# Determining the Concentration and Source of Lead in Chocolate Using Lead Isotopes

by

Jessica A. Thompson

Submitted to the Department of Earth, Atmospheric and Planetary Sciences

in Partial Fulfillment of the Requirements for the Degree of

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## ABSTRACT

Single-origin dark chocolate samples derived from cocoa grown in developing countries from around the world were analyzed to determine their lead concentrations and the isotopic composition of the lead. The lead isotope ratios were compared with published data from aerosols and volcanic rocks nearest to the cocoa growing regions. Samples from different countries and manufacturers were compared, and we conclude that the source of lead depends on the country of origin and not the manufacturer. Chocolates grown in the Northern Hemisphere usually had lead isotope ratios that matched the global atmospheric lead isotopic signature from the Northern Hemisphere. Chocolates grown in the Southern Hemisphere did not match the global signature, but rather more closely matched the lead isotopic signature from volcanic rocks in their respective countries, and had a lower average lead concentration than chocolates from the Northern Hemisphere. Soils from Venezuela were also analyzed, and confirmed the conclusion that atmospheric lead is the predominant source of bioavailable lead. Many of the chocolates also had lead concentrations below the limit of 0.1 ppm set by the FDA; however, one manufacturer, Dagoba, consistently had lead concentrations above the limit. The percent of cocoa in each chocolate bar was also compared with the lead concentrations, concluding that the concentration of lead is not necessarily dependent on the amount of cocoa in the bar.

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Title: Professor of Geology

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# Contents

Case Studies:

## List of Figures

## List of Tables

# Chapter 1. Introduction

Lead contamination from anthropogenic sources has increased since the industrial revolution. The most common sources of anthropogenic lead include the combustion of leaded gasoline, the production of steel and base metals, the mining and smelting of lead ores, and the combustion of coal. The global record of both natural and anthropogenic atmospheric transport and deposition of lead in the environment has been monitored using both lead concentrations and isotopic compositions in sediments, ice, soils, and peat bogs. Though lead is released into the environment through natural weathering of rocks, several analyses of variations in the isotopic composition of lead (Bollhofer 2000, 2001, 2002, Gulson 1981, Lima 2005) have shown a strong correlation between the increase in anthropogenic lead emissions and the amount and source of lead detected in the environment.

Lead contamination in food has become a "hot button" environmental issue, leading the U.S. Food and Drug Administration (FDA) and the World Health Organization (WHO) to set limits on the maximum allowable concentrations. More recently, concern about the amount of lead in chocolate has become an issue in the United States. In 2003, the American Environmental Safety Institute (AESI) even went so far as to sue the Hershey Food Corporation and Mars Incorporated, two well-known chocolate manufacturers, for dangerous levels of lead in their chocolate products (Morrison & Foerster, www.mofo.com/practice/practice/enivornmental/prop65/ overview.html). This case prompted an in-depth study of lead in chocolate and other candy products, focusing on whether the concentrations were high enough to cause danger to young children, who are more susceptible to lead poisoning than adults. The studies determined that the amount of lead in one chocolate bar is not enough to be dangerous; however, lead is a cumulative poison (O'Neill 1993). While a single chocolate bar may not contain enough lead to be concerned about, the average American consumes between 10-12 pounds of chocolate or chocolate products each year (http://www.rawcacao.com/). For the AESI case, some chocolates from common grocery stores in California were found to have concentrations as high as 0.11 μg/g or ppm, while most were around 0.01 μg/g (Heneman 2006). Using the high value, over the course of a year, the average American could ingest a total of 499 -599 μg of lead! Recently, a new trend has entered the market: single origin dark chocolate bars, specializing in rich chocolate flavors, served at trendy chocolate cafes (Kauffman 2005). These dark chocolates have a higher cocoa bean and cocoa fat content than milk chocolates, which are often diluted with milk, sugar and other ingredients. The higher cocoa bean and cocoa butter content (which has been suggested to have higher lead concentrations than cocoa beans (Mounicou 2003)) in dark chocolate means dark chocolates could, on average, a higher lead concentration.

The source(s) of lead contamination in chocolate are the subject of debate. Most manufactured chocolate comes from cacao plantations in developing countries situated within 20 degrees of the equator, where simple, non mechanized farming methods are often used to gather the cacao pods and remove the beans. The beans are laid out to ferment and dry under the sun before being shipped to more developed countries for further cleaning, roasting, and final processing. The long journey the beans take from cacao plant to manufactured chocolate bar allows for many possible sources of contamination along the way. A previous study (Rankin 2005) found a correlation between the isotopic composition of aerosol lead and that in chocolate, suggesting that contamination may occur while drying or fermenting under the sun. However, these studies also found a relatively low lead concentration in cocoa beans compared to the manufactured chocolate products, leading Rankin to conclude that most lead contamination occurs in the shipping or manufacturing process.

Lead isotopic ratios are a powerful tool for recognizing contamination and tracing the source(s) of lead in food, and these are important steps in minimizing human exposure. Though chocolate is widely consumed, neither the sources nor levels of lead contamination have been thoroughly studied. This study focuses on the analysis of both concentrations and the isotopic composition of lead in chocolate to constrain the source(s) of lead. In this study I compare the isotopic composition of Pb in various chocolate samples with those from aerosols measured at various stations around the world in the 1990s and early 2000s, as well as rock lead and soil isotopic compositions from the regions near where cacao plantations exist. This should allow one to distinguish between "naturally occurring" Pb and anthropogenic Pb to determine the source of lead in chocolate.

# Chapter 2. Background Information

## 2.1 Lead

### 2.1.1 Sources of Lead

Lead is a naturally occurring chemical element that occurs in concentrations from a few parts per billion (ppb) to tens's of parts per million (ppm) in most rocks and minerals. However Pb can also be highly concentrated by hydrothermal processes in ore deposits that are often mined for industrial uses. Terrestrial lead is a combination of the lead incorporated into the building blocks of the earth during condensation and accretion of our solar system, and lead that has formed due to radioactive decay of uranium and thorium. Primordial lead compositions have been obtained from metal or sulfide rich meteorites that contain very small amounts of uranium and thorium; thus their lead isotopic compositions have changed very little over the past 4.567 billion years, the conventionally accepted age of the earth. Lead is produced by the decay of three radioactive isotopes. They are $^{232}$Th, $^{235}$U, and $^{238}$U, which decay into $^{208}$Pb, $^{207}$Pb, and $^{206}$Pb, respectively. Although $^{204}$Pb is weakly radioactive ($T_{1/2}$= 1.4 x $10^{17}$ years) it is often used as a reference isotope because its quantities have remained essentially constant over earth history. Isotopic compositions of lead are usually expressed as ratios of the abundances of a lead isotope with a standard, such as $^{206}$Pb, to $^{204}$Pb. The primordial composition of different lead ratios is (Oversby 1970):

| | |
|---|---|
| $^{206}$Pb /$^{204}$Pb | 9.346 |
| $^{207}$Pb /$^{204}$Pb | 10.218 |
| $^{208}$Pb /$^{204}$Pb | 28.96 |

The isotopic composition of any lead is variable and depends on the U/Pb ratio of the mineral it resides in. For example, the isotopic composition of galena ore (PbS) does not change once it is precipitated because the U/Pb ratio is essentially zero. However, a zircon crystal has a very high U/Pb ratio and over time the U decays to Pb. In fact, since we know the rate of radioactive decay, this mineral can be used to calculate geological ages. Lead in ore minerals such as galena is scavenged from large volumes of rocks through which hydrothermal fluids circulate. Thus, the ore's isotopic composition reflects the age and U/Pb ratio of all the rocks that contributed Pb and the values are very much average values. This, however, is why each ore deposit tends to have a distinctive range of isotopic compositions and why we can then use that to trace Pb as it moves through the environment. Lead in minerals and rocks is released during chemical weathering (Faure 1991). Although there are small amounts of lead in the soil, the water, and the air, it tends to be in low concentration, is not a hazard to humans, and is referred to as "naturally occurring".

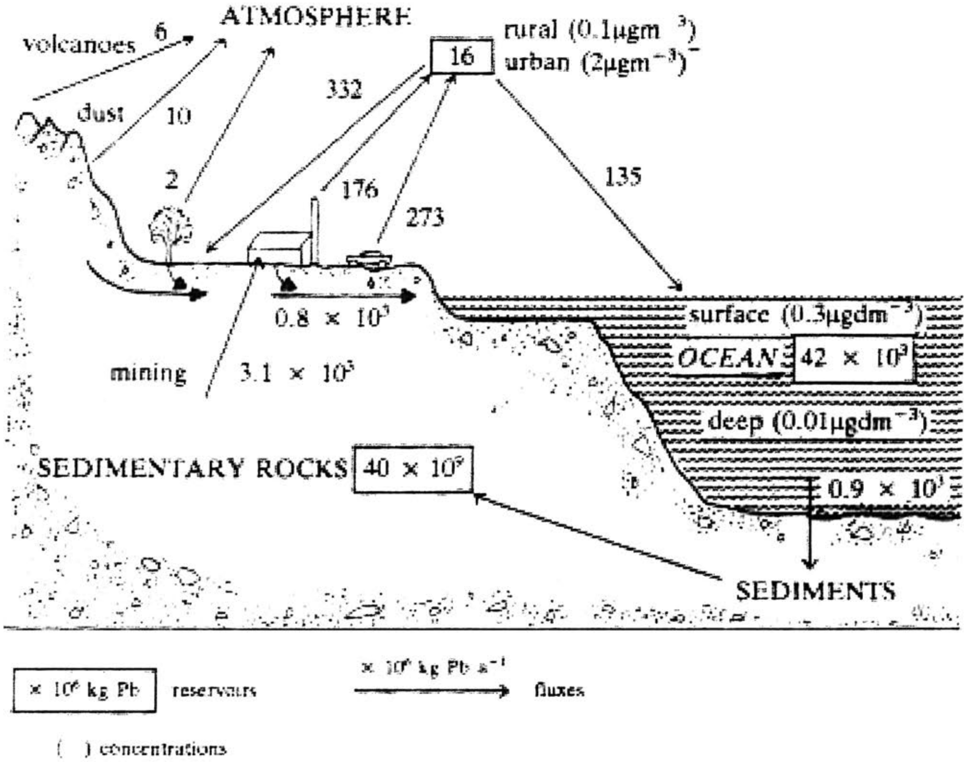


Figure 1: The lead cycle (from O'Neill 1993). A diagram showing the distribution and flows of lead in the environment.

Much of the lead in our environment today is anthropogenic. For example, natural sources only contribute about 4% to the total lead in the atmosphere (Faure 1991). Figure 1 illustrates the lead cycle, showing the flow and distribution of natural and anthropogenic sources within the environment. Natural lead in the atmosphere derives from windblown dust, plant exudates, forest fires, volcanic eruptions and seasalt spray (Faure 1991). A history of lead accumulation is recorded in snow, ice, lacustrine sediments, and plants (Faure 1991). In fact, Greenland ice cores have shown a 400 -fold increase in lead concentration between 800 BC and 1965 AD (O'Neill 1993). Anthropogenic sources of lead include the combustion of leaded gasoline, the production of steel and base metals, the mining and smelting of lead, and the combustion of coal (Faure 1991). There are two major uses of lead by humans: in lead-acid storage batteries, especially in motor vehicles, and in gasoline. Gasoline, though, proves to be the largest source of lead in the environment, particularly in developing countries where leaded gasoline is still used today.

The use of lead in gasoline as an anti-knock additive was common in the US for over 50 years until the mid 1970s when it was mandated that it be removed (Elsom 1992). Lead as a gasoline additive only contributes about 2.2 percent of the total lead use worldwide in contrast to 63 percent that is used in the manufacturing of batteries – yet it is the lead in gasoline additives that has had the largest effect on human health worldwide (Finkelman 1996); this is because vehicle emissions are directly into the environment. To combat this, as of 1996, two years after the Summit of the Americas where governments agreed to phase out leaded gasoline, 6 countries in the Western Hemisphere have completely removed the lead in gasoline, 13 countries have reduced to levels of 0.4 g/L, and 16 countries continue to sell leaded gasoline with levels of 0.8 g/L (Finkelman 1996). As of 2007, only 17 nations worldwide still regularly sell gasoline, mostly in the Middle East and Africa (http://www.lead.org.au/fs/fst27.html). However, though the amount of lead in gasoline is being reduced worldwide, the number of vehicles that operate on gasoline is increasing. In Venezuela, for example, lead levels in gasoline have been reduced from 0.84 g/L to 0.45 g/L in the late 1990s (Venezuela has recently passed laws to phase out leaded gasoline), but the number of vehicles has nearly tripled since the 1970s, creating a large increase in the total amount of lead released into the environment (3,763 to 5,492 tons/year) (Finkelman 1996). While leaded gasoline may no longer be a significant contributor to atmospheric lead in developed countries, in developing countries, some with higher lead concentrations in gasoline and economic and population increases, lead contamination from gasoline remains a serious issue.

### 2.1.2 Atmospheric Lead

Before the mid twentieth century, atmospheric pollutants were thought to affect only the areas immediately surrounding the emission sources (Elsom 1992). However we now understand that atmospheric contaminants may travel very long distances, and can affect locations far from the emission source (Elsom 1992). Atmospheric pollution can affect the quality of soils, water, vegetation, climate, animals, and buildings (Elsom 1992). While air pollution control measures have been put in place in many developed countries, most of the developing countries still lack proper plans to control or deal with air pollution, worried that such restrictions could negatively affect their economic and industrial growth (Elsom 1992).

As previously mentioned, leaded gasoline is a large contributor to lead aerosols in the atmosphere. Over 70% of the lead in combusted gasoline enters the atmosphere, and of this, 40% falls quickly nearby on roads or surrounding areas, 8% within average city limits of a few miles, and up to 24% becomes widely dispersed (Elsom 1992). This widely dispersed lead is caught in atmospheric circulation, where it has a residence time of 1-2 weeks and can travel thousands of kilometers (Elsom 1992). Figure 2 illustrates the paths some aerosol particles can take.

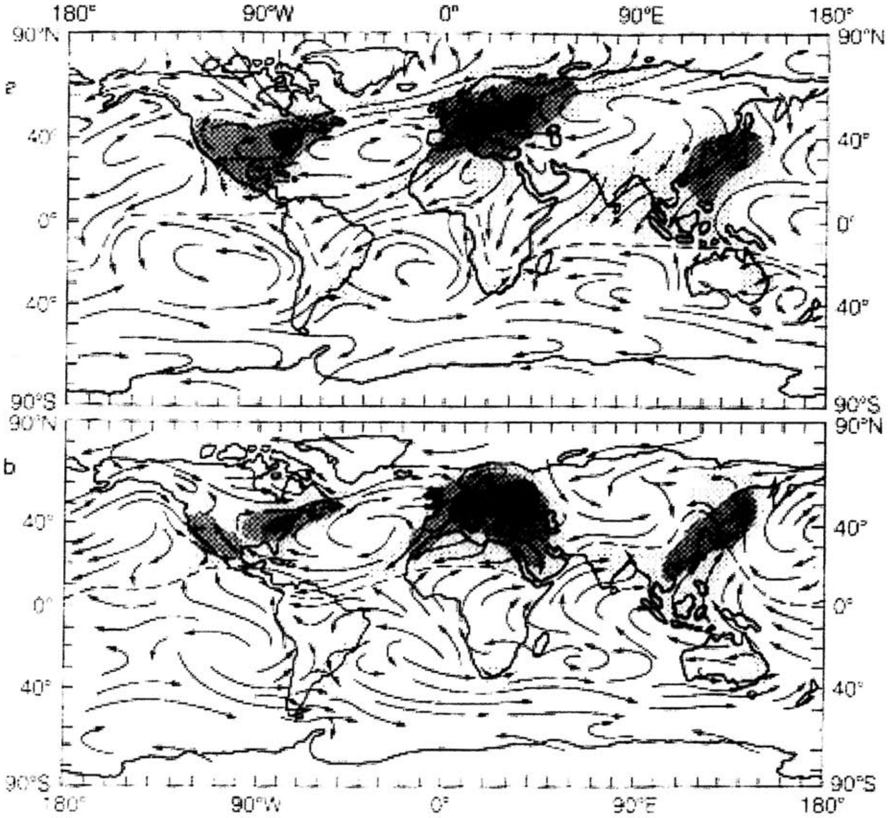


Figure 2: A map of the world, from Elsom 1992 , showing the atmospheric circulation of aerosols. The top image shows atmospheric circulation during January, and the bottom image shows atmospheric circulation during July.

Anthropogenic lead in the atmosphere accounts for 96-99% of the total atmospheric lead deposition (Erel 1997). The concentration of aerosol lead particles varies significantly with location, with large cities having 2-4 μg/cubic meter, and suburban and rural areas often having 2 μg/cubic meter or less (Elsom 1992).

### 2.1.3 Lead in Soils

Lead accumulates in soils from a combination of airfall deposition and bedrock weathering (Erel 1997). Lead is found in soil as a trace element in both primary and secondary silicate minerals. In soils, anthropogenic lead is often associated with organic matter, and is usually concentrated in the upper few centimeters (O'Neill 1993). Lead levels in soils vary a great deal, depending on atmospheric deposition and proximity to point sources of pollution. The residence time of lead in soil is between 17 and 80 years (Miller and Friedland 1994).

### 2.1.4 Lead as an Environmental Tracer

Lead isotopes can be used to fingerprint different sources of lead in the environment, including distinguishing between rock-derived and anthropogenic lead (Erel 1997). In general, the isotopic ratios of ore deposits, which are sources for anthropogenic uses through mining, are often less radiogenic than lead that is released from the weathering of rocks (Erel 1997) and large ore deposits have distinctive isotopic signals. This is a basis for distinguishing between anthropogenic lead (ie, lead from ore deposits) and so called "natural" lead. By analyzing the isotopic composition of lead in environmental samples, it is possible to trace the origin of the lead by comparing the ratio from the sample to known anthropogenic and natural ratios from previous studies.

Lead isotopes have another major attribute that make them an excellent proxy for tracing environmental pollutants, particularly in the United States. Lead's isotopic composition is not affected by changes in the geological environment, such as weathering (Gulson 1986), and therefore it can travel in the environment without changing its isotopic composition, allowing for it to be traced to a source.

There is a great deal of information in the isotopic composition of lead and over the years a number of plotting schemes have been developed to deconvolve multiple components in environmental lead samples. Lead isotopes are usually plotted in ratio/ratio space such as ($^{206}$Pb /$^{204}$Pb ) versus ($^{207}$Pb /$^{204}$Pb). When conducting environmental studies, this is useful to explore and constrain different sources of Pb. Figure 3 is an example of this, with the lead isotope compositions of various ore deposits and coal plotted with samples taken from lake Erie, Pettaquamscutt River and Chesapeake Bay demonstrating that the leads in the sediment samples are averages of industrial Pb (from Lima 2005).

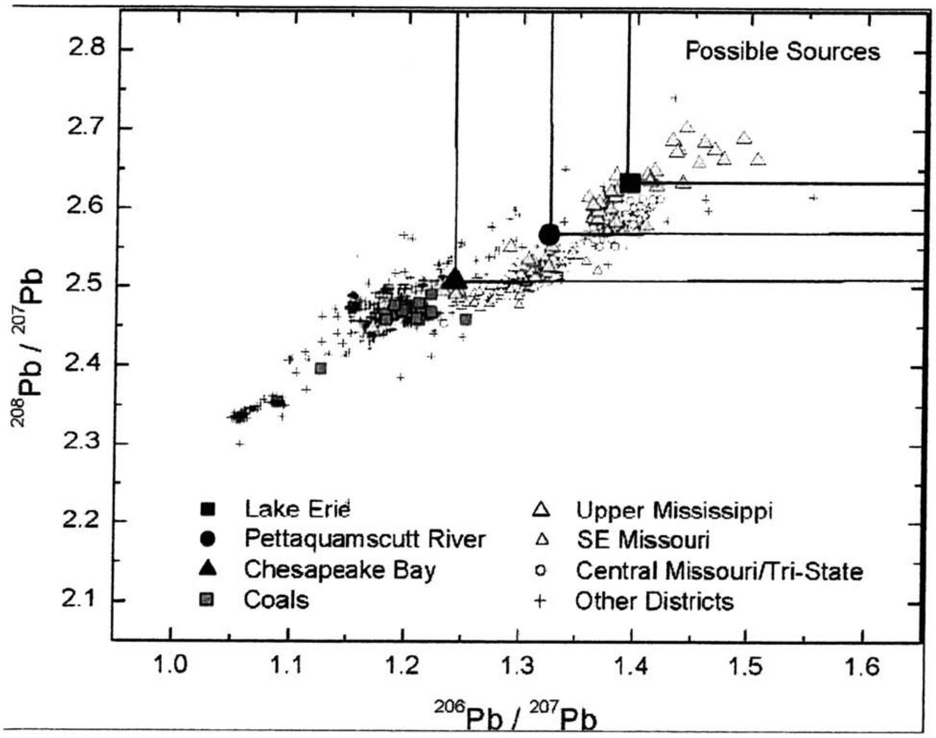


Figure 3: A plot of $^{206}$Pb /$^{207}$Pb and $^{208}$Pb /$^{207}$Pb isotope ratios from ores, coals and recent sediment samples, from Lima 2005.

