 | ,247 | ,004 | ,164 | ,704 | ,237 | ,143 | ,024 | ,004 | ,018 | ,605 | ,000 |
| | N | 15 | 13 | 15 | 13 | 15 | 10 | 15 | 11 | 15 | 15 | 15 | 11 | 15 | 15 | 6 | 15 | 15 | 13 | 15 | 15 | 14 | 15 |
| Cu | Corr Coeff | -,527 | ,648* | -,291 | -,576 | -,545 | ,952** | ,582 | 1,000 | -,145 | -,045 | ,173 | ,250 | -,036 | -,345 | ,086 | ,900** | -,100 | -,503 | ,009 | ,700* | ,318 | ,145 |
| | Sig. | ,096 | ,043 | ,385 | ,082 | ,083 | ,000 | ,060 | . | ,670 | ,894 | ,612 | ,516 | ,915 | ,298 | ,872 | ,000 | ,770 | ,138 | ,979 | ,016 | ,340 | ,670 |
| | N | 11 | 10 | 11 | 10 | 11 | 8 | 11 | 11 | 11 | 11 | 11 | 9 | 11 | 11 | 6 | 11 | 11 | 10 | 11 | 11 | 11 | 11 |
| Fe | Corr Coeff | -,236 | ,192 | -,086 | ,280 | ,604* | ,079 | -,539* | -,145 | 1,000 | -,489 | ,104 | ,709* | ,346 | ,650** | -,600 | -,129 | -,107 | ,170 | -,414 | -,171 | ,609* | -,346 |
| | Sig. | ,398 | ,529 | ,761 | ,354 | ,017 | ,829 | ,038 | ,670 | . | ,064 | ,713 | ,015 | ,206 | ,009 | ,208 | ,648 | ,704 | ,578 | ,125 | ,541 | ,021 | ,206 |
| | N | 15 | 13 | 15 | 13 | 15 | 10 | 15 | 11 | 15 | 15 | 15 | 11 | 15 | 15 | 6 | 15 | 15 | 13 | 15 | 15 | 14 | 15 |
| K | Corr Coeff | ,464 | ,154 | ,054 | -,253 | -,493 | -,721* | ,693** | -,045 | -,489 | 1,000 | -,293 | -,245 | -,475 | -,046 | ,200 | -,196 | ,643** | -,451 | ,939** | ,436 | -,666** | ,893** |
| | Sig. | ,081 | ,616 | ,850 | ,405 | ,062 | ,019 | ,004 | ,894 | ,064 | . | ,289 | ,467 | ,074 | ,869 | ,704 | ,483 | ,010 | ,122 | ,000 | ,104 | ,009 | ,000 |
| | N | 15 | 13 | 15 | 13 | 15 | 10 | 15 | 11 | 15 | 15 | 15 | 11 | 15 | 15 | 6 | 15 | 15 | 13 | 15 | 15 | 14 | 15 |

| | | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|-----------|------------|---------|-------|---------|---------|---------|--------|---------|--------|--------|---------|-------|--------|-------|--------|-------|--------|-------|---------|-------|---------|--------|--------|
| Mg | Corr Coeff | ,200 | -,319 | ,100 | ,346 | -,161 | -,079 | -,207 | ,173 | ,104 | -,293 | 1,000 | ,155 | -,029 | -,371 | -,086 | ,211 | ,100 | ,049 | -,425 | -,175 | ,160 | -,343 |
| | Sig. | ,475 | ,289 | ,723 | ,247 | ,567 | ,829 | ,459 | ,612 | ,713 | ,289 | . | ,650 | ,919 | ,173 | ,872 | ,451 | ,723 | ,873 | ,114 | ,533 | ,584 | ,211 |
| | N | 15 | 13 | 15 | 13 | 15 | 10 | 15 | 11 | 15 | 15 | 15 | 11 | 15 | 15 | 6 | 15 | 15 | 13 | 15 | 15 | 14 | 15 |
| Mn | Corr Coeff | -,700* | ,633 | -,773** | -,417 | ,000 | ,086 | ,382 | ,250 | ,709* | -,245 | ,155 | 1,000 | -,400 | ,055 | -,143 | ,491 | ,064 | -,527 | ,027 | ,527 | ,809** | ,309 |
| | Sig. | ,016 | ,067 | ,005 | ,265 | 1,000 | ,872 | ,247 | ,516 | ,015 | ,467 | ,650 | . | ,223 | ,873 | ,787 | ,125 | ,853 | ,117 | ,937 | ,096 | ,003 | ,355 |
| | N | 11 | 9 | 11 | 9 | 11 | 6 | 11 | 9 | 11 | 11 | 11 | 11 | 11 | 11 | 6 | 11 | 11 | 10 | 11 | 11 | 11 | 11 |
| Na | Corr Coeff | ,039 | -,104 | ,589* | ,220 | ,704** | ,358 | -,693** | -,036 | ,346 | -,475 | -,029 | -,400 | 1,000 | ,500 | ,600 | -,161 | -,232 | ,654* | -,511 | -,457 | ,125 | -,636* |
| | Sig. | ,889 | ,734 | ,021 | ,471 | ,003 | ,310 | ,004 | ,915 | ,206 | ,074 | ,919 | ,223 | . | ,058 | ,208 | ,567 | ,405 | ,015 | ,052 | ,087 | ,670 | ,011 |
| | N | 15 | 13 | 15 | 13 | 15 | 10 | 15 | 11 | 15 | 15 | 15 | 11 | 15 | 15 | 6 | 15 | 15 | 13 | 15 | 15 | 14 | 15 |
| P | Corr Coeff | ,121 | -,104 | ,200 | ,077 | ,693** | ,006 | -,379 | -,345 | ,650** | -,046 | -,371 | ,055 | ,500 | 1,000 | ,486 | -,582* | ,196 | ,462 | ,004 | -,279 | ,059 | -,050 |
| | Sig. | ,666 | ,734 | ,475 | ,803 | ,004 | ,987 | ,164 | ,298 | ,009 | ,869 | ,173 | ,873 | ,058 | . | ,329 | ,023 | ,483 | ,112 | ,990 | ,315 | ,840 | ,860 |
| | N | 15 | 13 | 15 | 13 | 15 | 10 | 15 | 11 | 15 | 15 | 15 | 11 | 15 | 15 | 6 | 15 | 15 | 13 | 15 | 15 | 14 | 15 |
| Pb | Corr Coeff | ,314 | -,429 | ,771 | -,429 | ,600 | ,200 | ,200 | ,086 | -,600 | ,200 | -,086 | -,143 | ,600 | ,486 | 1,000 | -,143 | ,486 | ,771 | ,200 | -,314 | ,029 | ,200 |
| | Sig. | ,544 | ,397 | ,072 | ,397 | ,208 | ,747 | ,704 | ,872 | ,208 | ,704 | ,872 | ,787 | ,208 | ,329 | . | ,787 | ,329 | ,072 | ,704 | ,544 | ,957 | ,704 |
| | N | 6 | 6 | 6 | 6 | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| S | Corr Coeff | -,568* | ,637* | -,518* | -,500 | -,407 | ,794** | ,325 | ,900** | -,129 | -,196 | ,211 | ,491 | -,161 | -,582* | -,143 | 1,000 | -,271 | -,582* | -,093 | ,589* | ,512 | -,004 |
| | Sig. | ,027 | ,019 | ,048 | ,082 | ,132 | ,006 | ,237 | ,000 | ,648 | ,483 | ,451 | ,125 | ,567 | ,023 | ,787 | . | ,328 | ,037 | ,742 | ,021 | ,061 | ,990 |
| | N | 15 | 13 | 15 | 13 | 15 | 10 | 15 | 11 | 15 | 15 | 15 | 11 | 15 | 15 | 6 | 15 | 15 | 13 | 15 | 15 | 14 | 15 |
| Si | Corr Coeff | ,379 | -,049 | ,046 | ,005 | -,100 | -,648* | ,396 | -,100 | -,107 | ,643** | ,100 | ,064 | -,232 | ,196 | ,486 | -,271 | 1,000 | -,055 | ,561* | ,071 | -,209 | ,564* |
| | Sig. | ,164 | ,873 | ,869 | ,986 | ,723 | ,043 | ,143 | ,770 | ,704 | ,010 | ,723 | ,853 | ,405 | ,483 | ,329 | ,328 | . | ,859 | ,030 | ,800 | ,474 | ,028 |
| | N | 15 | 13 | 15 | 13 | 15 | 10 | 15 | 11 | 15 | 15 | 15 | 11 | 15 | 15 | 6 | 15 | 15 | 13 | 15 | 15 | 14 | 15 |
| Sr | Corr Coeff | ,110 | -,564 | ,571* | ,345 | ,736** | -,119 | -,621* | -,503 | ,170 | -,451 | ,049 | -,527 | ,654* | ,462 | ,771 | -,582* | -,055 | 1,000 | -,401 | -,703** | -,042 | -,511 |