Plotting the $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb and $^{206}$Pb /$^{204}$Pb vs. $^{208}$Pb /$^{204}$Pb ratios is also useful in constraining sources. The isotopic compositions of ore deposits reflect the average U/Pb and Th/Pb ratio of the source from which the Pb is derived. The $^{206}$Pb /$^{204}$Pb vs $^{207}$Pb /$^{204}$Pb plot is for uranogenic Pb and the $^{206}$Pb /$^{204}$Pb or $^{207}$Pb /$^{204}$Pb vs $^{208}$Pb /$^{204}$Pb highlights differences in thorogenic Pb ($^{208}$Pb) vs uranogenic Pb. Using all three ratios allows better discrimination of environmental Pb samples. Figure 4 shows an example of how using both the $^{206}$Pb /$^{204}$Pb and $^{207}$Pb /$^{204}$Pb and $^{206}$Pb /$^{204}$Pb and $^{208}$Pb /$^{204}$Pb ratios is useful in constraining a source. By plotting the different ratios, the source of lead can be determined (in this case, sample series A is contaminated by "ore", whereas sample series B is not).

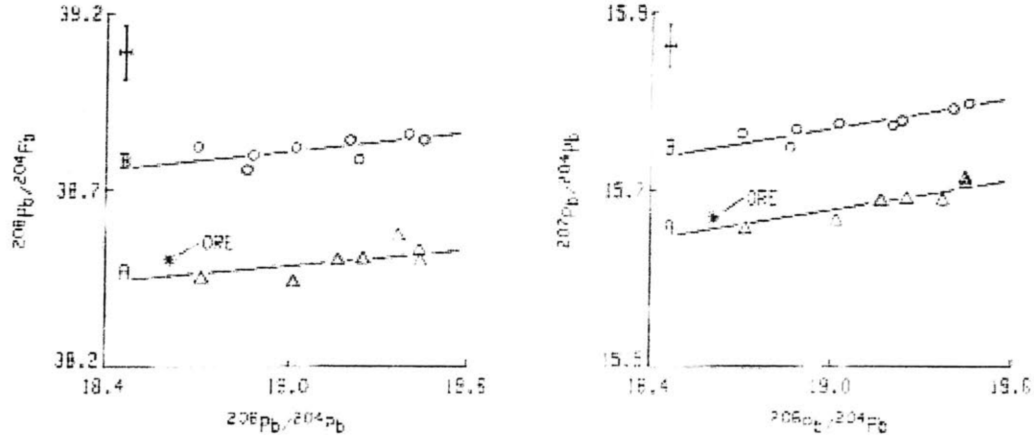


Figure 4. A plot of $^{206}$Pb /$^{204}$Pb and $^{207}$Pb /$^{204}$Pb and $^{206}$Pb /$^{204}$Pb and $^{208}$Pb /$^{204}$Pb ratios demonstrating the ability to constrain a source using lead isotope ratios (from Gulson 1986).

### 2.1.5 Health Effects of Lead Exposure

Most humans who are affected by lead poisoning have been exposed to lead from common household items (Faure 1991). These include paint, water pipes, solder, leaded gasoline, ammunition, storage batteries in automobiles, and glaze on pottery to name a few (Faure 1991). Those who work in a lead-rich environment, such as miners and smelters of lead, have a high risk of lead poisoning (Faure 1991). Young children are especially at risk for lead poisoning. Children with high exposure to lead at an early age are at risk for neurobehavioral disorders (Heneman 2006).

The World Health Organization has set the lead exposure standards at 3 μg per person per week for adults, and less than 1 μg per person per week for children and infants (O'Neill 1993). The rate of absorption of dietary lead is about 8 percent per year, but if lead enters the lungs, over 40 percent is absorbed. The absorbed lead enters the bloodstream, and is distributed throughout the body. The lead then accumulates in different locations throughout the body, most commonly in soft tissues, bones, and hair, for up to one month. Lead that accumulates in the bones can remain there for anywhere from 40-90 years (O'Neill 1993).

Lead is a cumulative poison, with over 90 percent of ingested lead retained in our bones (O'Neill 1993). It binds strongly to many molecules, such as amino acids, hemoglobin, many enzymes, RNA and DNA; it disrupts metabolic pathways and impairs blood synthesis, causing hypertension, hyperactivity, and brain damage (O'Neill 1993). Early lead poisoning symptoms include loss of appetite, colic, pallor, weight loss, fatigue, and irritability (Faure 1991).

The more serious symptoms of lead poisoning are well documented. Anemia, with a decrease in hemoglobin levels, is an early sign, usually occurring around blood levels of 50 μg/dl in adults, and 40 μg/dl in children (Betts 1973). Brain dysfunction begins at levels of 50-60 μg/dl in children and 60-70 μg/dl in adults. Acute or chronic encephalopathy and kidney damage occur at levels of 60-70 μg/dl in children and 80 μg/dl in adults (WHO 1977).

Average blood lead levels worldwide range from 6 μg/dl in Beijing, China, to 10-20 μg/dl in Europe, to 22 μg/dl in Mexico, to as low as 3 μg/dl in the United States (UK Royal Commission on Environmental Pollution 1983). Figure 5, from Lovei 1998, shows the drastic difference in blood lead levels between different cities worldwide. However, the blood lead levels have dropped considerably with the reduction of lead in gasoline. In the United States alone, average levels dropped from 15.8 μg/dl in the 1970s to 10 μg /dl in the 1980s (Elsom 1992), and even lower, to around 3 μg/dl in 1990 (USEPA), as depicted in Figure 6.

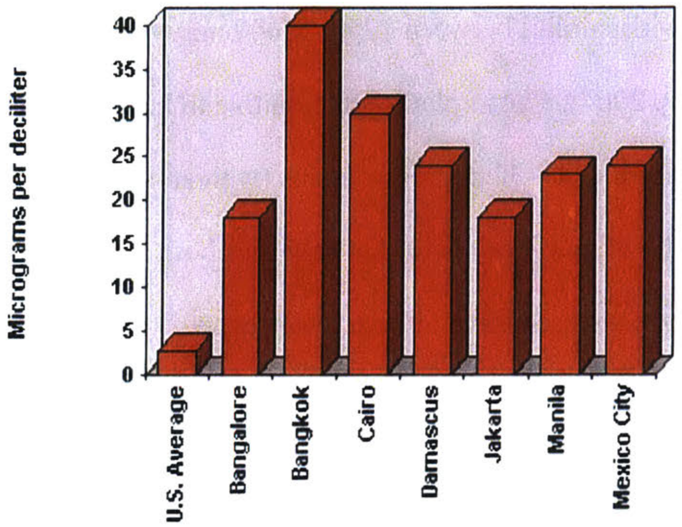


Figure 5: A graph from Lovei 1998, depicting the average blood levels for various cities around the world. The United States average is much lower compared to many other cities worldwide.

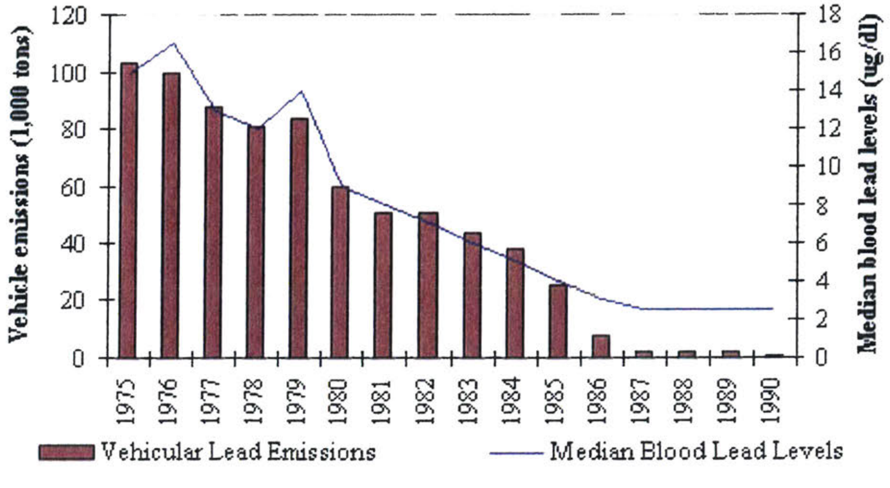


Figure 6: A graph showing the decrease in median blood lead levels and vehicular lead emissions in the United States from 1975-1990. The blood lead level drop coincides directly with the decrease in vehicular lead emissions (USEPA).

## 2.2 Chocolate & Cacao

### 2.2.1 Chocolate and Cacao Production

Chocolate is produced in the equatorial regions of the world (see Figure 7), limiting cultivation to between about 20 degrees north and south of the equator (Urquhart 1966). Cacao grows mostly in fertile soils with rainfall between 1250 – 3000 mm/year and a dry season of no longer than three months (Clay 2004). Cacao plants are sensitive to heat, sunlight and wind. They grow best between a temperature of 18-32 degrees Celsius (with an absolute minimum of 10 degrees C), and within the shelter of an older forest that serves as both a windbreak and shade from the sunlight. Cacao plants are also sensitive to both waterlogged soils and dry soils, and cannot be grown in previously cultivated soils (Clay 2004). In addition to these strict environmental conditions, cacao plants also only grow at low elevations (Urquhart 1966). Cacao plants themselves are usually between 12-15 meters in height. The plants begin producing pods after 2-3 years, and by 6-7 years will produce a full harvest (Beckett 2000). Flowers on the cacao plants grow into small pods (Figure 8), called cherelles, and take 5-6 months to develop into mature pods (Figure 9), each containing between 30-45 beans (Beckett 2000).

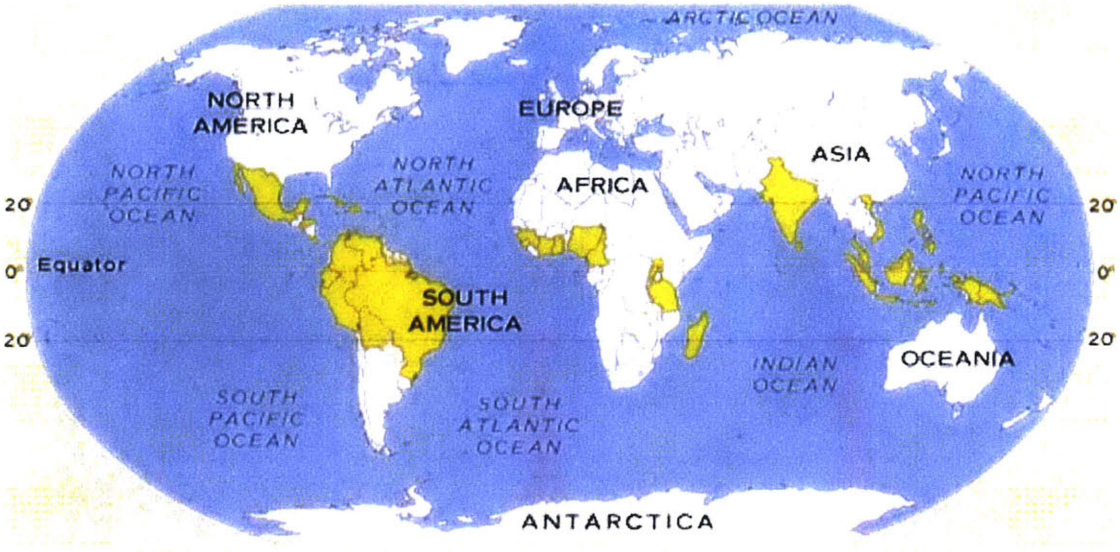


Figure 7: A map of the world, with major cacao-producing countries highlighted in yellow (from www.guittard.com). All cacao is grown near the equator, where there are warm temperatures, low elevations, and a fair amount of rainfall.

Many cacao plantations harvest cacao pods every 1-4 weeks over a period of a few months. The pods are opened, often with a machete, and the beans are removed. When first removed, the beans are covered in a white pulp that must be removed by hand. The beans are fermented, sometimes by placing them within a pile of banana leaves on the ground (Figure 10). Fermentation differs for each type of cacao, and varies between 1 and 6 days. After the beans are fermented, they are laid out to dry in the sun (Figure 11). They are usually spread out on mats, trays, or terraces in a layer about 100 mm thick, and are raked at intervals throughout the day. In Venezuela, the beans are sometimes laid out to dry along the side of the road (M. Perignon, personal communication). If drying takes more than one day or if it rains, the beans are heaped up and covered at night. Laying the beans out leads to risk of contamination from the surroundings, atmosphere, and from farm and wild animals wandering through them.

Figure 8: Cacao pods while still attached to the tree. These are cut from the tree with a machete before being opened and the cocoa beans removed (Urquhart 1966).

Figure 9: Cocoa beans in a cacao pod. The beans are still surrounded by a white pulp that must be removed before further processing (http://chocolate.org/health/cocoa-beans.jpg).

The dried beans are stored in jute sacks, and usually transported by ship, where care is taken to prevent moisture from entering the sacks, which causes the beans to mold and lose their flavor.

Upon arrival to the country where they will be processed, the beans are cleaned and their shells removed. After all impurities, such as stones and dust, have been removed from the beans, they are roasted and ground for further processing.

Figure 10: Cacao pods fermenting on top of banana leaves (Urquhart 1966).

There are three main varieties of cacao plants: the criollo, forastero, and trinitarios, each associated with a different quality of cocoa produced from the fruits. Forastero produces a rich chocolate flavor suitable for milk chocolate while criollo and trinitarios produce a more bitter flavor that is usually blended (Lass 1985). The different flavors of the beans also depend on the climate and soil conditions as well (Beckett 2000).

Figure 11: Cocoa beans after they have been removed from the cacao pod (http://www.gourmetsleuth.com/images/cocoabeans.jpg ).

There are three main cocoa producing areas in the world. These include countries such as Ghana and the Ivory Coast in Western Africa, South-East Asia, and South America. Currently, over 40% of the world's cocoa is grown in the Ivory Coast, and 14% is grown in Ghana (Beckett 2000).

### 2.2.2 Lead in Chocolate

Chocolate, like many foods, contains trace metals, such as lead and cadmium. However, the amount of the trace metal that is available for the body to retain is quite small (Mounicou 2003). A study by Mounicou concluded that less than 10 percent of the lead in ingested chocolate was retained the body.

The Food and Agriculture Organization (FAO) and the World Health Organization have set the limit of lead concentrations in cocoa, either powder or beans, to 1 μg/g, or 1 ppm (Heneman

2006). The FDA has set the limit at 0.1 ppm for chocolate products. Almost all chocolates that have been analyzed worldwide have been found to fall well below this limit (Heneman 2006).

## 2.3 Previous Studies of Lead in Chocolate

Recent studies have shown that chocolate manufactured in the United States has relatively low levels of lead, often ranging from 0.01 µg/g to 0.0965 µg/g (Heneman 2006). A study performed by a Swiss research group in 2002 tested chocolate products worldwide, and also found them to fall well below the limit set by the FAO and the WHO (Heneman 2006). However, other studies have shown that there is contamination in manufactured chocolate products, despite the fact that previously analyzed cocoa beans have some of the lowest reported lead concentrations among all foods worldwide (Taylor 2005). An analysis of the cocoa bean shells by Rankin *et al* 2005 indicated elevated lead concentrations. While the average concentration for cocoa beans was around 0.512 ng/g or ppb, cocoa bean shells had values as high as 160 ng/g (Rankin 2005). Even higher were the lead concentrations in manufactured cocoa and chocolate products, which had values as high as 230 ng/g and 70 ng/g, respectively, shown in Figure 12 (Rankin 2005).

A previous study by Mounicou *et al* 2003 found that the amount of lead in cocoa products changes during the processing. During the roasting of the beans, the concentration rises due to water loss, but during the cleaning and grinding that follows, there is a large (fivefold) decrease in the concentration (Mounicou 2003). After further processing, the concentration drops further, but increases during the production of cocoa butter (Mounicou 2003).

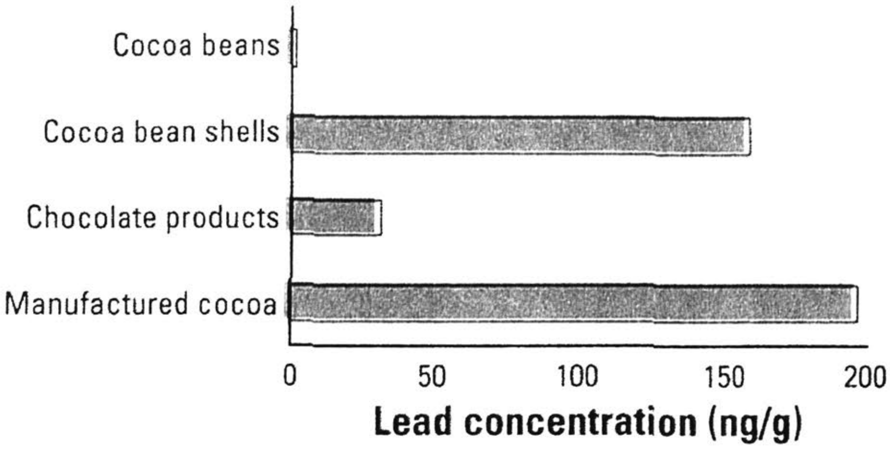


Figure 12: (from Rankin, 2005) A graph showing the differences between manufactured cocoa products, cocoa beans, and shells.

It has been proposed that much of the lead in the chocolate products is from atmospheric deposition onto growing cacao plants which is dominated by lead from gasoline (Rankin 2005). Previous studies have shown that cocoa bean shells have higher lead concentrations, protecting the cocoa bean while in the shell, and suggesting that contamination must occur later in the process, possibly during the fermentation and drying of the cocoa beans, after the bean is removed from the pod (Rankin 2005). If the pods or shells are imperfectly removed and crushed with the beans, the resulting product could have elevated lead despite a relatively small volume of shell.

The isotopic composition of lead in manufactured chocolate products is within the range of industrial lead aerosols, providing evidence that most of the contamination occurs during the transportation of the cocoa beans, and final processing and manufacturing of the bean, cocoa, and chocolate products (Rankin 2005).

One possible pathway for contamination is from atmospheric deposition on the cocoa beans while drying. Here the beans are outside of the protective pod and exposed to the air. A study by Bollhofer concluded that most of the lead found in the atmosphere is from coal burning, mining and smelting operations, and gasoline emissions because of a close lead isotopic match between the atmospheric signature and these sources (Bollhofer *et al* 2005).

While Rankin's study presents a strong argument for contamination of chocolate during the drying and manufacturing process given the high concentrations of lead in manufactured products, there are several points that need to be considered before accepting his thesis. First, the cocoa shells have much higher concentrations of lead, by an order of magnitude, than the beans. During the harvesting and manufacturing of cocoa, the beans are removed from the shells and go through various cleaning stages to remove the excess shells before final processing. However, it is impossible to remove all of the shells – a few are bound to slip through, and be processed with the remaining cocoa beans. Because the shells have such a higher concentration of lead than the beans, just a small amount of shells mixed in with the beans could greatly change the concentration of the end product. Another point to consider is that sizes of the samples that Rankin measured for lead concentrations. A bean or a shell is significantly smaller, and the lead less concentrated, than an average-sized candy bar, so then naturally it would have less lead in it. It is difficult to draw a conclusion about the concentrations of lead in manufactured cocoa products and cocoa beans if the cocoa content or addition of ingredients is not taken into account. Also, Rankin reports lead concentrations and ratios of manufactured products that are milk chocolate. The additional ingredients in milk chocolate lower the overall concentration of lead, and Rankin fails to mention how various kinds of chocolate might be affected. Dark chocolate, with a higher concentration of cocoa, is likely to have a higher concentration of lead. Finally, Rankin's analysis of manufactured chocolate is only from companies that purchase beans from multiple continents, thereby creating a mixture of various cocoa beans, while the cocoa beans he analyzed in his study are only from a few farms in Nigeria. Comparing the two values yields inconclusive results. Rankin's study, currently the most well known analysis of lead in chocolate, requires more information before anything conclusive can be determined.