| | Sig. | ,721 | ,071 | ,041 | ,298 | ,004 | ,779 | ,024 | ,138 | ,578 | ,122 | ,873 | ,117 | ,015 | ,112 | ,072 | ,037 | ,859 | . | ,174 | ,007 | ,897 | ,074 |
| | N | 13 | 11 | 13 | 11 | 13 | 8 | 13 | 10 | 13 | 13 | 13 | 10 | 13 | 13 | 6 | 13 | 13 | 13 | 13 | 13 | 12 | 13 |
| Ti | Corr Coeff | ,304 | ,231 | -,157 | -,467 | -,464 | -,479 | ,689** | ,009 | -,414 | ,939** | -,425 | ,027 | -,511 | ,004 | ,200 | -,093 | ,561* | -,401 | 1,000 | ,561* | -,455 | ,921** |
| | Sig. | ,271 | ,448 | ,576 | ,108 | ,081 | ,162 | ,004 | ,979 | ,125 | ,000 | ,114 | ,937 | ,052 | ,990 | ,704 | ,742 | ,030 | ,174 | . | ,030 | ,102 | ,000 |
| | N | 15 | 13 | 15 | 13 | 15 | 10 | 15 | 11 | 15 | 15 | 15 | 11 | 15 | 15 | 6 | 15 | 15 | 13 | 15 | 15 | 14 | 15 |
| V | Corr Coeff | -,279 | ,588* | -,464 | -,725** | -,693** | ,539 | ,600* | ,700* | -,171 | ,436 | -,175 | ,527 | -,457 | -,279 | -,314 | ,589* | ,071 | -,703** | ,561* | 1,000 | ,059 | ,496 |
| | Sig. | ,315 | ,035 | ,081 | ,005 | ,004 | ,108 | ,018 | ,016 | ,541 | ,104 | ,533 | ,096 | ,087 | ,315 | ,544 | ,021 | ,800 | ,007 | ,030 | . | ,840 | ,060 |
| | N | 15 | 13 | 15 | 13 | 15 | 10 | 15 | 11 | 15 | 15 | 15 | 11 | 15 | 15 | 6 | 15 | 15 | 13 | 15 | 15 | 14 | 15 |
| Zn | Corr Coeff | -,714** | ,441 | -,495 | -,161 | ,345 | ,517 | -,152 | ,318 | ,609* | -,666** | ,160 | ,809** | ,125 | ,059 | ,029 | ,512 | -,209 | -,042 | -,455 | ,059 | 1,000 | -,301 |

| | | Al | As | Ba | C | Ca | Co | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|-----------|-------|-----------|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|----------|-----------|-----------|-----------|----------|-----------|----------|-----------|-----------|-----------|----------|-----------|-----------|
| | Sig. | ,004 | ,152 | ,072 | ,618 | ,227 | ,154 | ,605 | ,340 | ,021 | ,009 | ,584 | ,003 | ,670 | ,840 | ,957 | ,061 | ,474 | ,897 | ,102 | ,840 | . | ,296 |
| | N | 14 | 12 | 14 | 12 | 14 | 9 | 14 | 11 | 14 | 14 | 14 | 11 | 14 | 14 | 6 | 14 | 14 | 12 | 14 | 14 | 14 | 14 |
| | Corr | | | | | | | | | | | | | | | | | | | | | | |
| | Coeff | ,204 | ,357 | -,307 | -,407 | -,439 | -,661* | ,811** | ,145 | -,346 | ,893** | -,343 | ,309 | -,636* | -,050 | ,200 | -,004 | ,564* | -,511 | ,921** | ,496 | -,301 | 1,000 |
| Zr | Sig. | ,467 | ,231 | ,265 | ,168 | ,101 | ,038 | ,000 | ,670 | ,206 | ,000 | ,211 | ,355 | ,011 | ,860 | ,704 | ,990 | ,028 | ,074 | ,000 | ,060 | ,296 | . |
| | N | 15 | 13 | 15 | 13 | 15 | 10 | 15 | 11 | 15 | 15 | 15 | 11 | 15 | 15 | 6 | 15 | 15 | 13 | 15 | 15 | 14 | 15 |

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 7 Complete result of the Spearman correlation analysis of the 9 samples deposited under euxinic conditions (Gabon).