# Chapter 3. Methods

## 3.1 Preparation of the samples

### 3.1.1 Preparation of the chocolate samples

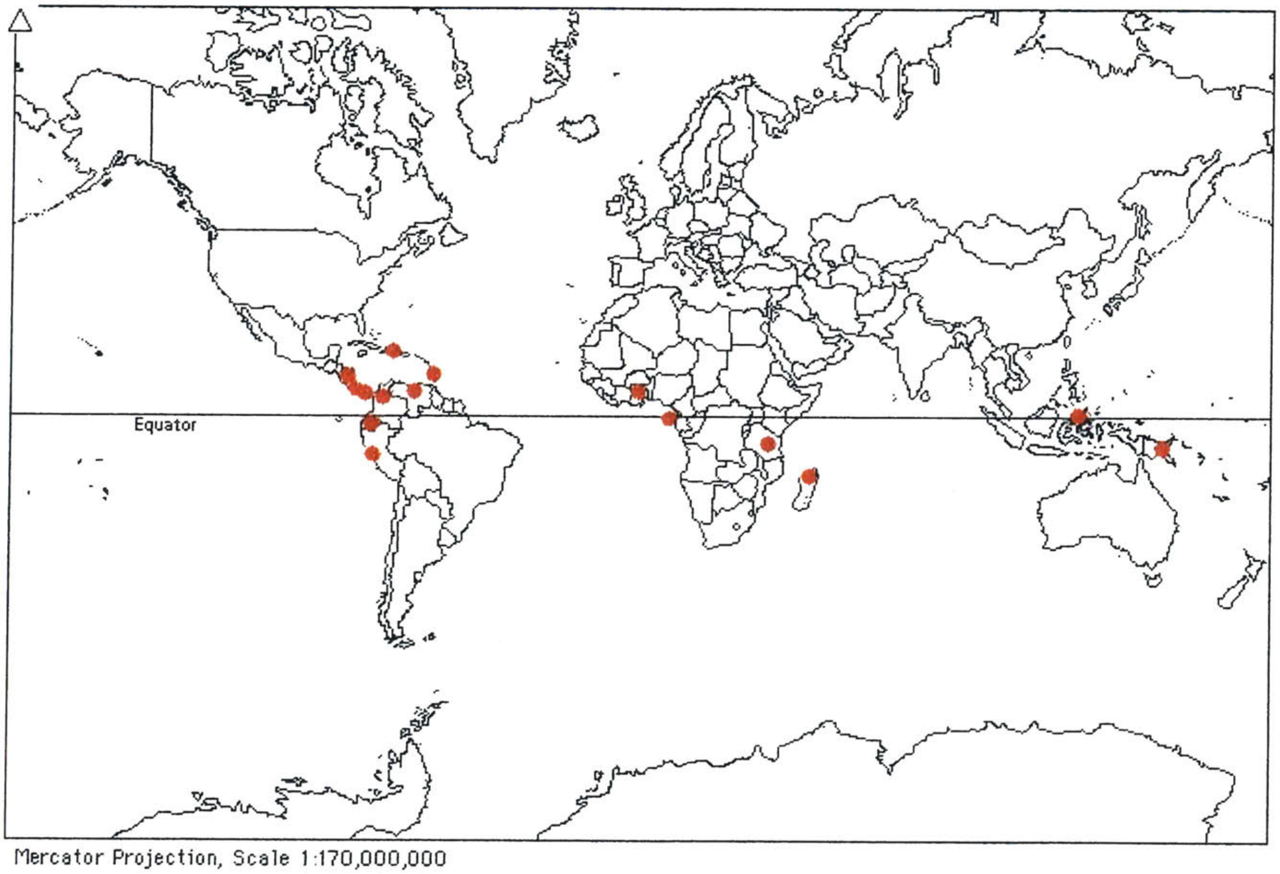


Figure 13. Map of locations (shown as red dots) of chocolate samples that were analyzed during this study.

A variety of chocolates from developing, cacao-producing countries around the world were bought at a local grocery store. The locations of samples are shown on a map in Figure 13. The chocolate samples were prepared for analysis. First, a clean, empty silica glass crucible, with cover, was weighed for each sample. Approximately 1 gram of chocolate was added to each crucible, broken into smaller pieces. The chocolate samples were then heated in a muffle furnace to remove organics for 2 hours at 80 degrees C, 2 hours at 200 degrees C, and 12 hours at 600 degrees C. The samples were then cooled.

After the samples cooled, 1 mL concentrated nitric acid was added to each sample and the samples were dried down on hot plate. The purpose of the nitric acid steps is to oxidize the remaining organics, to take the entire sample into solution, and to homogenize the sample. This step was repeated after the samples had dried down. Then 0.2 mL concentrated nitric acid was added to each beaker, along with 3 aliquots of 100 μL hydrogen peroxide. The peroxide reacted with each sample before the next aliquot was added. This step should have destroyed all remaining organic matter in the sample. The nitric acid – peroxide solution was then dried down. Two mL 6 M HCl were added to each beaker, the beakers capped, and placed on the hot plate to reflux for at least an hour. This step washed the spatter that collected on the walls of the beaker back into the sample solution and converted the sample to chloride form. After the samples refluxed for more than an hour, the caps were removed and the samples dried down.

When the samples were dry, the crucibles were taken off the hot plate and cooled. The samples were taken up in 0.5 mL 1.1 M HBr. The samples were spiked with a $^{208}$Pb spike. The weight of the crucible with the HBr-sample solution was recorded. A clean Teflon beaker, with 3 drops of $^{208}$Pb spike, was also weighed, recording the weight of the spike. Fifty μL of the HBr-sample solution was pipetted into the Teflon beaker, and the total sample weight recorded. These spiked samples were used for concentration determinations by isotope dilution (ID). The samples were dried down, and then taken up in 0.5 mL 1.1 HBr. The remaining unspiked HBr solution was transferred into clean Teflon beakers for isotopic composition (IC) analysis.

### 3.1.2 Preparation of the soil samples

Each soil sample was analyzed in duplicate. For each soil sample, approximately 0.3 g of soil was weighed into a 5 mL centrifuge tube. Two empty tubes were used for blanks, and processed along with the samples. Three mL reagent ethanol were added to each centrifuge tube. The tubes were placed in an ultrasonic bath for approximately 5 minutes to mix the ethanol with the soil. The tubes were then placed in a hot water bath on a hot plate for an hour. Twice during this 1 hour period, the tubes were removed and placed into the ultrasonic bath for 5 minutes. After one hour, the samples were centrifuged for 5 minutes. For each sample, a clean, empty, 15 mL Teflon beaker was labeled and weighed. The ethanol was pipetted from the centrifuge tube into the beaker as cleanly as possible. The beaker was capped and set aside. Three mL hot MQ water were added to each centrifuge tube. The samples were placed in the ultrasonic bath for at least 5 minutes to suspend the solids in the water. The centrifuge tubes were placed into the hot water bath for 15 minutes. After 15 minutes, the samples were put into the ultrasonic bath for 5 minutes. The samples were centrifuged for another 5 minutes. The water was pipetted from the centrifuge tubes and added to the ethanol extracted in step 4. The ethanol – water mixture was allowed to cool for at least 1 hour, then the Teflon beakers with the extracted liquid were weighed. Three mL hot 1 M HCl were added to the solid residue from the ethanol extraction and the centrifuge tubes placed into the ultrasonic bath for 10 minutes, making sure that all of the soil was suspended in the acid. After 10 minutes, the samples were centrifuged for 5 minutes. For each sample, a clean, empty, 15 mL Teflon beaker was labeled and weighed. The HCl was pipetted from the centrifuge tube into the beaker. The beaker was capped and set aside. Three mL hot MQ water were added to each centrifuge tube. The samples were placed in the ultrasonic bath for at least 10 minutes to suspend the solids in the water. After 10 minutes, the samples were centrifuged for 5 minutes and the water was pipetted from the centrifuge tubes and added to the HCl extracted in step 9. The HCl – water mixture cooled for at least 1 hour, then the Teflon beakers with the extracted liquid were weighed. MQ water was used, sparingly, to transfer the solid residue as completely as possible to clean, weighed 15 mL Teflon beakers. The samples were dried. When the samples were dry, the beakers and sample were weighed, then 3 mL fresh aqua regia were added to each sample. The beakers were capped lightly and placed on the hot plate to reflux for at least 8 hours. The beakers were left loosely capped overnight to prevent buildup of gases in the beaker. After 8+ hours of reaction, the samples were transferred to 15 mL centrifuge tubes and centrifuged for 5 minutes. For each sample, a clean, empty, 15 mL Teflon beaker was labeled and weighed. The aqua regia was pipetted from the centrifuge tube into the beaker. The beaker was capped and set aside. Ten mL hot MQ water were added to the residue. The tube was mixed with the vortex mixer to suspend the solids, and set aside for 10 minutes. After 10 minutes, the tube was mixed on the vortex mixer again, then centrifuged for 5 minutes. The water from the centrifuge tube was added to the Teflon beakers with the aqua regia. The samples cooled for at least 1 hour, then were weighed. MQ water was used to suspend the solids, and transfer the residues to clean, weighed, 15 mL Teflon beakers. The samples were dried, then 1 mL conc. nitric acid and 3 mL conc. hydrofluoric acid were added to each. The beakers were capped and refluxed on the hot plate at least 8 hours. The samples were dried down. One mL conc. nitric acid was added to each, then the samples were dried down again. This process was repeated twice more. Then 3 mL 6 M HCl were added to each sample, and the beakers were capped and refluxed for 8 hours. The samples were then aliquotted for IC and ID. The IC aliquot is used to determine the isotopic composition of the Pb, and the ID aliquot is used for calculating the concentration of Pb by adding a known amount of $^{208}$Pb. For IC, 1 mL of each solution was aliquotted. For ID, 0.1 ml of each solution was spiked with 2-3 drops of $^{208}$Pb spike. An ID solution weight and a spike weight were recorded, and the samples pipetted into clean, labeled beakers. The samples were then prepared for column chemistry. The samples were dried down, and 1 mL conc. nitric acid was added to each, then the samples were dried down again. 0.2 mL conc. nitric acid were added to each, then 3 increments of 100 μL of hydrogen peroxide were added to each. The samples were allowed to react with the peroxide before the next increment was added. The samples were then dried down, and 1 mL 6 M HCl was added, and the beakers were capped and refluxed for an hour. After being dried down, 0.5 mL 1.1 M HBr were added to each.

### 3.2 Column Chemistry

Pb column chemistry was performed for both the IC and ID samples. First, 120 μL columns were cleaned with MQ-water and set up using Eichrom AG1-X8, 200-400 mesh Cl- form resin. The columns were cleaned twice using 1000 μL of 6 M HCl, and equilibrated using 500 μL of 1.1 M HBr. The samples were then loaded into the columns, with small plastic cups placed underneath to catch the waste. The empty Teflon beakers with 2 mL 6 M HCl were placed on the hot plate to be cleaned while continuing with columns. A drop wise rinse was performed by dropping one drop of 1.1 M HBr through the columns 5 times, followed by a bulk rinse using 500 μL of 1.1 M HBr. The bulk rinse was repeated, this time using 500 μL of 2 M HCl. The samples were eluted using 1000 μL of 6 M HCl to extract the lead, then dried down on the hot plate using 1 μL of 0.1M $H_3PO_4$.

Once the samples were dried down, they were loaded on Re filaments for analysis in the mass spectrometer.

### 3.3 Mass Spectrometry

The chocolate samples were analyzed using a TIMS (Thermal Ionization Mass Spectrometer). Mass spectrometers are instruments that measure the abundance of isotopes through separating charged atoms based on mass dependent deflection in a magnetic field. The sample, loaded on a Re filament in silica gel and phosphoric acid, is heated until it is volatized, causing ionization of the atoms into vapor. The ions are accelerated into a beam, and enter a magnetic field that deflects the ions into certain paths depending on their mass. The heavier ones are deflected less than the lighter ones.

### 3.4 Possible Sources of Error

During the course of this study we were concerned with identifying and quantifying the sources of uncertainty in both the concentrations and isotopic compositions of lead in the chocolate samples. The concentration data are dependent on quantitative extraction of all the Pb from the chocolate, weighing errors, and sample heterogeneity. Apparent variations in lead content could be caused if extraction were not 100%. The simplest way to quantify this is by replicate analyses which are a measure of the heterogeneity of Pb in individual bars as well as our ability to extract the Pb. Based on our data this is about 0.16-0.20 %.

The use of thermal ionization mass spectrometry for measuring isotopic ratios results in relatively small error bars on all isotopic ratios (in general $< 0.05$ %) which in most cases is many times smaller than the variability in composition that we are interpreting. Sources of uncertainty in Pb isotopic measurements include total processing blank (includes incineration, digestion, and chemical separation), uncertainty in mass dependent fractionation, and counting statistics.

# Chapter 4. Data

A total of 52 analyses was done on 41 different chocolate and cacao products. These represent 13 different distributors and manufacturers, and cacao sources in 15 different countries. Additionally, 16 analyses were done on two soil samples. Samples and raw data are listed in the appendix.

# Chapter 5. Discussion

## 5.1 Comparison of Data in Determining the Source of Lead

The typical one sigma analytical uncertainties for the TIMS isotopic analyses in this study are $^{206}Pb/^{204}Pb = 0.008$, $^{207}Pb/^{204}Pb = 0.010$, $^{208}Pb/^{204}Pb = 0.033$, based on analysis of NBS 981 Pb standard (N=36).

### 5.1.1 Same Location

Chocolate samples that are from the same geographic location but manufactured by different companies were found to have similar lead isotopic ratios. These data, shown in Figure 14, suggest that the lead in chocolate is a function of the location where the beans are grown. With a few exceptions, the lead isotopic composition of chocolates from the same country plot near each other, which is expected if they have the same source of lead. A comparison of the manufacturers follows in a later section.

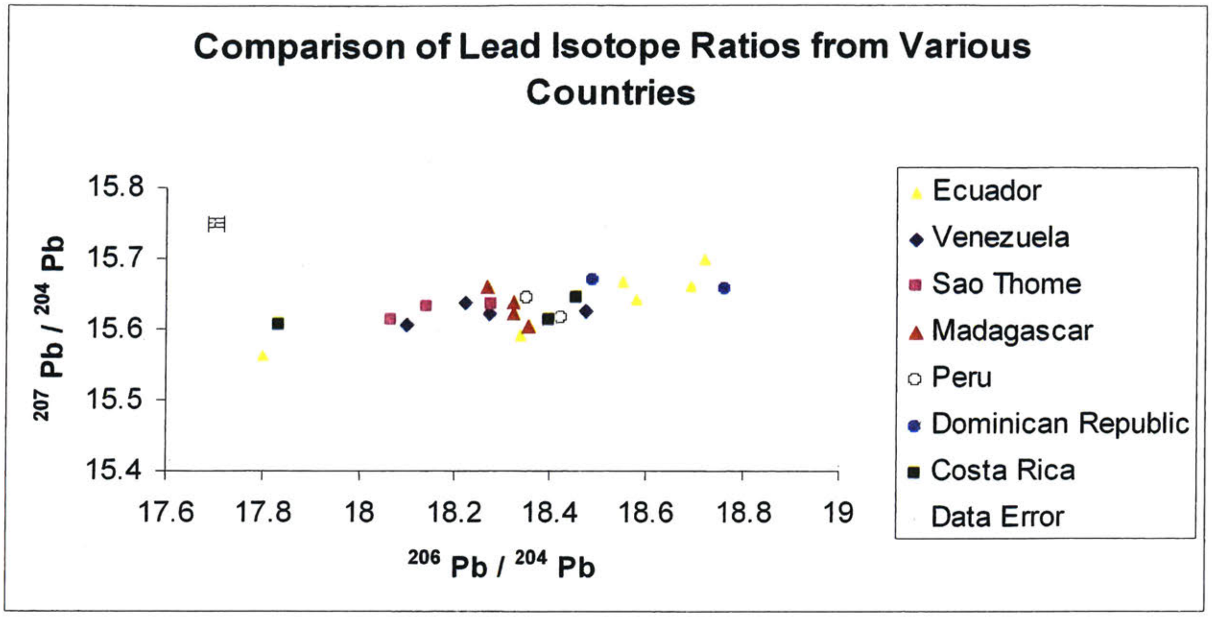


Figure 14: A plot showing the $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb ratios of chocolate samples, labeled by the country where the beans were grown. The data suggest that chocolate from each locality has a distinctive isotopic composition. With only a few exceptions, such as two data points from Ecuador and one from Costa Rica, all of the ratios from chocolate from the same location plot relatively close to each other. A small data point showing the typical one sigma analytical uncertainty is shown.

### 5.1.2 Same Manufacturer

#### 5.1.2.1 Temporal Sample Heterogeneity: Same Product, Different Bar

Several of the chocolates were processed and analyzed a second time to determine if the manufacturer is the source of lead contamination, and to check the reproducibility of the first analysis. These samples are from the same manufacturer and the same product, but a different chocolate bar, purchased in two locations and at different times, probably representing different cacao crops. The lead isotopic compositions are listed in Table 1, and the differences in the ratios are shown in Table 2. Neither is within a typical one sigma analytical uncertainty of one another, suggesting that the lead in the chocolate products varies significantly, probably due to the different cacao crops and plantations used throughout the years. More work will be necessary to understand this.

Table 1: A table showing the $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ ratios of two chocolate bars where the samples were run a second time to check reproducibility and determine if the manufacturer is a possible source of contamination. The difference between replicates in the case of Los Rios #2 is quite large, suggesting heterogeneous distribution of Pb throughout time.

| Sample Number | Sample Name | $^{206}Pb/^{204}Pb$ | $^{207}Pb/^{204}Pb$ | $^{208}Pb/^{204}Pb$ |
|---|---|---|---|---|
| T 1273 | Dagoba Pacuare Costa Rica | 18.459 | 15.645 | 38.435 |
| T 1288 | Dagoba Pacuare #2 Costa Rica | 18.402 | 15.611 | 38.276 |
| T 1274 | Dagoba Los Rios Ecuador | 18.338 | 15.590 | 38.181 |
| T 1289 | Dagoba Los Rios #2 Ecuador | 17.796 | 15.561 | 37.591 |

Table 2: Table showing the differences in the $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ ratios from "replicate" chocolate bars

| Sample | $^{206}Pb/^{204}Pb$ | $^{207}Pb/^{204}Pb$ | $^{208}Pb/^{204}Pb$ |
|---|---|---|---|
| Newman's Own Costa Rica- minimum | 0.059 | 0.005 | 0.006 |
| Newman's Own Costa Rica-maximum | 0.173 | 0.033 | 0.155 |
| Dagoba Pacuare Costa Rica | 0.057 | 0.034 | 0.159 |
| Dagoba Los Rios Ecuador | 0.542 | 0.029 | 0.590 |

### 5.1.2.2 Sample Heterogeneity: Same Product, Same Bar, Different Analysis Time

Analysis of a suite of samples was repeated in order to compare with previous measurements that were done in April of 2004. These samples are from the same manufacturer, and the same chocolate bar of the same product. Results are shown in Table 3 and Figure 15. The differences between the ratios of samples are shown in Table 4. However, they agree within two-sigma analytical uncertainty for all ratios except $^{208}Pb/^{206}Pb$ suggesting reasonably good reproducibility despite the complex dissolution and separation procedure. The discrepancy needs further investigation.

Table 3: A table showing the data plotted in Figure 15. The JF samples were analyzed in April of 2004, and the T samples were analyzed in 2007.

| Sample Number | Sample Name | $^{206}Pb/^{204}Pb$ | $^{207}Pb/^{204}Pb$ | $^{208}Pb/^{204}Pb$ |
|---|---|---|---|---|
| T 1220(2) | Chocovic Ocumare Venezuela | 18.221 | 15.637 | 38.248 |
| JF 2(2) | Chocovic Ocumare Venezuela | 18.180 | 15.608 | 38.101 |
| T 1221(2) | Chocovic Guaranda Ecuador | 18.552 | 15.666 | 38.614 |
| JF 3(2) | Chocovic Guaranda Ecuador | 18.541 | 15.648 | 38.601 |
| T 1222 | Trader Joe's, Sao Tome | 18.144 | 15.630 | 38.149 |
| JF 4(2) | Trader Joe's, Sao Tome | 18.163 | 15.623 | 38.149 |
| T 1223 | 365 Organic Swiss, Dominican Republic | 18.493 | 15.669 | 38.754 |
| JF 7 | 365 Organic Swiss, Dominican Republic | 18.481 | 15.648 | 38.394 |
| T 1224 | Newman's Own #2, Costa Rica/Panama | 18.631 | 15.665 | 38.427 |
| JF 8 | Newman's Own #2, Costa Rica/Panama | 18.583 | 15.627 | 38.282 |
| T 1224(2) | Newman's Own #2, Costa Rica/Panama | 18.533 | 15.634 | 38.266 |
| JF 1(2) | Newman's Own #1, Costa Rica/Panama | 18.706 | 15.660 | 38.421 |
| T 069(3) | Newman's Own #1, Costa Rica/Panama | 18.690 | 15.648 | 38.384 |

Table 4: Table of difference between the ratios of the samples analyzed in 2004 and 2007.

| Manufacturer | $^{206}Pb/^{204}Pb$ | $^{207}Pb/^{204}Pb$ | $^{208}Pb/^{204}Pb$ |
|---|---|---|---|
| Chocovic Ocumare Venezuela | 0.041 | 0.029 | 0.147 |
| Chocovic Guaranda Ecuador | 0.011 | 0.018 | 0.013 |
| Trader Joe's, Sao Tome | 0.019 | 0.007 | 0.000 |
| 365 Organic Swiss, Dominican Republic | 0.012 | 0.021 | 0.360 |
| Newman's Own #1, Costa Rica/Panama | 0.098 | 0.038 | 0.161 |
| Newman's Own #2, Costa Rica/Panama | 0.016 | 0.012 | 0.037 |

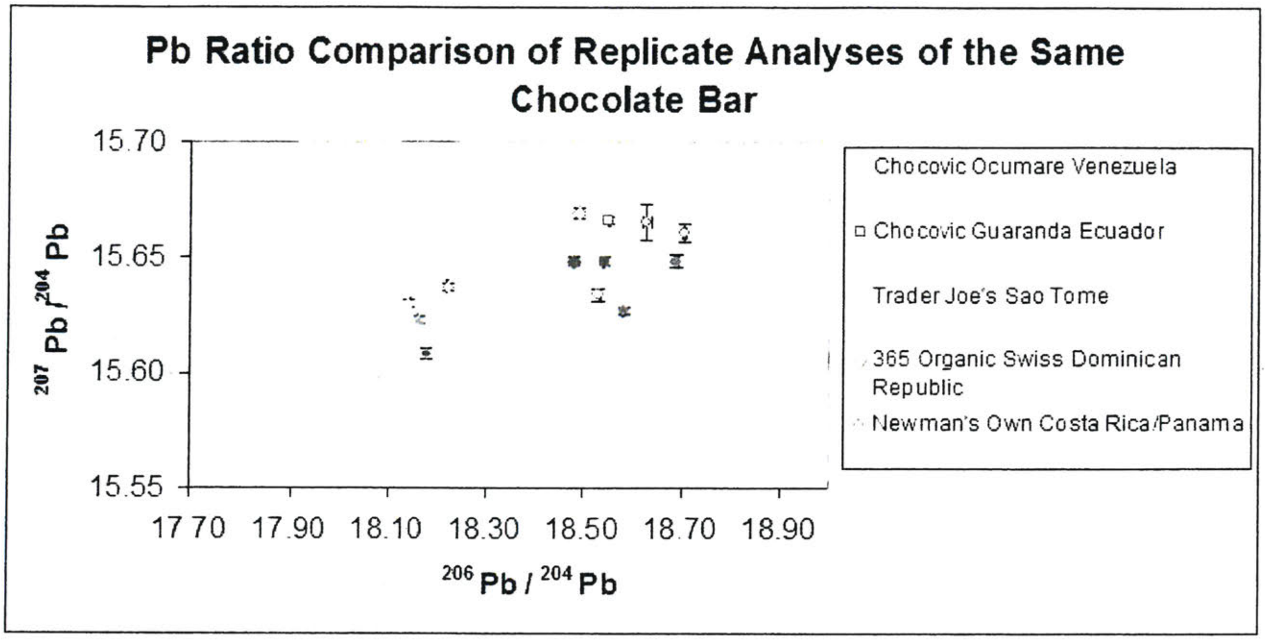


Figure 15: A plot of $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb from analyses of different chips from the same chocolate bar. The samples analyzed in 2004 are in solid shapes, and the samples analyzed in 2006/2007 are in open shapes. The chocolate bars analyzed in April of 2004 have relatively similar ratios to the chocolate bars analyzed in 2007.

### 5.1.2.3 Different Product

There does not appear to be any simple relationship between the manufacturer or distributor of a particular chocolate product and its isotopic composition. Target is considered a distributor because they market chocolates from an unknown manufacturer. All of the major manufacturers whose chocolates were analyzed in this study were compared by plotting the $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb (Figure 16), and no relationship between the same manufacturers was observed, with the scatter appearing random, well outside the typical one sigma analytical uncertainty of the mass spectrometer (also shown in Figure 16). It can be concluded, then, that the different manufacturers are not the predominant cause for the varying lead isotope ratios in chocolate and that the observed Pb is not introduced during making of the confections.

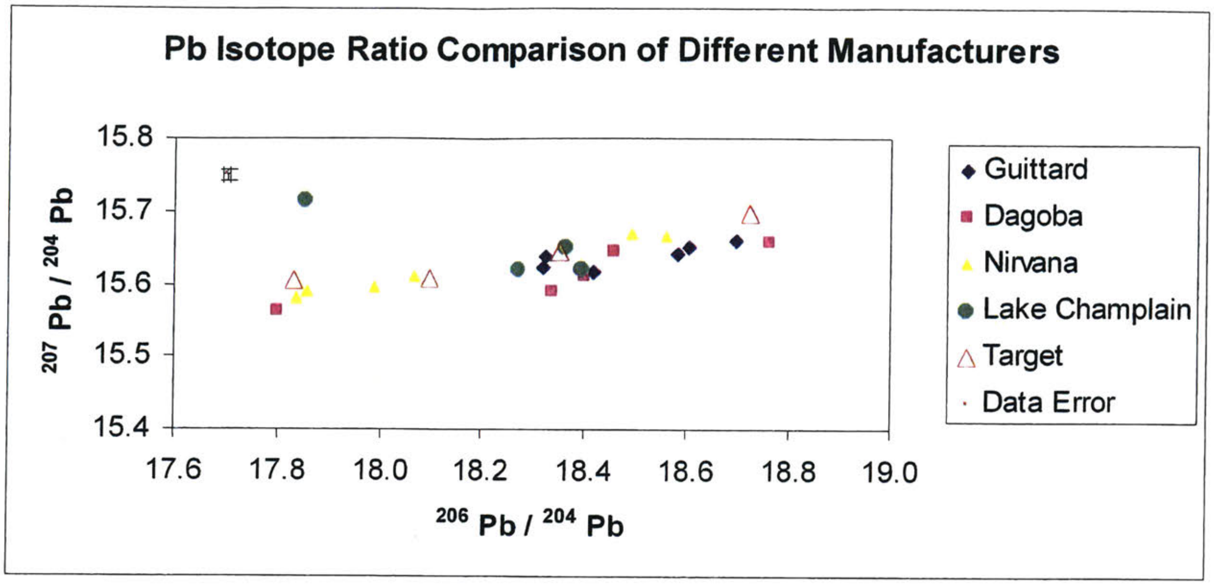


Figure 16. Pb isotope ratio comparison of the major different manufacturers on a $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb plot. Target markets chocolates from an unknown manufacturer. There appears to be no significant relationship between the isotopic composition of Pb and the different manufacturers, suggesting that the lead contamination is not introduced during processing.

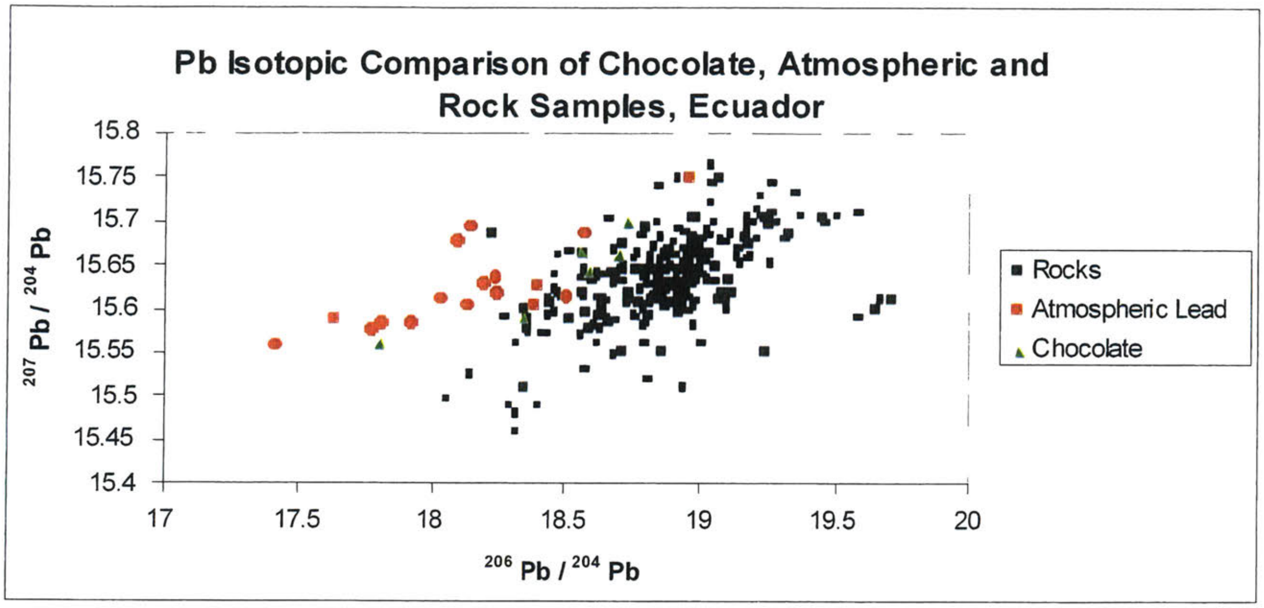
## 5.2 Comparison of Data to Literature to Determine the Source of Lead

The isotopic composition of chocolate samples was compared with published atmospheric lead data from Bollhofer (2000, 2001, 2002) as well as other geochemical studies. The atmospheric data that are used in comparison were collected by Bollhofer in the 1990s and early 2000s through an air filtration system. The aerosol samples were collected using small diaphragm-type aquarium pumps that were modified to invert air flow and the air passed through a filter with 0.45 µm pores (Bollhofer 1999). However, these data are somewhat limited, as only a few dozen cities throughout the world were sampled for his analysis. In this comparison, the atmospheric ratios measured in neighboring countries are used to determine if a relationship exists between the isotopic composition of atmospheric lead and the chocolate. For example, for Ecuadorian chocolate, the atmospheric data we compare it with include data collected in Quito, but also in other South American countries like Brazil and Argentina. In addition to atmospheric data from nearby countries, some of the countries with enough data points are also plotted with the isotopic signature from their respective hemisphere, to help constrain if the source is from the global atmospheric lead, or local industrial activities.

The Pb isotope ratios of chocolates are also compared to those of the volcanic rocks in the country of origin. The assumption is made that volcanic rocks from the surrounding area will be weathered and contribute significantly to the lead in the soil in the regions where the cacao plants are grown. Therefore, while we try to compare the ratios of the chocolates to those of the rocks within the areas surrounding cacao plantations, in some cases a wide distribution of volcanic rocks from throughout the countries is used to gain a more accurate view of the lead in the soils due to the lack of Pb literature on lead isotopes in the soils were the cacao is grown. The cacao plantations may be tens to hundreds of kilometers from the locations for which Pb isotopic data are available for either rocks or aerosols; there are no site-specific Pb isotopic data for areas near cacao plantations. The lead ratios from volcanic rocks are also used because there is very little literature and previous studies done on the lead and contamination of soils in many of the developing countries where cacao is produced, specifically in the exact locations of cacao plantations. Several case studies follow for countries where data were available for atmospheric lead and lead from rocks.

## Ecuador

The Pb isotopic composition of various chocolate samples from Ecuador can be compared with isotopic data from both the surrounding volcanic rocks in the Andes and atmospheric lead collected during the 1990s in South America. The $^{206}$Pb /$^{204}$Pb and $^{207}$Pb /$^{204}$Pb and $^{206}$Pb /$^{204}$Pb and $^{208}$Pb /$^{204}$Pb ratios were plotted against one another for comparison in Figure 17. The plot shows an overlap between the isotopic composition of the chocolate samples and the atmospheric lead. The volcanic rock samples, composed of samples collected throughout Ecuador, are distinctly different from the chocolate samples. The atmospheric lead, when plotted, appears to have nearly similar isotope ratios to that of the chocolate, though there are a few chocolates that have a higher $^{206}$Pb /$^{204}$Pb than most of the aerosols, suggesting another source of lead is likely present. When plotted with the Northern Hemisphere atmospheric lead in Figure 18, the chocolates again lie near them, and the trend line nearly matches, though the trend line is skewed because of a chocolate with a low $^{206}$Pb /$^{204}$Pb ratio (Dagoba). Disregarding this point, the trend line is no longer such a close match, again suggesting there is likely another source of lead. The conclusion, based on the previously stated assumptions regarding soil and atmospheric lead data, is that the lead in chocolate from Ecuador is derived mostly from atmospheric sources, though there is likely another source present. Further studies are necessary to understand this in greater depth.



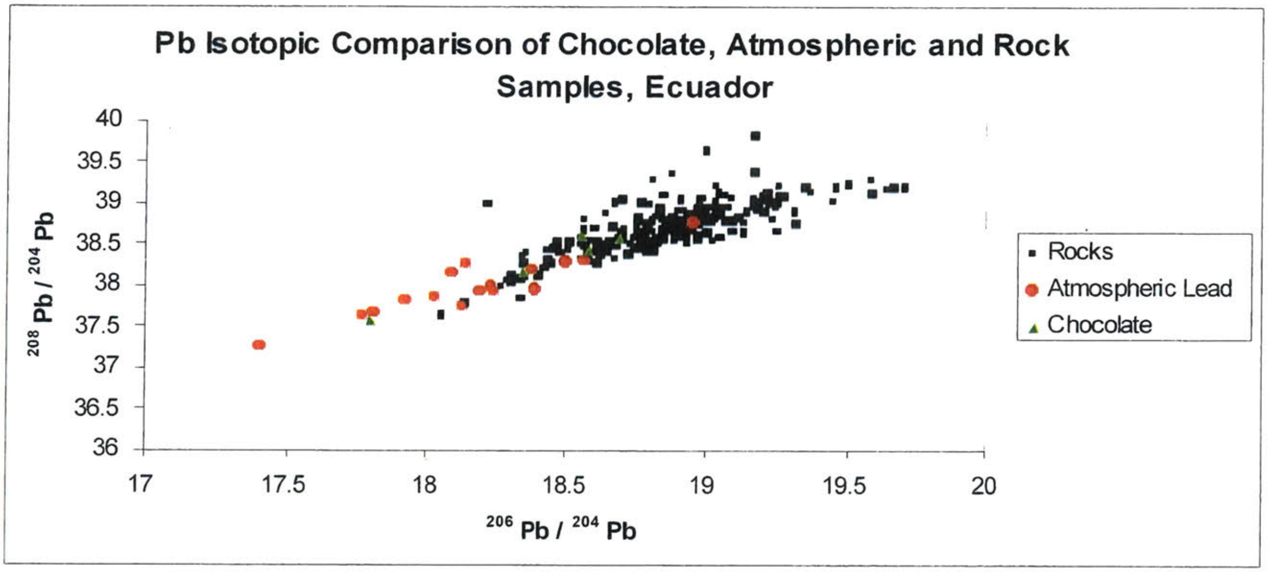


Figure 17: Plot of lead isotope ratios comparing $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb (top) and $^{206}$Pb /$^{204}$Pb vs. $^{208}$Pb /$^{204}$Pb (bottom)of the chocolate samples from Ecuador, the atmospheric lead measured in Quito and various other South American countries, and the volcanic rocks in Ecuador (that are assumed to contribute to the lead in the soils).

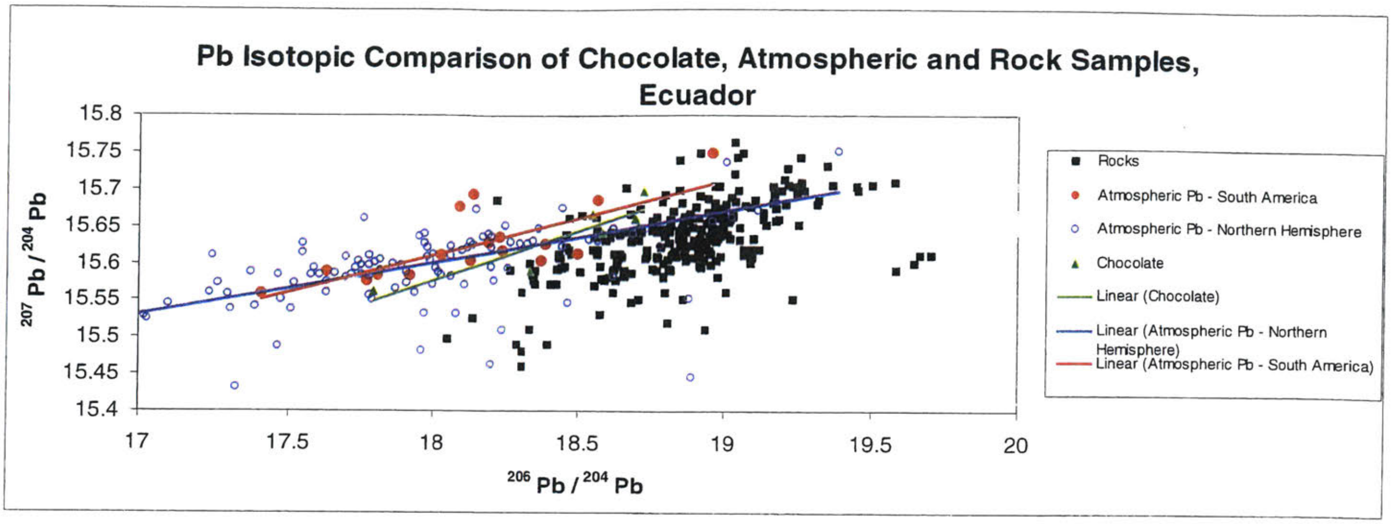
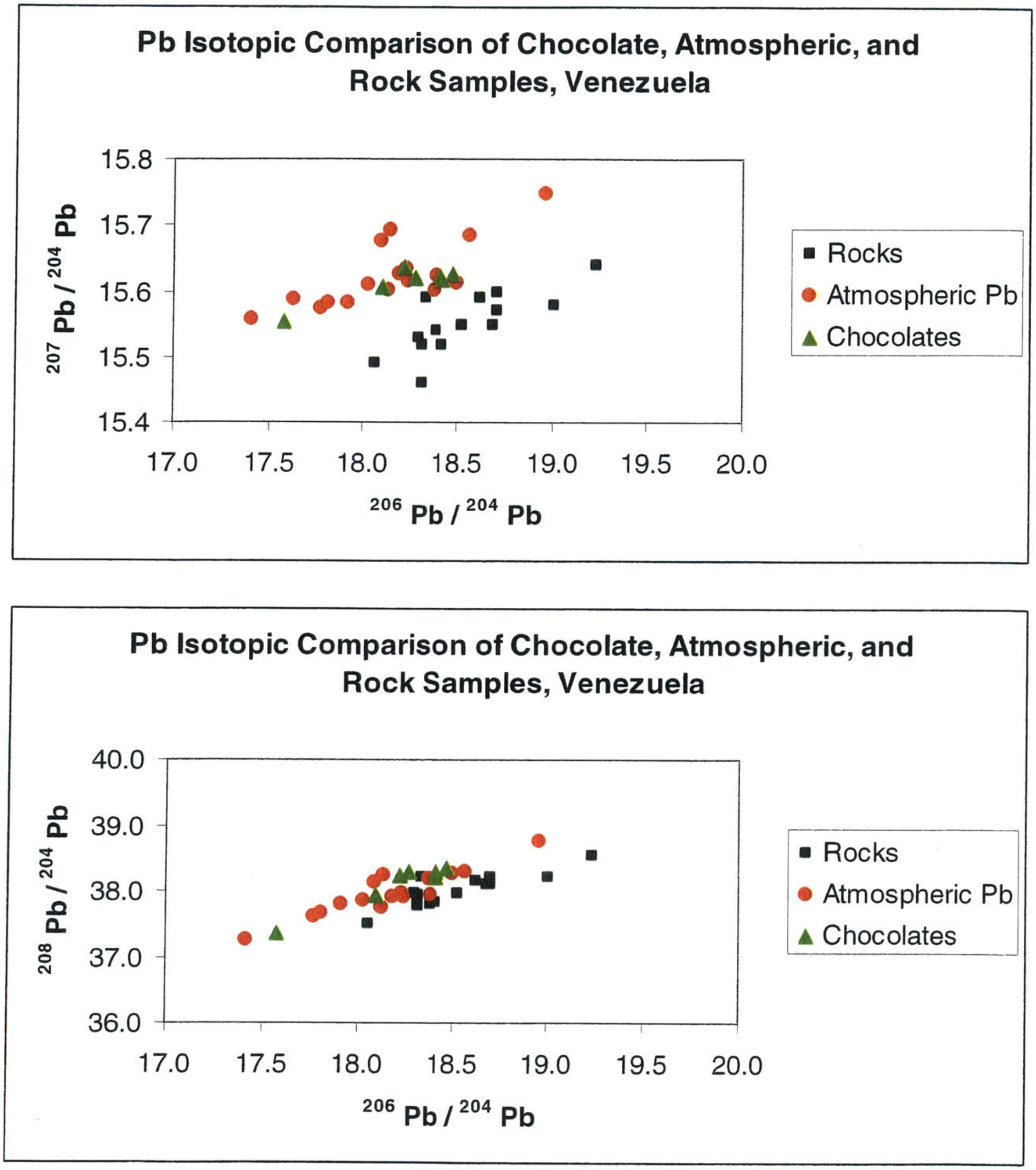


Figure 18: A plot of the $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb ratios for Ecuador, shown above, with the addition of linear trend lines for the local atmospheric data (red), Northern Hemisphere isotopic data (blue) and chocolate data (green).