| | | Al | As | Ba | C | Ca | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|----|------------|--------|---------|--------|-------|--------|---------|-------|-------|--------|--------|-------|--------|-------|---------|-------|-------|-------|--------|---------|-------|--------|
| Al | Corr Coeff | 1,000 | ,500 | ,667 | ,667 | -,317 | ,095 | ,543 | ,683* | ,817** | -,033 | -,017 | ,850** | ,550 | -,400 | -,067 | -,367 | ,143 | ,900** | ,429 | ,214 | ,643 |
| | Sig. | . | ,391 | ,071 | ,071 | ,406 | ,823 | ,266 | ,042 | ,007 | ,932 | ,966 | ,004 | ,125 | ,600 | ,865 | ,332 | ,736 | ,001 | ,397 | ,610 | ,119 |
| | N | 9 | 5 | 8 | 8 | 9 | 8 | 6 | 9 | 9 | 9 | 9 | 9 | 9 | 4 | 9 | 9 | 8 | 9 | 6 | 8 | 7 |
| As | Corr Coeff | ,500 | 1,000 | ,700 | ,400 | -,900* | 1,000** | -,500 | ,900* | ,900* | -,300 | ,300 | ,500 | ,900* | -,500 | -,100 | ,200 | ,900* | ,800 | 1,000** | ,100 | ,800 |
| | Sig. | ,391 | . | ,188 | ,600 | ,037 | . | ,667 | ,037 | ,037 | ,624 | ,624 | ,391 | ,037 | ,667 | ,873 | ,747 | ,037 | ,104 | . | ,873 | ,104 |
| | N | 5 | 5 | 5 | 4 | 5 | 5 | 3 | 5 | 5 | 5 | 5 | 5 | 5 | 3 | 5 | 5 | 5 | 5 | 4 | 5 | 5 |
| Ba | Corr Coeff | ,667 | ,700 | 1,000 | ,536 | -,738* | ,607 | -,600 | ,714* | ,643 | -,619 | -,095 | ,595 | ,619 | -,200 | -,429 | ,024 | ,405 | ,857** | ,657 | ,286 | ,464 |
| | Sig. | ,071 | ,188 | . | ,215 | ,037 | ,148 | ,285 | ,047 | ,086 | ,102 | ,823 | ,120 | ,102 | ,800 | ,289 | ,955 | ,320 | ,007 | ,156 | ,493 | ,294 |
| | N | 8 | 5 | 8 | 7 | 8 | 7 | 5 | 8 | 8 | 8 | 8 | 8 | 8 | 4 | 8 | 8 | 8 | 8 | 6 | 8 | 7 |
| C | Corr Coeff | ,667 | ,400 | ,536 | 1,000 | -,690 | ,036 | ,086 | ,286 | ,833* | -,619 | -,500 | ,714* | ,476 | -,800 | -,643 | -,190 | ,000 | ,786* | ,300 | -,179 | ,371 |
| | Sig. | ,071 | ,600 | ,215 | . | ,058 | ,939 | ,872 | ,493 | ,010 | ,102 | ,207 | ,047 | ,233 | ,200 | ,086 | ,651 | 1,000 | ,021 | ,624 | ,702 | ,468 |
| | N | 8 | 4 | 7 | 8 | 8 | 7 | 6 | 8 | 8 | 8 | 8 | 8 | 8 | 4 | 8 | 8 | 7 | 8 | 5 | 7 | 6 |
| Ca | Corr Coeff | -,317 | -,900* | -,738* | -,690 | 1,000 | -,833* | ,257 | -,417 | -,517 | ,817** | ,617 | -,550 | -,167 | ,800 | ,550 | ,167 | -,190 | -,467 | -,886* | ,000 | -,786* |
| | Sig. | ,406 | ,037 | ,037 | ,058 | . | ,010 | ,623 | ,265 | ,154 | ,007 | ,077 | ,125 | ,668 | ,200 | ,125 | ,668 | ,651 | ,205 | ,019 | 1,000 | ,036 |
| | N | 9 | 5 | 8 | 8 | 9 | 8 | 6 | 9 | 9 | 9 | 9 | 9 | 9 | 4 | 9 | 9 | 8 | 9 | 6 | 8 | 7 |