## Venezuela

The isotopic compositions of chocolates from Venezuela are plotted against the atmospheric lead measured in South American countries, such as Brazil, Ecuador, and Colombia, and volcanic rocks from the country in Figure 19. While there is some overlap in the isotope ratios of the chocolates, atmosphere, and the rocks, the chocolates clearly lie much closer, and in a few cases right on top of, the atmospheric lead. This strongly suggests that the lead found in the chocolates from Venezuela is from atmospheric contamination rather than from the soils that formed through the weathering of the volcanic rocks. However, there is a limited amount of Pb literature data from Venezuela, which should be taken into account when constraining the source. The rock data that are used are not from locations near cacao plantations, so the data might not be comparable.



Pb Isotopic Comparison of Chocolate, Atmospheric, and Rock Samples, Venezuela

Pb Isotopic Comparison of Chocolate, Atmospheric, and Rock Samples, Venezuela

Figure 19: Plot of lead isotope ratios comparing $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb (top) and $^{206}$Pb /$^{204}$Pb vs. $^{208}$Pb /$^{204}$Pb (bottom) of the chocolate samples from Venezuela, the atmospheric lead measured South American countries, and the volcanic rocks in Venezuela (that are assumed to contribute to the lead in the soils). The plot clearly shows the lead isotope ratios fall within the range of atmospheric lead rather than the lead ratios from the volcanic rocks in the region.

However, Venezuela is located in the Northern Hemisphere, so the isotopic compositions were also plotted with atmospheric data measured in Europe, North America and the Caribbean, and northwestern Africa, to compare the global Northern Hemisphere isotopic signature with what might be contributing locally. The results are plotted in Figure 20. Several trend lines were fitted to the South American atmospheric data, the Northern Hemisphere atmospheric data, and the chocolates. The trend line of the chocolates matches almost exactly that of the Northern Hemisphere, suggesting much of the lead is a result from the global lead signature instead of from locally derived sources. However, because it is not an exact match, there is likely a small contribution from local industrial activities.

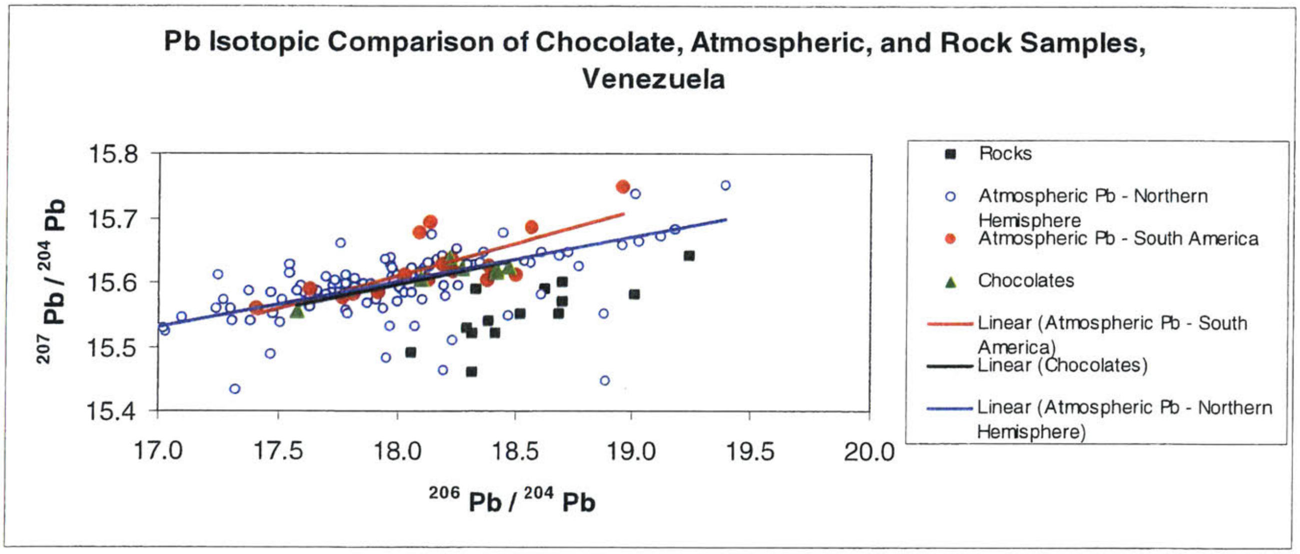
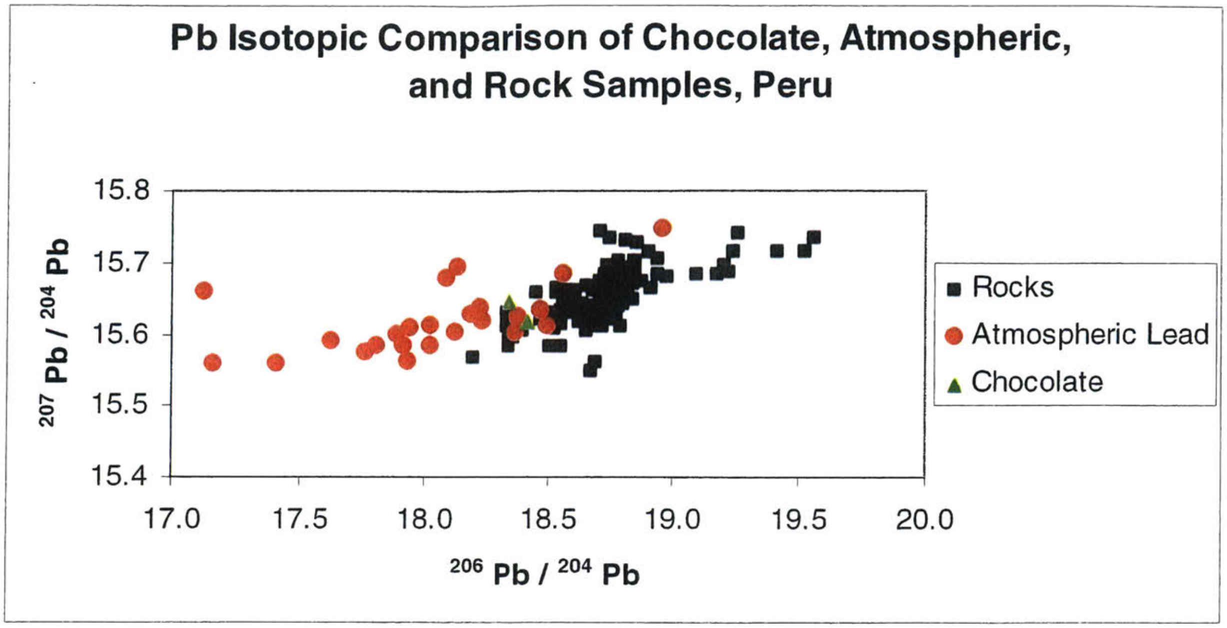


Figure 20. A plot of the lead isotope ratios comparing $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb of the chocolate samples from Venezuela, the atmospheric lead measured in South American countries and the Northern Hemisphere, and the volcanic rocks in Venezuela. Trend lines have been added to show how the chocolates match closely with the isotopic signature from the Northern Hemisphere.

## Peru

The Pb isotope ratios of rocks, chocolates and the atmospheric data measured in South American countries were plotted in Figure 21. The data points overlap, however, the chocolate samples are plotted almost on top of a few of the atmospheric lead data points. While this plot doesn't quite show a clear relationship between the chocolate and the atmospheric lead, it doesn't rule out this possibility entirely either. Part of this ambiguity may be because there are three different cacao growing regions in the Peru – along the Pacific coast, in the Amazon basin, and in the valley between the western and eastern ranges of the Andes. Each of these three locations have distinct weather patterns and atmospheric circulations, and distinct geologic histories, so it is difficult to compare all of the chocolates with the aerosol and rock data without knowing specific locations of cacao plantations. The conclusion for the chocolate samples from Peru is that the source of lead in chocolate could be from either the atmosphere or the soil (formed by the weathering of the rocks), or possibly a mixture of the two. Additional work near the locations of the cacao plantations would need to be done to make a more insightful interpretation.



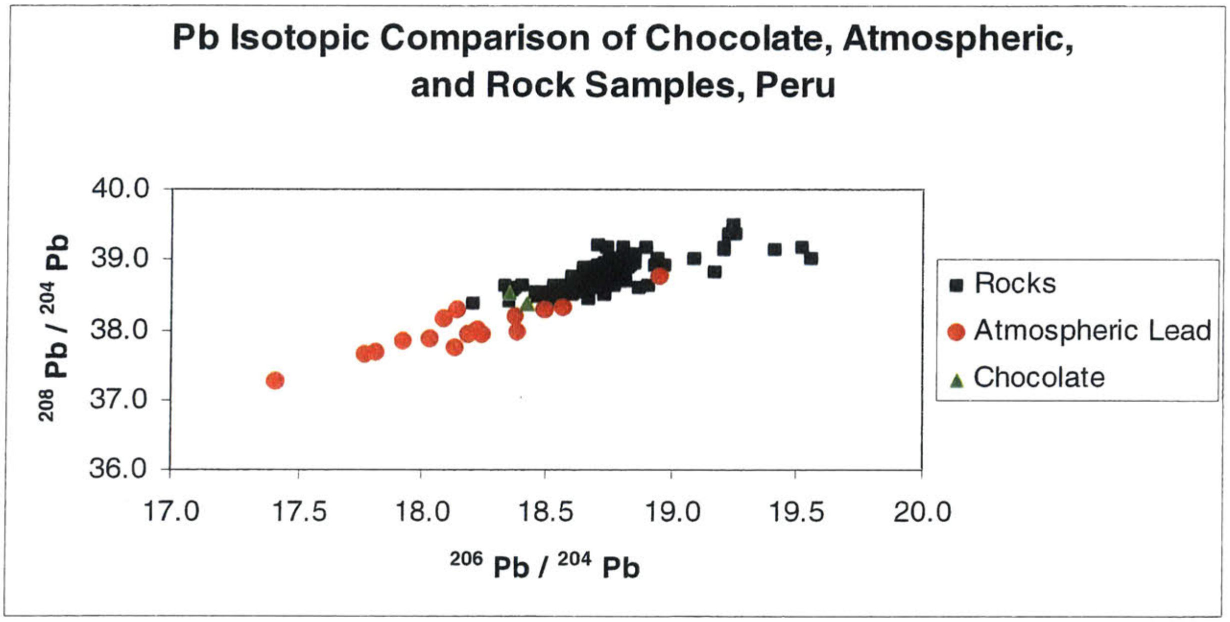


Figure 21: Plot of lead isotope ratios comparing $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb (top) and $^{206}$Pb /$^{204}$Pb vs. $^{208}$Pb /$^{204}$Pb (bottom) of the chocolate samples from Peru, the atmospheric lead measured in South American countries, and the volcanic rocks in Peru (that are assumed to contribute to the lead in the soils). The overlap of data points leads to an ambiguous conclusion for constraining the source of lead.

## Central America & the Caribbean

The isotopic composition of chocolate from Central America and the Caribbean was plotted against atmospheric data measured in several Central American countries and the volcanic rocks in various Central American and Caribbean countries in Figure 22. The plot clearly shows the relationship between the atmospheric lead and the chocolate samples, as both of them have distinctly different lead ratios than the rock samples. While the atmospheric lead was not measured directly in every country, it can be assumed that the isotopic composition of the aerosols does not vary significantly within Central America. The same local atmospheric, chocolate, and rock data are also plotted with the Northern Hemisphere atmospheric lead data in Figure 23. The lead in the chocolate samples from these Central American countries and the Caribbean is clearly consistent with data from the atmosphere. However, additional work is needed to make a better interpretation, with more site-specific data near cacao plantations.

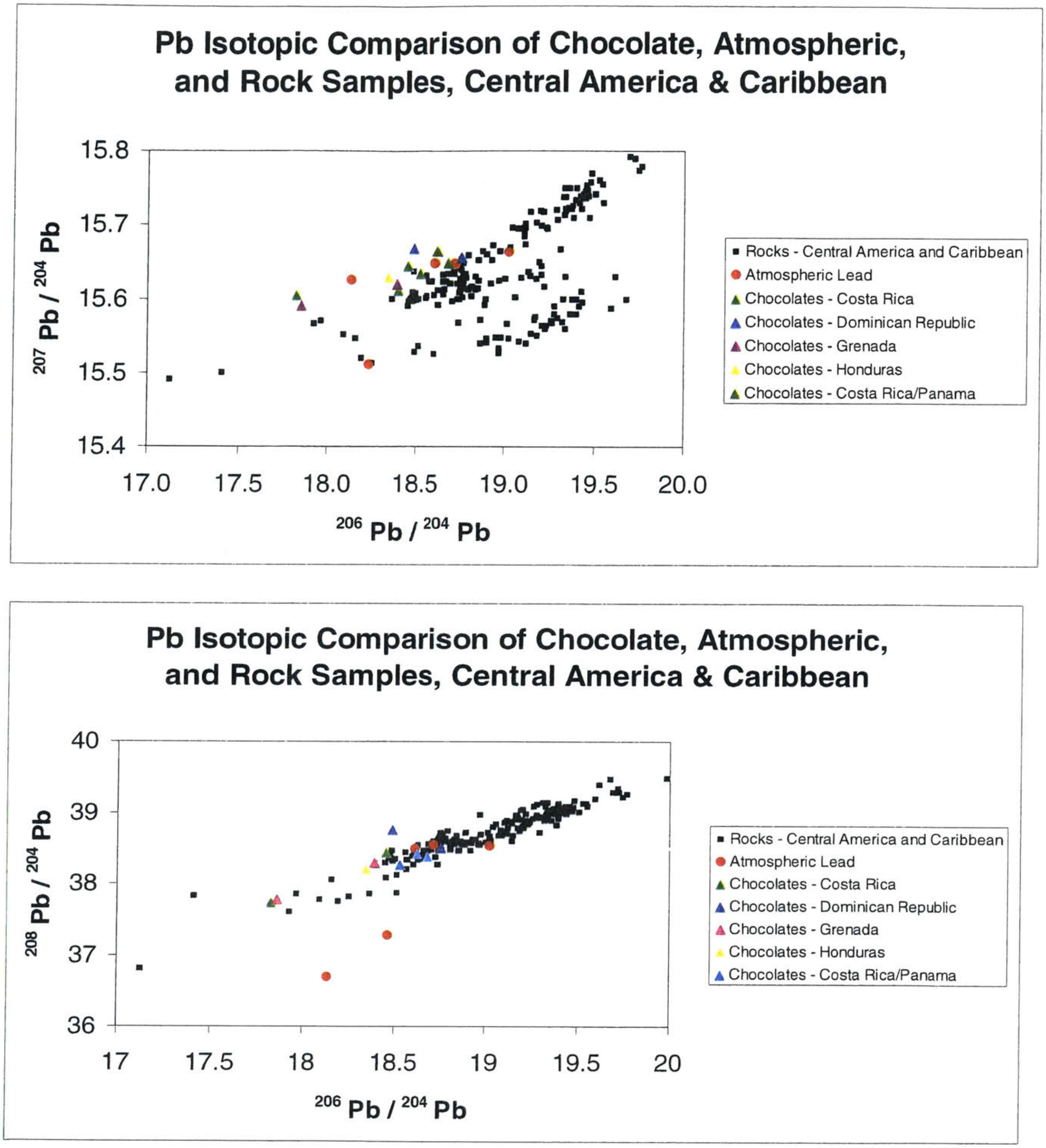


Figure 22: Plot of lead isotope ratios comparing $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb (top) and $^{206}$Pb /$^{204}$Pb vs. $^{208}$Pb /$^{204}$Pb (bottom) of the chocolate samples from Central America and the Caribbean, the atmospheric lead measured in Central American countries, and the volcanic rocks in Central America and the Caribbean (that are assumed to contribute to the lead in the soils). Again, the isotope ratios from the chocolates lie much closer to the atmospheric lead than to the rocks.

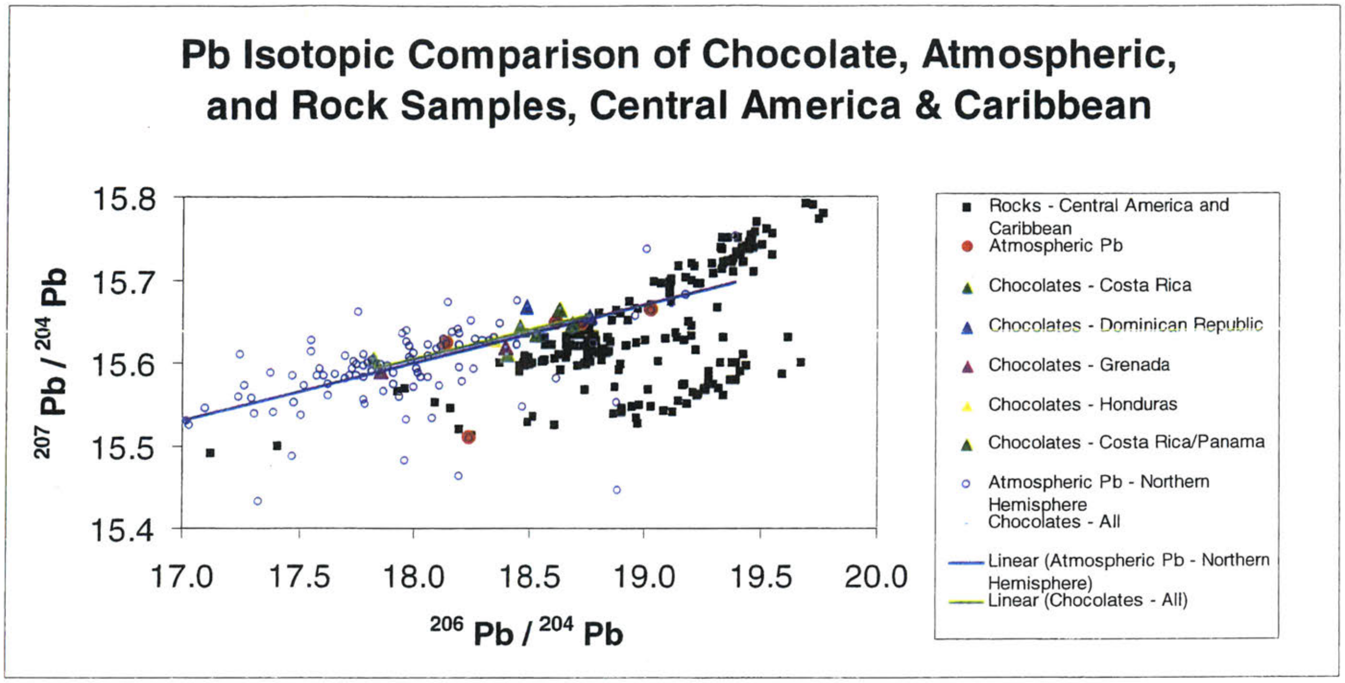


Figure 23: A plot of the $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb ratios, in addition to the atmospheric data from the Northern Hemisphere. Linear trend lines have been added to the Northern Hemisphere Pb data (blue) and the chocolate data (green). The trend lines are essentially identical; the isotopic signature from the chocolates matches that of the Northern Hemisphere, suggesting that the source of lead is from the global lead emissions rather than just local sources.