| Cr | Corr Coeff | ,095 | 1,000** | ,607 | ,036 | -,833* | 1,000 | -,086 | ,381 | ,238 | -,476 | -,429 | ,119 | ,000 | -,200 | -,095 | ,024 | ,750 | ,238 | ,943** | -,107 | ,643 |
| | Sig. | ,823 | . | ,148 | ,939 | ,010 | . | ,872 | ,352 | ,570 | ,233 | ,289 | ,779 | 1,000 | ,800 | ,823 | ,955 | ,052 | ,570 | ,005 | ,819 | ,119 |
| | N | 8 | 5 | 7 | 7 | 8 | 8 | 6 | 8 | 8 | 8 | 8 | 8 | 8 | 4 | 8 | 8 | 7 | 8 | 6 | 7 | 7 |
| Cu | Corr Coeff | ,543 | -,500 | -,600 | ,086 | ,257 | -,086 | 1,000 | -,086 | ,086 | ,257 | ,086 | ,371 | ,086 | 1,000** | ,486 | -,543 | -,400 | ,029 | -,200 | ,200 | ,600 |
| | Sig. | ,266 | ,667 | ,285 | ,872 | ,623 | ,872 | . | ,872 | ,872 | ,623 | ,872 | ,468 | ,872 | . | ,329 | ,266 | ,505 | ,957 | ,800 | ,747 | ,285 |
| | N | 6 | 3 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 3 | 6 | 6 | 5 | 6 | 4 | 5 | 5 |
| Fe | Corr Coeff | ,683* | ,900* | ,714* | ,286 | -,417 | ,381 | -,086 | 1,000 | ,717* | ,033 | ,150 | ,617 | ,450 | -,400 | -,167 | ,183 | ,619 | ,783* | ,829* | -,143 | ,607 |
| | Sig. | ,042 | ,037 | ,047 | ,493 | ,265 | ,352 | ,872 | . | ,030 | ,932 | ,700 | ,077 | ,224 | ,600 | ,668 | ,637 | ,102 | ,013 | ,042 | ,736 | ,148 |
| | N | 9 | 5 | 8 | 8 | 9 | 8 | 6 | 9 | 9 | 9 | 9 | 9 | 9 | 4 | 9 | 9 | 8 | 9 | 6 | 8 | 7 |
| K | Corr Coeff | ,817** | ,900* | ,643 | ,833* | -,517 | ,238 | ,086 | ,717* | 1,000 | -,183 | -,083 | ,767* | ,667* | -,800 | -,250 | -,150 | ,167 | ,917** | ,943** | ,024 | ,750 |
| | Sig. | ,007 | ,037 | ,086 | ,010 | ,154 | ,570 | ,872 | ,030 | . | ,637 | ,831 | ,016 | ,050 | ,200 | ,516 | ,700 | ,693 | ,001 | ,005 | ,955 | ,052 |
| | N | 9 | 5 | 8 | 8 | 9 | 8 | 6 | 9 | 9 | 9 | 9 | 9 | 9 | 4 | 9 | 9 | 8 | 9 | 6 | 8 | 7 |
| Mg | Corr Coeff | -,033 | -,300 | -,619 | -,619 | ,817** | -,476 | ,257 | ,033 | -,183 | 1,000 | ,783* | -,283 | ,100 | ,800 | ,783* | ,167 | -,071 | -,200 | -,086 | ,214 | -,250 |
| | Sig. | ,932 | ,624 | ,102 | ,102 | ,007 | ,233 | ,623 | ,932 | ,637 | . | ,013 | ,460 | ,798 | ,200 | ,013 | ,668 | ,867 | ,606 | ,872 | ,610 | ,589 |
| | N | 9 | 5 | 8 | 8 | 9 | 8 | 6 | 9 | 9 | 9 | 9 | 9 | 9 | 4 | 9 | 9 | 8 | 9 | 6 | 8 | 7 |

| | | Al | As | Ba | C | Ca | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|-----------|------------|--------|---------|--------|-------|--------|--------|---------|-------|--------|-------|-------|----------|-------|----------|-------|-------|-------|-------|-------|-------|-------|
| Mn | Corr Coeff | -,017 | ,300 | -,095 | -,500 | ,617 | -,429 | ,086 | ,150 | -,083 | ,783* | 1,000 | -,267 | ,467 | ,600 | ,583 | ,500 | ,000 | -,050 | ,200 | ,571 | ,036 |
| | Sig. | ,966 | ,624 | ,823 | ,207 | ,077 | ,289 | ,872 | ,700 | ,831 | ,013 | . | ,488 | ,205 | ,400 | ,099 | ,170 | 1,000 | ,898 | ,704 | ,139 | ,939 |
| | N | 9 | 5 | 8 | 8 | 9 | 8 | 6 | 9 | 9 | 9 | 9 | 9 | 9 | 4 | 9 | 9 | 8 | 9 | 6 | 8 | 7 |
| Na | Corr Coeff | ,850** | ,500 | ,595 | ,714* | -,550 | ,119 | ,371 | ,617 | ,767* | -,283 | -,267 | 1,000 | ,267 | -1,000** | -,250 | -,517 | -,071 | ,750* | ,429 | ,071 | ,607 |
| | Sig. | ,004 | ,391 | ,120 | ,047 | ,125 | ,779 | ,468 | ,077 | ,016 | ,460 | ,488 | . | ,488 | . | ,516 | ,154 | ,867 | ,020 | ,397 | ,867 | ,148 |
| | N | 9 | 5 | 8 | 8 | 9 | 8 | 6 | 9 | 9 | 9 | 9 | 9 | 9 | 4 | 9 | 9 | 8 | 9 | 6 | 8 | 7 |
| P | Corr Coeff | ,550 | ,900* | ,619 | ,476 | -,167 | ,000 | ,086 | ,450 | ,667* | ,100 | ,467 | ,267 | 1,000 | ,400 | ,150 | ,200 | ,167 | ,667* | ,429 | ,690 | ,536 |
| | Sig. | ,125 | ,037 | ,102 | ,233 | ,668 | 1,000 | ,872 | ,224 | ,050 | ,798 | ,205 | ,488 | . | ,600 | ,700 | ,606 | ,693 | ,050 | ,397 | ,058 | ,215 |
| | N | 9 | 5 | 8 | 8 | 9 | 8 | 6 | 9 | 9 | 9 | 9 | 9 | 9 | 4 | 9 | 9 | 8 | 9 | 6 | 8 | 7 |
| Pb | Corr Coeff | -,400 | -,500 | -,200 | -,800 | ,800 | -,200 | 1,000** | -,400 | -,800 | ,800 | ,600 | -1,000** | ,400 | 1,000 | ,800 | -,200 | -,200 | -,400 | -,500 | ,800 | -,400 |
| | Sig. | ,600 | ,667 | ,800 | ,200 | ,200 | ,800 | . | ,600 | ,200 | ,200 | ,400 | . | ,600 | . | ,200 | ,800 | ,800 | ,600 | ,667 | ,200 | ,600 |
| | N | 4 | 3 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 |
| S | Corr Coeff | -,067 | -,100 | -,429 | -,643 | ,550 | -,095 | ,486 | -,167 | -,250 | ,783* | ,583 | -,250 | ,150 | ,800 | 1,000 | -,183 | -,310 | -,300 | -,086 | ,619 | -,071 |
| | Sig. | ,865 | ,873 | ,289 | ,086 | ,125 | ,823 | ,329 | ,668 | ,516 | ,013 | ,099 | ,516 | ,700 | ,200 | . | ,637 | ,456 | ,433 | ,872 | ,102 | ,879 |
| | N | 9 | 5 | 8 | 8 | 9 | 8 | 6 | 9 | 9 | 9 | 9 | 9 | 9 | 4 | 9 | 9 | 8 | 9 | 6 | 8 | 7 |
| Si | Corr Coeff | -,367 | ,200 | ,024 | -,190 | ,167 | ,024 | -,543 | ,183 | -,150 | ,167 | ,500 | -,517 | ,200 | -,200 | -,183 | 1,000 | ,548 | -,117 | ,257 | -,238 | ,250 |
| | Sig. | ,332 | ,747 | ,955 | ,651 | ,668 | ,955 | ,266 | ,637 | ,700 | ,668 | ,170 | ,154 | ,606 | ,800 | ,637 | . | ,160 | ,765 | ,623 | ,570 | ,589 |
| | N | 9 | 5 | 8 | 8 | 9 | 8 | 6 | 9 | 9 | 9 | 9 | 9 | 9 | 4 | 9 | 9 | 8 | 9 | 6 | 8 | 7 |
| Sr | Corr Coeff | ,143 | ,900* | ,405 | ,000 | -,190 | ,750 | -,400 | ,619 | ,167 | -,071 | ,000 | -,071 | ,167 | -,200 | -,310 | ,548 | 1,000 | ,405 | ,486 | -,310 | ,393 |