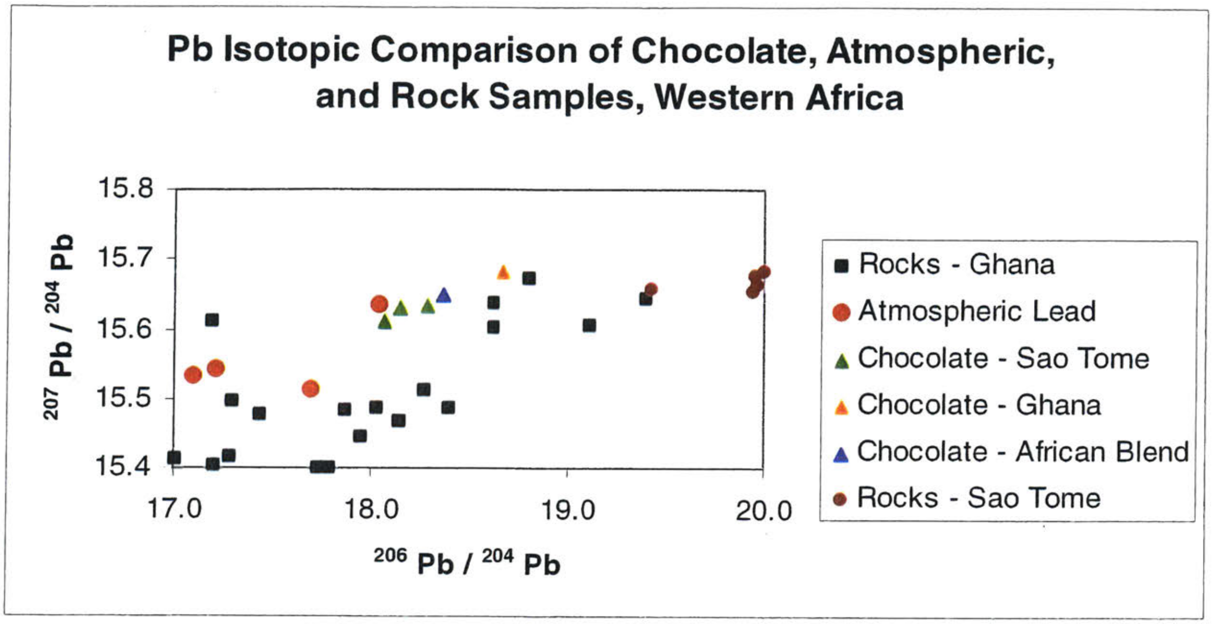
## Western Africa

The Pb isotope ratios of the chocolate samples from countries in western Africa, like Sao Tome, Ghana, and an African Blend from the Ivory Coast and other countries are plotted in Figure 24 against the atmospheric data measured in various countries from western Africa, like Ghana, Cameroon, Senegal, and Morocco, and rock samples from Ghana, Principe and Sao Tome. Here, the plot clearly shows a distinct difference between the isotope ratios of the rocks and chocolates. The lead isotope ratios are also plotted with the Northern Hemisphere isotopic signature in

Figure 25. The trend line of the chocolates is similar to that of the atmospheric lead, strongly suggesting much of the lead is from the atmosphere. However, western Africa is a very geologically complex area, with a large variety of rocks. Additional work on the soil or rocks at cacao plantation locations is necessary to draw a more conclusive interpretation.

## Southeast Asia

The lead isotope ratios of the chocolates from two countries in Southeast Asia, Java and Papua, were plotted against the lead isotope ratios from Java and Papua, and the atmospheric lead isotope ratios measured in several Southeast Asian countries, like Malaysia, Indonesia, and Vietnam in Figure 26. The chocolates plot close to the atmospheric lead and along the same trend, again leading to the conclusion that the lead in chocolates from Java and Papua is likely from atmospheric lead.



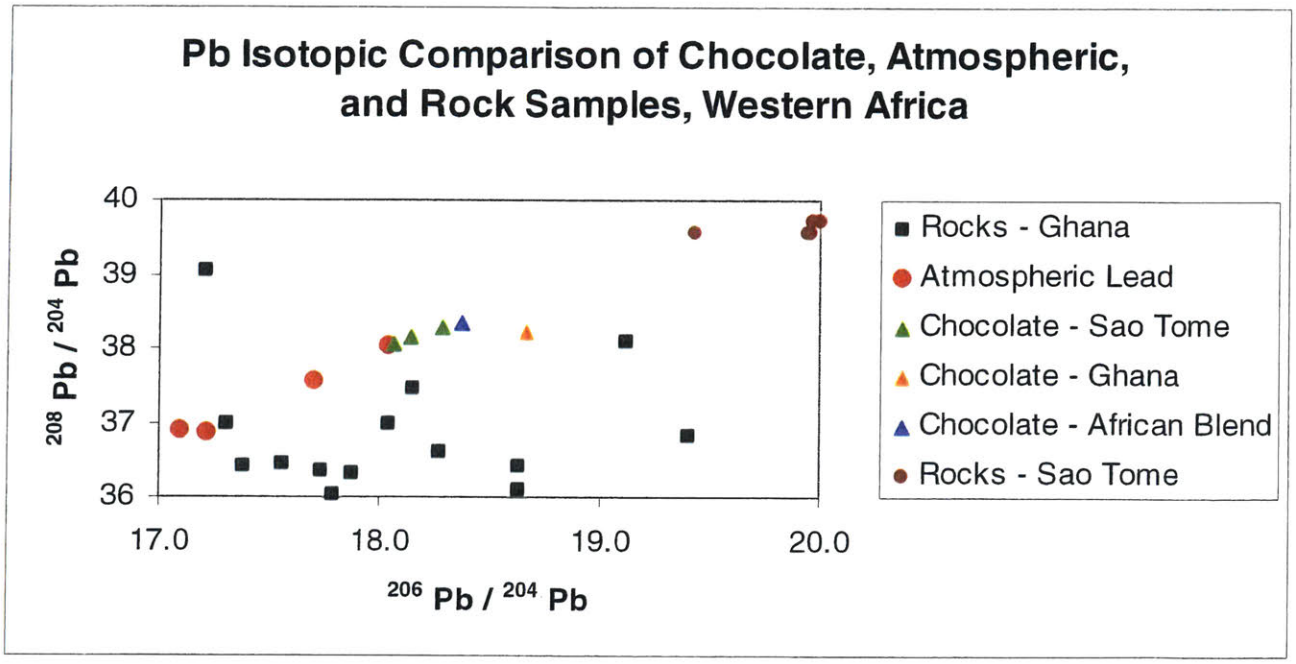


Figure 24: Plot of lead isotope ratios comparing $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb (top) and $^{206}$Pb /$^{204}$Pb vs. $^{208}$Pb /$^{204}$Pb (bottom) of the chocolate samples from Western Africa, the atmospheric lead measured in West African countries, and the volcanic rocks in Ghana and Prinicipe and Sao Tome (that are assumed to contribute to the lead in the soils). The plot clearly shows the chocolate lead isotope ratios are much closer to the atmospheric lead than to the rocks.

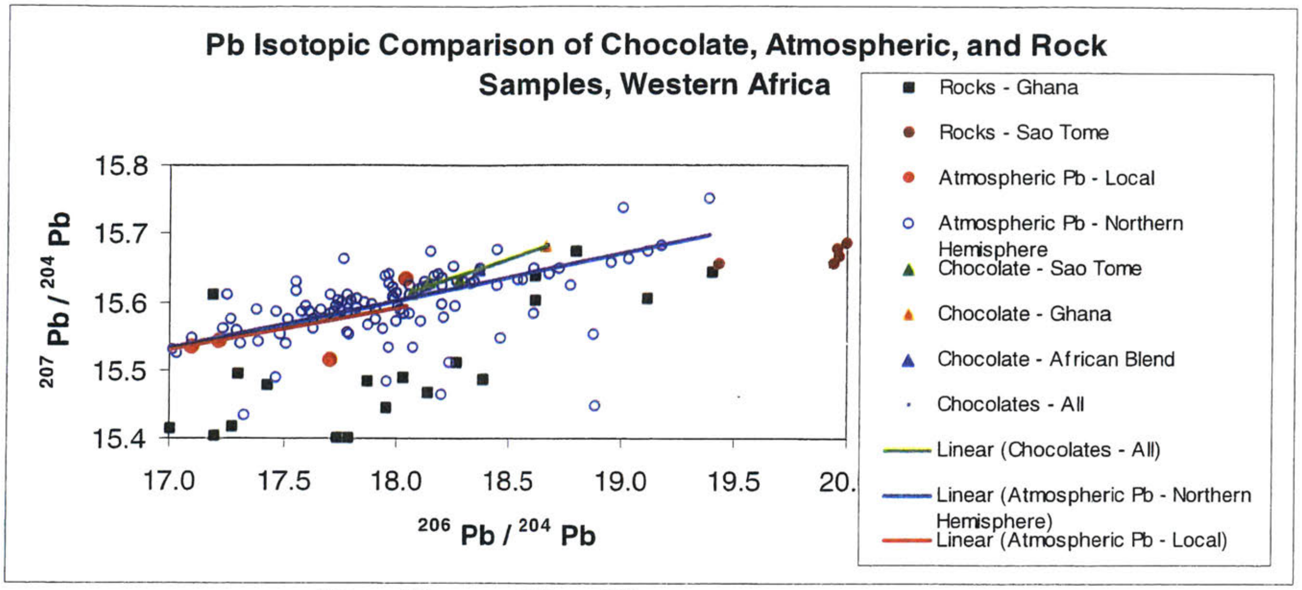
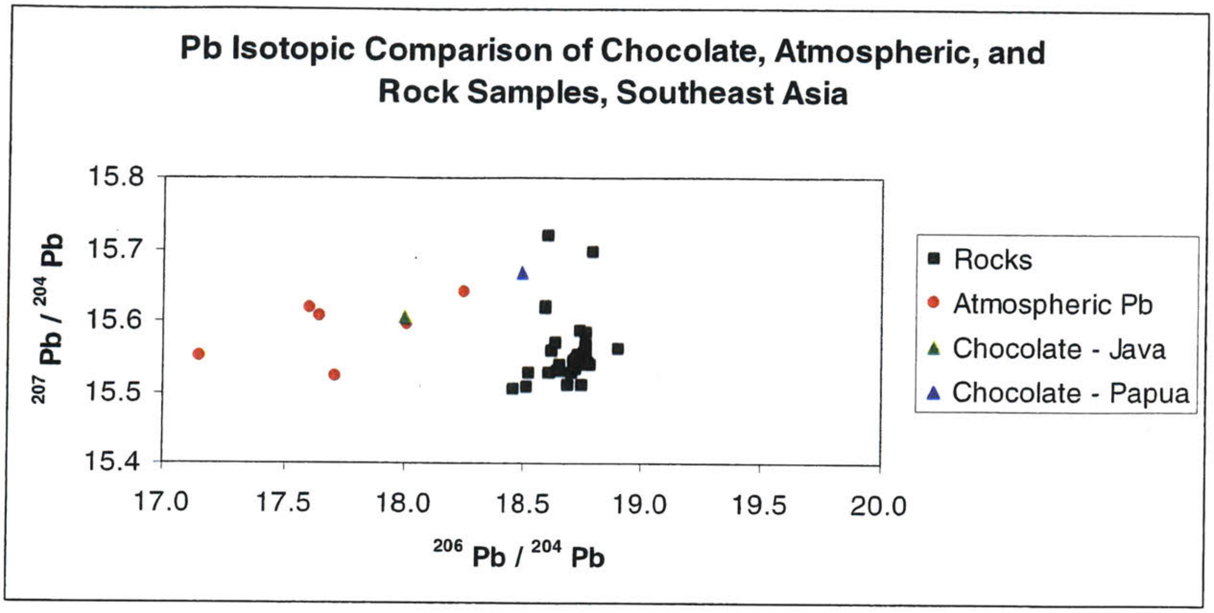


Figure 25: A plot of the $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb of the chocolates, atmospheric, and rock data, with the Northern Hemisphere atmospheric signature. Three trend lines have also been added, one of the Northern Hemisphere atmospheric lead, one for local atmospheric lead, and another for the chocolates. The trend line of the chocolates is most similar to that of the atmospheric data.

### Eastern Africa & Madagascar

The isotopic composition of chocolates from Tanzania and Madagascar was compared with the atmospheric lead isotope ratios collected in South Africa and other southeast African countries and the rock isotope ratios from Tanzania and Madagascar in Figure 27. The isotope ratios from the chocolates plot within the range of isotope ratios from the rocks, opposite from what is expected given the results from all of the other countries and chocolates analyzed thus far.



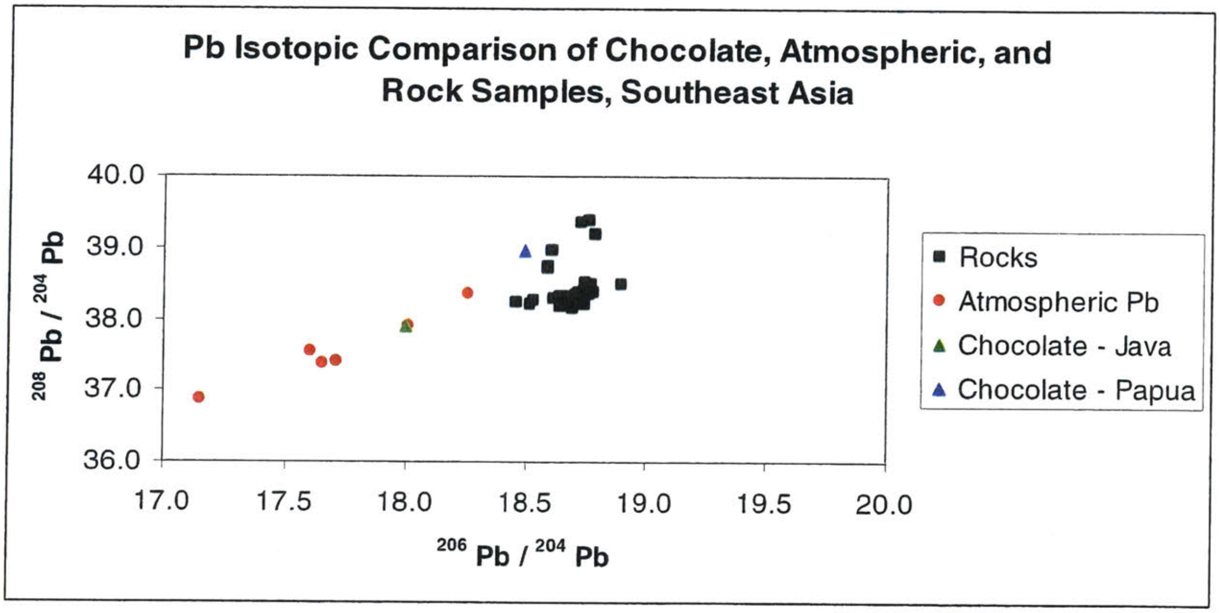


Figure 26: Plot of lead isotope ratios comparing $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb (top) and $^{206}$Pb /$^{204}$Pb vs. $^{208}$Pb /$^{204}$Pb (bottom) of the chocolate samples from Southeast Asia to the atmospheric lead measured in Indonesia, Malaysia, and other Southeast Asian countries, and the volcanic rocks in Papua, Java, and Indonesia (that are assumed to contribute to the lead in the soils). The plot clearly shows the lead isotope ratios from the chocolate from Java are much closer to the atmospheric lead than to the rocks, and both chocolates lie along the same trend as the atmospheric lead.

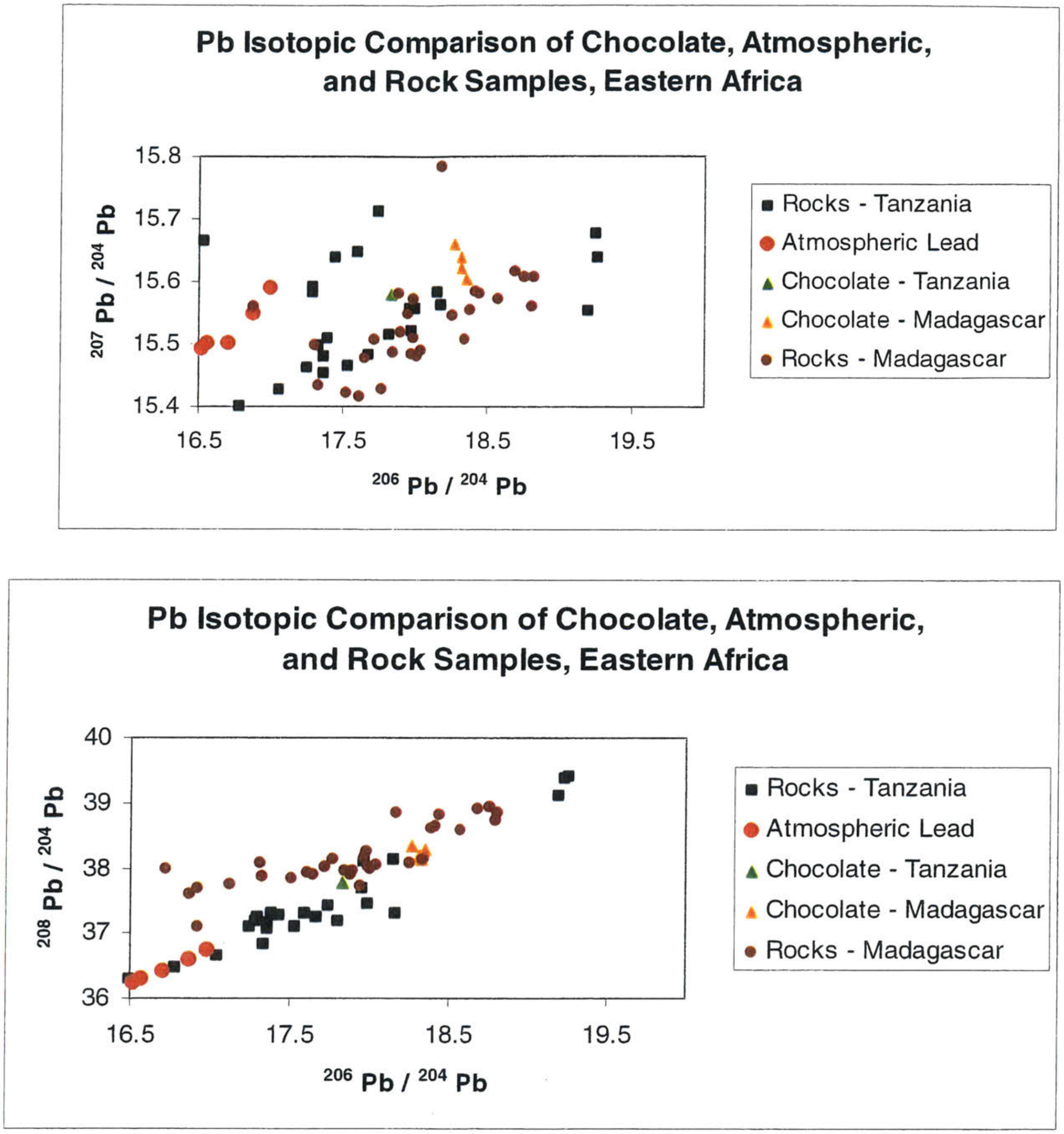
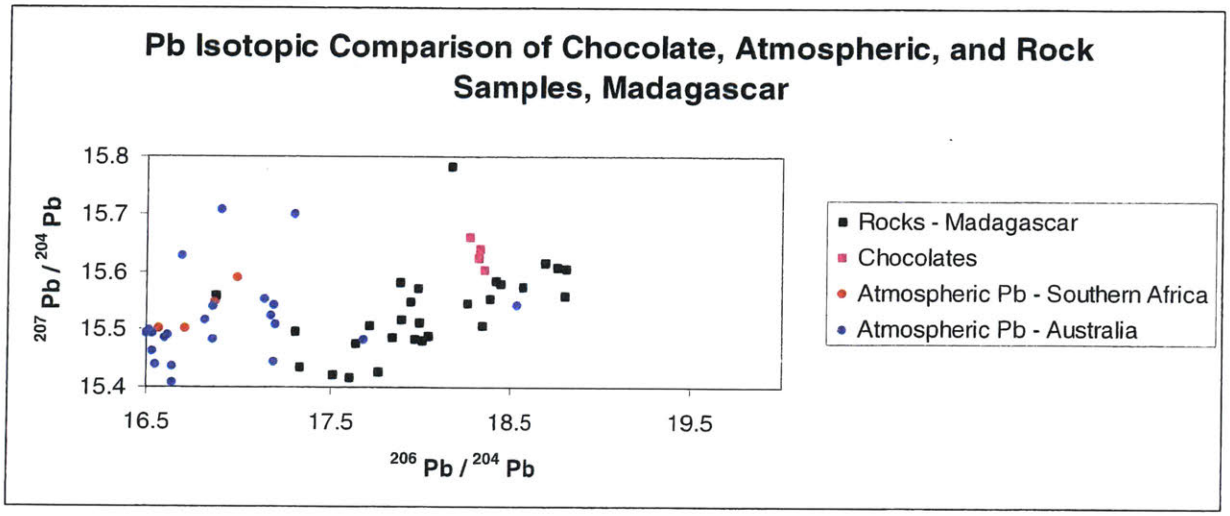


Figure 27: Plot of lead isotope ratios, comparing $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb (top) and $^{206}$Pb /$^{204}$Pb vs. $^{208}$Pb /$^{204}$Pb (bottom) of the chocolate samples from Eastern Africa, the atmospheric lead measured in South Africa, Zimbabwe, and other southeast African countries, and the volcanic rocks in Tanzania and Madagascar (that are assumed to contribute to the lead in the soils). The chocolate lead isotope ratios from both Tanzania and Madagascar are close to the ratios of the rocks.

In order to understand better why the predominant lead source in Eastern African chocolates is from the rocks and not atmospheric emissions, the chocolates from Madagascar are also plotted against the atmospheric lead measured in Australia, because for part of the year the trade winds bring aerosols from there across the Indian Ocean to Madagascar. The results are plotted in Figure 28, this time without the Tanzania chocolates or rocks. The chocolates from Madagascar still plot nowhere near the atmospheric lead measured in Southeastern Africa or Australia.



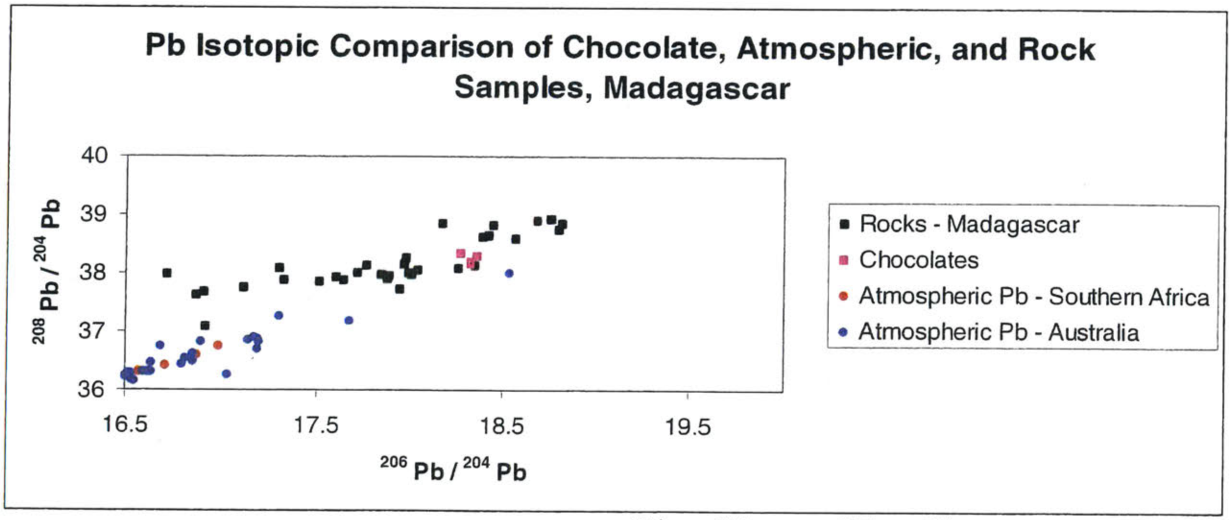


Figure 28: Plot of lead isotope ratios comparing $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb (top) and $^{206}$Pb /$^{204}$Pb vs. $^{208}$Pb /$^{204}$Pb (bottom) of the chocolate samples from Madagascar, the atmospheric lead measured in South Africa, Zimbabwe, and other southeast African countries, and Australia, and the volcanic rocks in Madagascar (that are assumed to contribute to the lead in the soils). The chocolate lead isotope ratios from Madagascar still plot close to the ratios of the rocks.

The results from Madagascar can be explained by examining the country's atmospheric emissions and comparing it to a country where the chocolates closely match the atmospheric emissions, like Venezuela. Madagascar switched to unleaded gasoline in 2001, after the signing of the Dakar Declaration calling for Sub-Sahara African countries to phase out leaded gasoline (Burke 2004). Venezuela switched to unleaded gasoline more recently, with some areas still using leaded gasoline as of 2005. While both countries no longer widely use leaded gasoline, lead deposited on the soils can remain there for 17- 80 years. The total amount of lead emissions while both countries used leaded gasoline is an important factor in determining the lead remaining in soils. Based on 2006 estimates, Madagascar had approximately 106,000 vehicles total, whereas Venezuela had 2,264,300 vehicles, almost 21 times more than Madagascar (www.worldbank.org). This drastic difference, in addition to the occasional use of leaded gasoline in Venezuela, could provide enough of a difference in lead emissions to explain the difference in the source of lead in the chocolates.

Madagascar and Tanzania are located in the Southern Hemisphere, away from the developed nations' industrial activities in the Northern Hemisphere. This lack of additional lead in the atmosphere could also be the difference in the source of lead measured in the chocolates.

## 5.3 Comparison of Data for Determining Concentration of Lead

Most of the chocolates examined in this study had relatively low lead concentrations, all well below the limit of 1 ppm recommended by the WHO, but a few are above the limit of 0.1 ppm set by the FDA. A plot of cumulative frequency of the concentrations, in ppb, is shown in Figure 29 and is a good way to show the large range in concentrations. The average concentration of all of the chocolates is 44 ppb, at the 75th percentile. The median concentration is 28 ppb, which indicates how skewed the data are from the high lead concentrations from Dagoba. A few of the chocolates, though, have significantly higher concentrations. Many of the chocolates that have higher concentrations are from South America, specifically Ecuador, Venezuela, and Peru. However, the data show that the concentration of lead doesn't depend on location within South America. There are several other chocolates from Ecuador, Venezuela and Peru that have lower concentration. Instead, the data suggest that the concentration depends strongly on the manufacturer of the chocolate. Another graph with the average lead concentrations for each manufacturer is shown in Figure 30. Again, Dagoba consistently has higher concentrations of lead when compared with the other main manufacturers in this study. The chocolates from Ecuador and Peru produced by Dagoba have the highest concentrations of all of the chocolates analyzed. This suggests that either lead contaminants are introduced during the manufacturing process, or that the plantations where Dagoba purchases its beans have contamination either in their harvesting or transporting procedures. El Rey also has high concentrations, but only two samples from this manufacturer were analyzed, both from Venezuela, so it is difficult to determine if the cause of high concentrations is because of the location or the manufacturer, or a combination of the two.

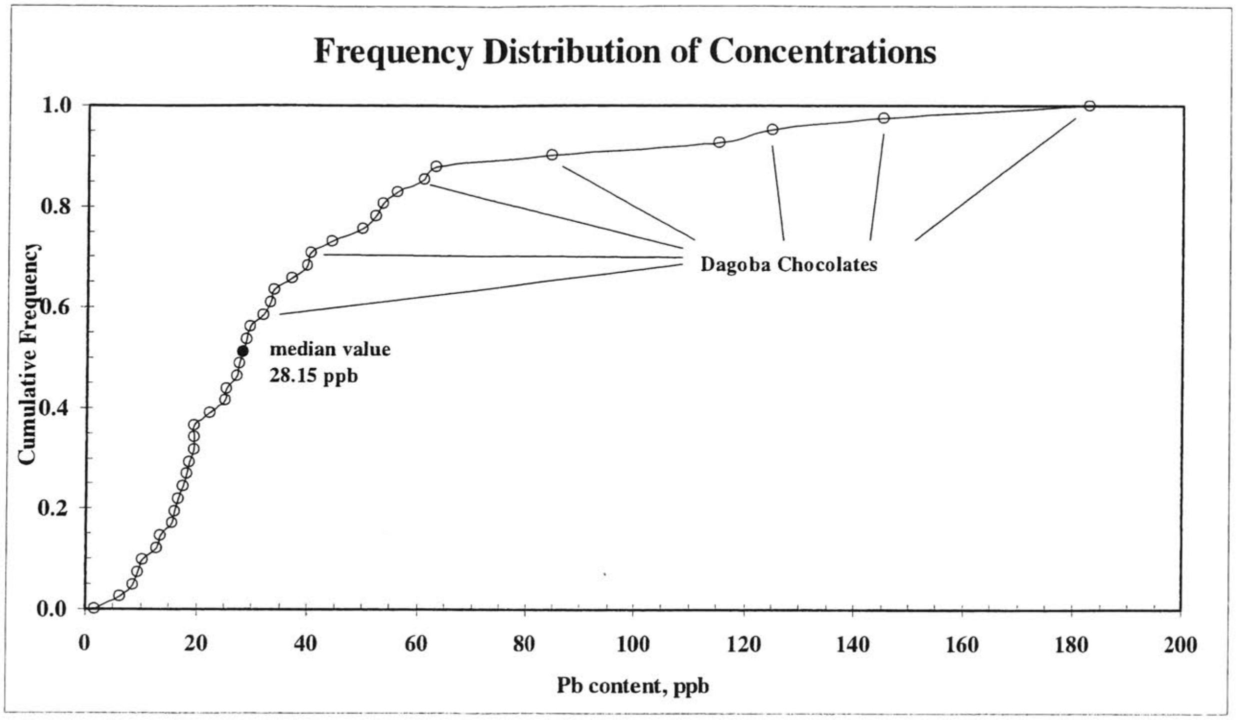


Figure 29: A graph showing the cumulative frequency of the concentration of lead in chocolate. The Dagoba chocolates have the highest lead concentrations.

The concentration in the cocoa beans is much less than the concentration in any of the chocolates (about 1.62 ppb compared to an average of 44 ppb for the chocolates), and indicates the same as previous studies that determined cocoa beans have among the lowest of all lead concentrations in foods.

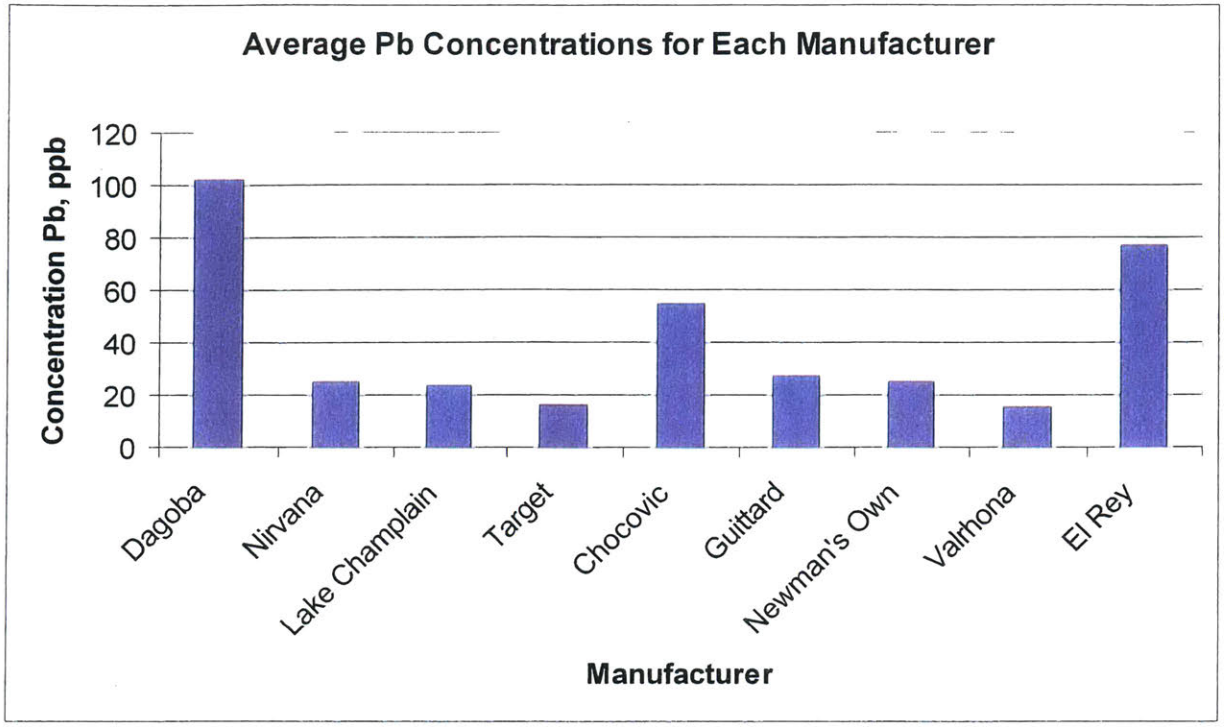


Figure 30: A bar graph showing the average concentrations of lead, in ppb, for each manufacturer. Dagoba has the highest average concentration, followed by El Rey. The chocolates that Target distributes from an unknown manufacturer have the lowest average concentration.

As previously mentioned, the concentration of lead in chocolate is expected to increase as the percentage of dark cocoa increases because the darker the chocolate the more cocoa beans and cocoa butter (and less other ingredients, such as milk and sugar that are present in milk chocolate, that dilute the lead concentration). The concentration of lead in chocolate bars is plotted against their percentage of cocoa in Figure 31. If we ignore the Dagoba analyses, there is a weak trend of increasing Pb with % cocoa but with a great deal of scatter. A large number of chocolates still have relatively low concentrations of lead despite having high cocoa concentration. The concentration of lead seems to depend more on the manufacturer or more likely the source of cocoa beans, and less on the percentage of cocoa in the chocolate bar.

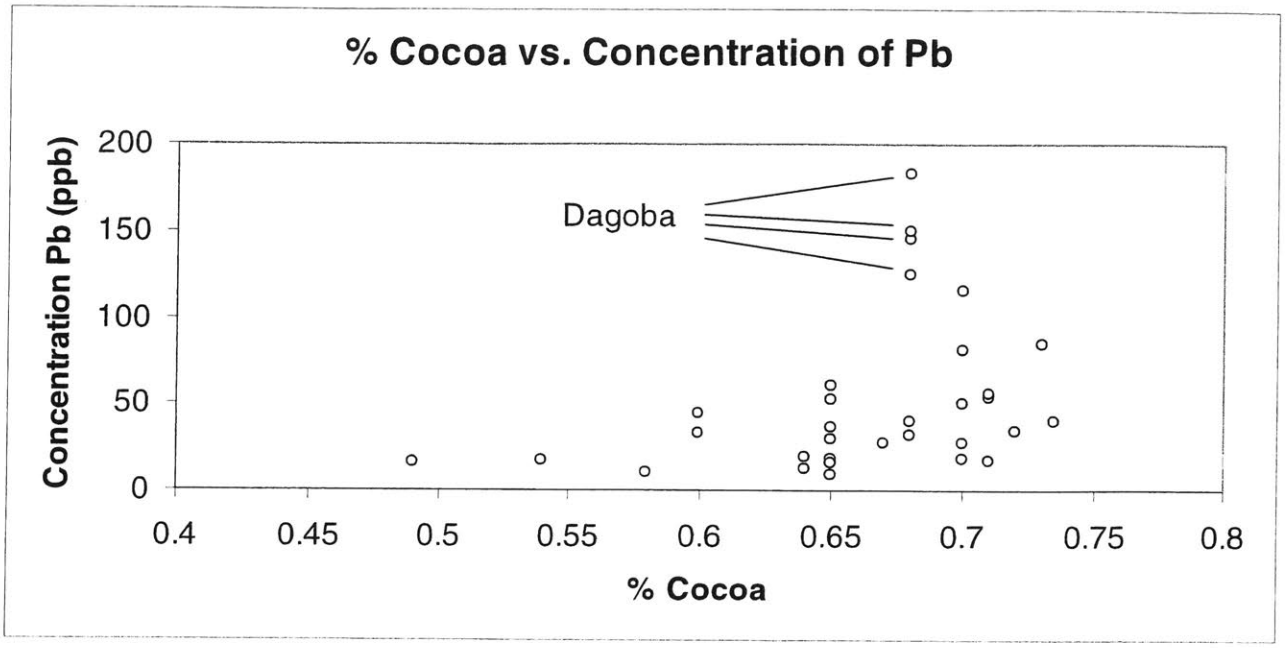


Figure 31: A plot of the percentage of cocoa and the concentration of lead in a chocolate bar. There is a weak correlation between the percentage of cocoa and the lead concentration. The plot shows the conclusion that manufacturer or location is a more important contributor than the percentage of cocoa.

The concentrations of lead in chocolates from the countries where the source was dominated by atmospheric lead (Northern Hemisphere) and by rocks or soils (Southern Hemisphere) reveals that chocolates from countries where the source of lead was dominated by rocks have a lower average lead concentration. The average concentration where the lead in chocolate mostly matched the atmospheric signature is 43.6 ppb, whereas the average concentration for chocolates where the lead was considered "naturally occurring" is 28 ppb.

## 5.4 Soil Analysis from Venezuela

Two soil samples were taken from Hacienda San Jose, in Benitez county, Paria, Sucre state, Venezuela, shown in Figure 32. Hacienda San Jose is near where Hacienda Bucare chocolates are produced. The samples were taken on January 2nd, 2007. The samples were taken at depths of 0 cm (surface) and 30 cm, about 100 meters from the side of the road (M. Perignon, personal communication).

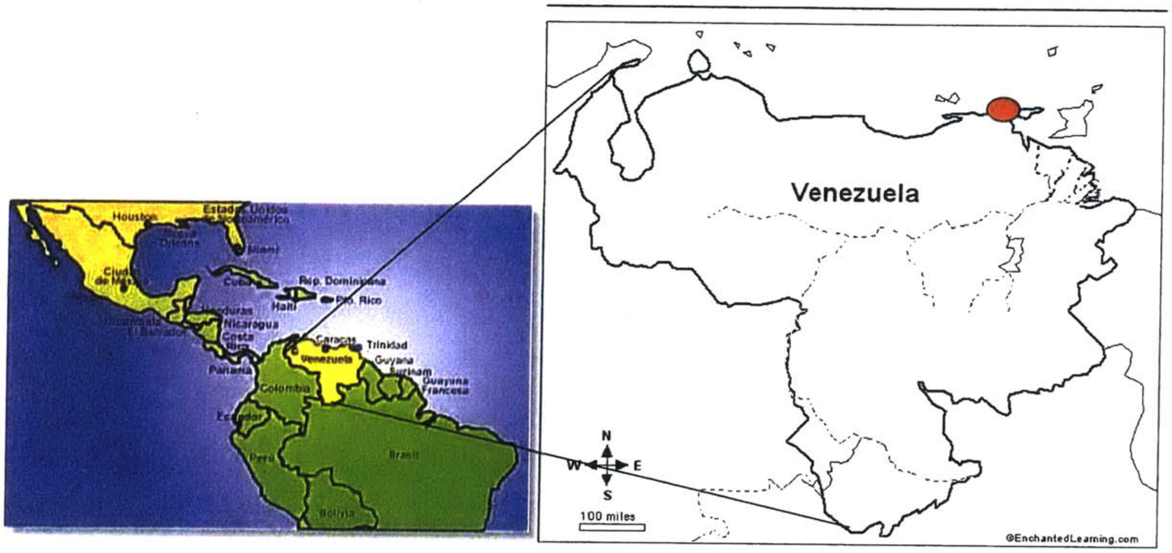


Figure 32: A map of Venezuela, showing the location of the soil samples.

As previously mentioned, the samples were treated with four different leach steps in an attempt to isolate labile from rock Pb. The steps in order of increasing aggressiveness are: ethanol, 1 M hydrochloric acid (HCl), aqua regia, and hydrofluoric acid (HF). Ethanol removes the most loosely bound lead, lead that likely has been deposited through atmospheric deposition or other contamination, as does HCl. Aqua regia breaks down organic matter, and HF removes the lead that is the most tightly bound in silicates, usually representing the lead that is from natural weathering in the rocks. The lead extracted in ethanol, HCl, and aqua regia is considered bioavailable to the plants.

### 5.4.1 Lead Isotope Ratios in Soil

The $^{206}Pb/^{204}Pb$ ratios are plotted in Figure 33 against depth. The ethanol, HCl, and aqua regia fractions all have a lower ratio than the HF lead isotope ratio, demonstrating the low $^{206}Pb/^{204}Pb$ ratio of the bioavailable lead that would be found in plants and organics grown in that soil. Also, the surface soils are all shifted toward lower $^{206}Pb/^{204}Pb$ ratios, which are characteristic of atmospheric lead, suggesting some surface soil contamination. However, the $^{206}Pb/^{204}Pb$ ratios of the Venezuelan chocolates are still lower than those of the soils, and much closer to the low ratios expected of atmospheric lead. The lead in chocolate could be the result of some mixing between atmospheric lead and local lead from the soils.