| | Sig. | ,736 | ,037 | ,320 | 1,000 | ,651 | ,052 | ,505 | ,102 | ,693 | ,867 | 1,000 | ,867 | ,693 | ,800 | ,456 | ,160 | . | ,320 | ,329 | ,456 | ,383 |
| | N | 8 | 5 | 8 | 7 | 8 | 7 | 5 | 8 | 8 | 8 | 8 | 8 | 8 | 4 | 8 | 8 | 8 | 8 | 6 | 8 | 7 |
| Ti | Corr Coeff | ,900** | ,800 | ,857** | ,786* | -,467 | ,238 | ,029 | ,783* | ,917** | -,200 | -,050 | ,750* | ,667* | -,400 | -,300 | -,117 | ,405 | 1,000 | ,771 | ,071 | ,607 |
| | Sig. | ,001 | ,104 | ,007 | ,021 | ,205 | ,570 | ,957 | ,013 | ,001 | ,606 | ,898 | ,020 | ,050 | ,600 | ,433 | ,765 | ,320 | . | ,072 | ,867 | ,148 |
| | N | 9 | 5 | 8 | 8 | 9 | 8 | 6 | 9 | 9 | 9 | 9 | 9 | 9 | 4 | 9 | 9 | 8 | 9 | 6 | 8 | 7 |
| V | Corr Coeff | ,429 | 1,000** | ,657 | ,300 | -,886* | ,943** | -,200 | ,829* | ,943** | -,086 | ,200 | ,429 | ,429 | -,500 | -,086 | ,257 | ,486 | ,771 | 1,000 | ,086 | ,714 |
| | Sig. | ,397 | . | ,156 | ,624 | ,019 | ,005 | ,800 | ,042 | ,005 | ,872 | ,704 | ,397 | ,397 | ,667 | ,872 | ,623 | ,329 | ,072 | . | ,872 | ,111 |
| | N | 6 | 4 | 6 | 5 | 6 | 6 | 4 | 6 | 6 | 6 | 6 | 6 | 6 | 3 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Zn | Corr Coeff | ,214 | ,100 | ,286 | -,179 | ,000 | -,107 | ,200 | -,143 | ,024 | ,214 | ,571 | ,071 | ,690 | ,800 | ,619 | -,238 | -,310 | ,071 | ,086 | 1,000 | ,036 |
| | Sig. | ,610 | ,873 | ,493 | ,702 | 1,000 | ,819 | ,747 | ,736 | ,955 | ,610 | ,139 | ,867 | ,058 | ,200 | ,102 | ,570 | ,456 | ,867 | ,872 | . | ,939 |
| | N | 8 | 5 | 8 | 7 | 8 | 7 | 5 | 8 | 8 | 8 | 8 | 8 | 8 | 4 | 8 | 8 | 8 | 8 | 6 | 8 | 7 |
| Zr | Corr Coeff | ,643 | ,800 | ,464 | ,371 | -,786* | ,643 | ,600 | ,607 | ,750 | -,250 | ,036 | ,607 | ,536 | -,400 | -,071 | ,250 | ,393 | ,607 | ,714 | ,036 | 1,000 |

| | Al | As | Ba | C | Ca | Cr | Cu | Fe | K | Mg | Mn | Na | P | Pb | S | Si | Sr | Ti | V | Zn | Zr |
|------|-----------|-----------|-----------|----------|-----------|-----------|-----------|-----------|----------|-----------|-----------|-----------|----------|-----------|----------|-----------|-----------|-----------|----------|-----------|-----------|
| Sig. | ,119 | ,104 | ,294 | ,468 | ,036 | ,119 | ,285 | ,148 | ,052 | ,589 | ,939 | ,148 | ,215 | ,600 | ,879 | ,589 | ,383 | ,148 | ,111 | ,939 | . |
| N | 7 | 5 | 7 | 6 | 7 | 7 | 5 | 7 | 7 | 7 | 7 | 7 | 7 | 4 | 7 | 7 | 7 | 7 | 6 | 7 | 7 |

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

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