The $^{206}Pb/^{204}Pb$ and $^{207}Pb/^{204}Pb$ isotope ratios of the soil are plotted in Figure 34 with the atmospheric, chocolate, and rock lead isotope ratios previously discussed. Though the soil does have a different signature than the rocks, it also has a different signature than the chocolates as well. However, it does lie along roughly the same mixing line as the chocolates and the atmospheric lead, suggesting that there might be a small component of soil lead present in the chocolates. For the most part, though, the lead isotopic signature in the chocolates is dominated by the atmospheric lead.

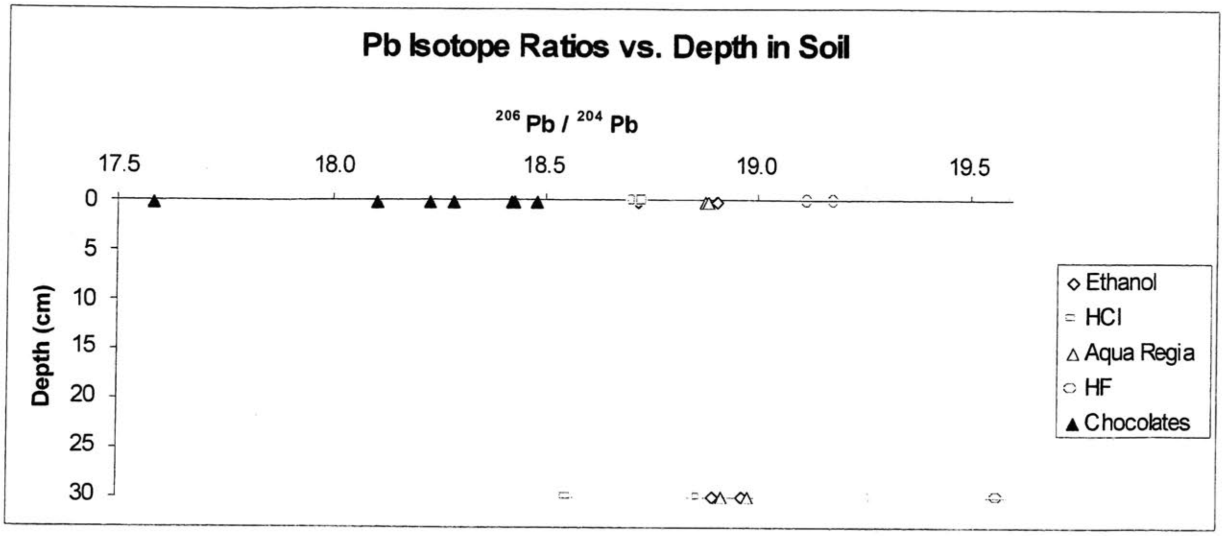
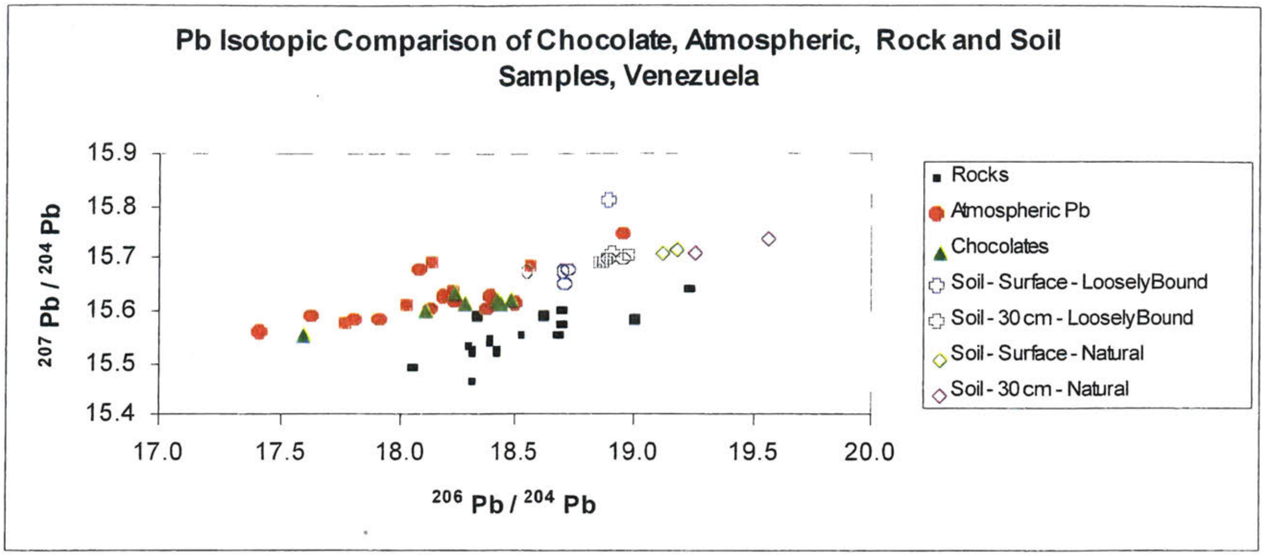


Figure 33: A plot of the $^{206}$Pb /$^{204}$Pb ratio with depth. The HF fraction, which represents the most tightly bound lead in the soil, has higher ratios, which matches more closely with that of the rocks. The chocolates are plotted at the surface, and show that the lead isotope ratios of the chocolates do not match those of the soils.



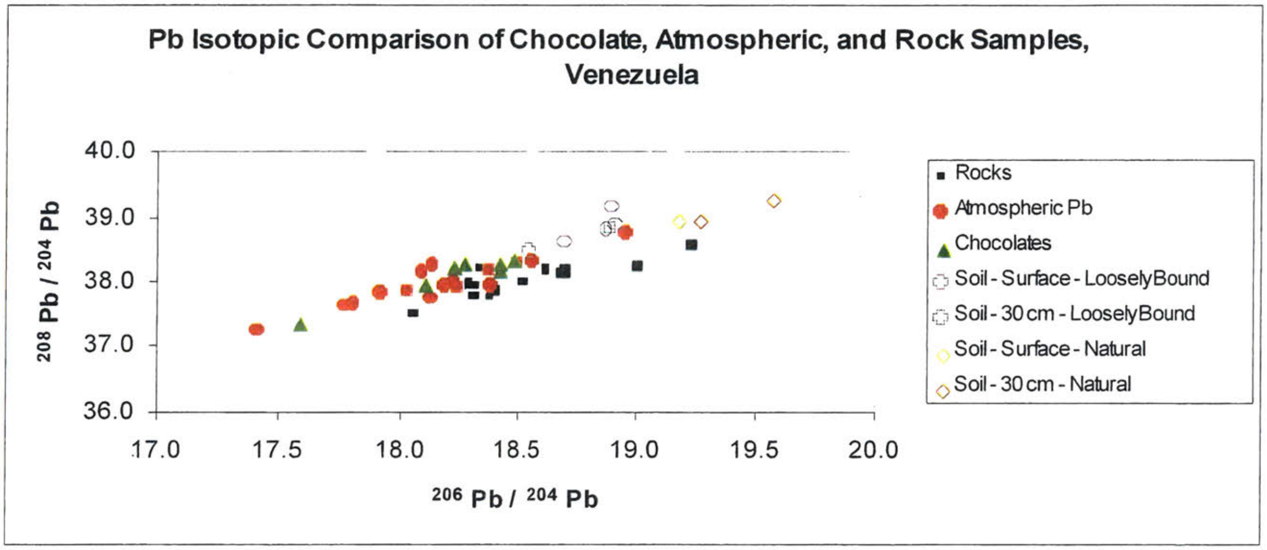


Figure 34: A plot of the $^{206}$Pb /$^{204}$Pb vs. $^{207}$Pb /$^{204}$Pb (top) and $^{206}$Pb /$^{204}$Pb vs. $^{208}$Pb /$^{204}$Pb (bottom) ratios comparing the chocolate, atmospheric, rock and soil samples. The soil has a different isotopic signature, suggesting that perhaps there is a small mixing component involved, but that much of the lead in chocolate is not from the soils.

### 5.4.2 Lead Concentrations in Soil

The lead concentrations in the soils from Venezuela were also analyzed. For both the surface and the 30 cm deep soils, most of the lead is located in the bioavailable fraction of the soil – the loosely bound Pb in the ethanol, HCl, and aqua regia fractions. A summary of the concentrations, and the percentage of lead, is shown in Tables 5 and 6. While the acids remove some of the more tightly bound lead, this amount is assumed to be minimal compared to the remaining lead in the sample. For example, the HCl would leach some lead out of the tightly bound HF fraction, but not to significantly change the overall percentages.

Table 5: A table showing the concentration of lead in the different fractions at the surface, and the percent of the total lead in the soil sample at the surface. Most of the lead (~96%) is located in the bioavailable fraction of the soil.

| Surface Soils | | |
|---|---|---|
| fraction | Concentration of Pb (ppb) | % of total Pb |
| Ethanol | 159 | 0.83 |
| HCl | 1570 | 8.17 |
| Aqua Regia | 16800 | 87.4 |
| HF | 689 | 3.59 |

Table 6: A table showing the concentration of lead in the different fractions at 30 cm depth, and the percent of the total lead in the soil sample at 30 cm depth. Most of the lead (~97%) is in the bioavailable fraction of the soil.

| 30 cm Soils | | |
|---|---|---|
| fraction | Concentration of Pb (ppb) | % of total Pb |
| Ethanol | 377 | 2.20 |
| HCl | 1170 | 6.87 |
| Aqua Regia | 15100 | 88.3 |
| HF | 455 | 2.66 |

The high concentrations of bioavailable lead in the soil suggests that some of the lead in chocolate may have been from the soils, but the previous conclusion from comparing the lead isotope ratios indicates only a small amount is from the soils because not all bioavailable lead is taken up by plants.

The total lead content of these soils, at 20 ppm or less, is considered normal for soils. The amounts of lead in the soils are not an indicator of significant aerosol lead contamination, nor are the isotopic ratios consistent with significant aerosol contamination. There is likely an aerosol component, because the mobile lead fraction has a lower $^{206}Pb/^{204}Pb$ ratio than the HF fraction. The aerosol component is not the dominant source.

## 5.5 Implications

The average concentration of the lead in chocolate was shown to fall below the maximum limit of 1 ppm of lead allowed in cocoa and cocoa products set by the FAO and the WHO, and below the limit of 0.1 ppm set by the FDA. While the average amount of lead in a single chocolate bar may not be harmful, it is important to remember that lead is a cumulative poison, and can remain in the body for years after it's been ingested. The average American consumes 10-12 lbs of chocolate a year, equivalent to between 110 and 130 average-sized chocolate bars. This adds up to 824 μg per year of lead if consuming Dagoba dark chocolate! There is a recommended limit of 3 μg of lead per person per week set by the WHO, but an average-sized chocolate bar (41 g) has as high as 7.46 μg total Pb. This is more than twice as much as the weekly limit. And, if over 8 percent of the lead ingested remains in the body, with 90 percent of that in the bones, this contributes significantly to the amount of lead stored in the body. The data suggest that consumers need to be aware of the lead concentration in the chocolate they are eating, and be cautious of eating large amounts of dark chocolate from certain manufacturers.

The Dagoba chocolates have an average concentration of lead that is above the recommended maximum. Recently, Dagoba issued a recall of chocolates sold during the fall of 2006 and spring of 2007 due to levels of lead that were found to be above the FDA-approved limit (http://www.dagobachocolate.com/recall/#progress). Through a thorough analysis of the harvesting and manufacturing process, they determined the lead contamination occurred between harvesting the pods, and before being shipped to the manufacturing facility in Oregon, claiming contamination occurred during transport or at the processing facility in Ecuador. The chocolates analyzed in this study were not from the chocolate batch recalled; they were from a batch manufactured after the recall. This raises a few questions. Is this really an isolated incident only involving a single facility in Ecuador? How can they prevent it from happening again? If they claimed to have fixed the problem, why do the chocolates still have high lead concentrations? Also, Dagoba only recalled Los Rios chocolates from Ecuador, in addition to a few others not analyzed in this study, but several other chocolates produced by Dagoba from other countries were still found to be above the FDA limit. Why were these chocolates not recalled?

The data from this study suggest that atmospheric lead is the dominant source of lead in chocolate. Bollhofer (2000, 2001) concluded that much of the lead in the atmosphere today is likely due to the emissions of leaded gasoline. The use of leaded gasoline is still common only in some developing countries due to the actions taken at the Summit of the Americas in 1994 and the United Nations Commission on Sustainable Development in 1995 (Finkelman 1996), but the number of vehicles has significantly increased over the last few decades, leading to increased lead in the atmosphere. However, will stopping the use of leaded gasoline solve the problem of lead contamination from atmospheric deposition in foods? There are still other sources, such as smelting operations and mines that will contribute locally.

Almost all of the countries where the chocolate lead isotope ratios matched closely with the atmospheric lead isotope ratios are located in the Northern Hemisphere. The few countries where no concrete conclusion could be made, like Peru and Ecuador, and where the lead in chocolate was concluded to be from the rocks, such as Tanzania and Madagascar, are located in the Southern Hemisphere. The difference in the lead isotopic signature of the Northern and Southern Hemisphere is different due to the industrial activities of the developed nations, and it is interesting to note how much the emissions that result from these activities are affecting countries worldwide.

The soil data, though limited, indicate that there is likely some mixing in the lead found in chocolate from Venezuela, though the isotopic signature is dominated by the atmospheric lead. Also, the soil from Venezuela had a detectable amount of anthropogenic Pb contamination.

# Chapter 6. Conclusions

An analysis of chocolate samples from around the world was conducted to determine the primary source of lead in chocolate. Previous studies by Rankin suggested that perhaps the lead in chocolate was from atmospheric anthropogenic lead, and these studies confirm that idea. The lead isotope ratios from $^{206}$Pb /$^{204}$Pb, $^{207}$Pb /$^{204}$Pb, and $^{208}$Pb /$^{204}$Pb suggest that the lead in chocolate from almost every country in the Northern Hemisphere most often matched the global lead isotopic signature. Though local sources likely also have an effect, the correlation between the chocolates and the global signature strongly indicates that much of the contamination is a result of lead emissions from industrialized nations in the Northern Hemisphere. However, Tanzania and Madagascar are special cases, where perhaps their low total lead emissions, and location in the Southern Hemisphere, away from industrialized nations, have prevented a large amount of atmospheric contamination. The dominant source of lead in chocolate from these countries is primarily from the rocks and soils in the environment. Other countries in the Southern Hemisphere but near the equator, such as Peru, and even Ecuador, had ambiguous results about the dominant source of lead.

The average concentration of lead in chocolate was also found to be well below the standards set by the WHO and the FAO and below the standards in the United States set by the FDA. One manufacturer, Dagoba, was found to have consistently high levels of lead, with their average lead concentration being above the limit allowed by the FDA. The chocolates from countries in the Northern Hemisphere, where the isotopic signature was found to match that of the global atmospheric lead signature, had an average higher lead concentration than chocolates from countries in the Southern Hemisphere where the isotopic signature matched the isotopic signature of the rocks. This further supports the conclusion that the lead in chocolate is predominantly from atmospheric sources.

The soils analyzed suggested that perhaps a small amount of the lead came from aerosols, though it was not the dominant source. Neither the amount of lead, nor the isotope ratios, indicated that aerosols were a significant contributor to the lead in the soil. The concentrations of lead in the soil were found to be within normal ranges.

# Chapter 7. Future Studies

Future studies on chocolate are necessary to determine an exact source of lead, and exactly where in the harvesting and processing of the cocoa beans the contamination occurs. While this study has shown that the source of lead is from anthropogenic atmospheric lead, there are likely several other sources of anthropogenic lead contamination along the way. An analysis of the chocolate after every step of harvesting and processing could reveal where most of the contamination occurs. Also, a more detailed study of the soils from the cacao plantations, at different depths, could reveal more information about the sources and mixing of the lead in chocolate.

Another interesting aspect to study would be the difference in lead absorption of the different cacao plants. Is criollo affected the same as trinitario? Do the chocolates from these different hybrids have different lead concentrations? Also, do organic chocolates, considered to be natural, have less lead than other chocolates? Dagoba is considered an organic chocolate, and its chocolates had higher concentrations of lead. A more detailed analysis of organic vs. non-organic chocolates would be interesting to study.

Answering these questions, and continuing further studies of cacao, chocolate, and lead, will allow for a better understanding of how much the industrial activities of the developed world are felt worldwide.

# Appendix

## List of Samples & Raw Data

The following is a list of samples, including the sample number, manufacturer name, location where cacao beans were grown, and the type of sample.

| MIT # | Sample Name and Location | Type |
|---|---|---|
| T 671(2) | Dagoba Conacado Dominican Republic | chocolate |
| T 672(2) | Nirvana Santo Domingo | chocolate |
| T 673(2) | Nirvana Tanzania | chocolate |
| T 674(2) | Nirvana Sao Thome | chocolate |
| T 675(2) | Nirvana Grenada | chocolate |
| T 676(2) | Nirvana Papua | chocolate |
| T 677(2) | Nirvana Java | chocolate |
| T 678(2) | Lake Champlain Sao Thome | chocolate |
| T 679(2) | Lake Champlain Grenada | chocolate |
| T 680(2) | Lake Champlain Venezuela | chocolate |
| T 681(2) | Lake Champlain African Blend | chocolate |
| T 1180(2) | Target Venezuela 49% | chocolate |
| T 1181(2) | Target Ghana 58% | chocolate |
| T 1182(2) | Target Costa Rica 64% | chocolate |
| T 1183(2) | Target Peru 64% | chocolate |
| T 1184(2) | Target Ecuador 71% | chocolate |
| T 1185(4) | Navitas cocoa beans Peru | cocoa beans |
| T 1220(2) | Chocovic Ocumare Venezuela | chocolate |
| T 1221(2) | Chocovic Guaranda Ecuador | chocolate |
| T 1222 | Trader Joe's, Sao Tome | chocolate |
| T 1223 | 365 Organic Swiss, Dominican Republic | chocolate |
| T 1224 | Newman's Own #2, Costa Rica/Panama | chocolate |
| T 1245 | Guittard - Ecuador | chocolate |
| T 1246 | Valrhona Manjari Madagascar | chocolate |
| T 1247 | Valrhona Guanaja Honduras Caribbean | chocolate |
| T 1248 | El Rey Carenero Venezuela (Gran Saman) | chocolate |
| T 1249 | Guittard - Madagascar Criollo | chocolate |
| T 1273 | Dagoba Pacuare Costa Rica | chocolate |
| T 1274 | Dagoba Los Rios Ecuador | chocolate |
| T 1275 | Dagoba Milagros Peru | chocolate |

| MIT # | Sample Name | Date Analyzed | 206/204 | % SE | 207/204 | % SE | 208/204 | % SE | Pb (ng) (2) | Sample Wt. (g) | Pb (ppb) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T 671(2) | Dagoba Conacado Dominican Republic | 2/1/07 | 18.763 | 0.004 | 15.657 | 0.004 | 38.505 | 0.004 | 84.91 | 1.0038 | 84.59 |
| T 672(2) | Nirvana Santo Domingo | 2/1/07 | 18.558 | 0.031 | 15.665 | 0.032 | 38.396 | 0.032 | 27.61 | 1.0164 | 27.17 |
| T 673(2) | Nirvana Tanzania | 2/1/07 | 17.833 | 0.008 | 15.579 | 0.008 | 37.773 | 0.008 | 50.14 | 1.0065 | 49.82 |
| T 674(2) | Nirvana Sao Thome | 2/2/07 | 18.065 | 0.006 | 15.612 | 0.006 | 38.070 | 0.006 | 27.67 | 1.0066 | 27.49 |
| T 675(2) | Nirvana Grenada | 2/1/07 | 17.857 | 0.021 | 15.590 | 0.023 | 37.769 | 0.022 | 44.95 | 1.0145 | 44.30 |
| T 676(2) | Nirvana Papua | 2/1/07 | 18.492 | 0.009 | 15.669 | 0.009 | 38.972 | 0.009 | 10.40 | 1.0102 | 10.30 |
| T 677(2) | Nirvana Java | 2/2/07 | 17.988 | 0.031 | 15.595 | 0.031 | 38.412 | 0.031 | 6.531 | 1.0541 | 6.20 |
| T 678(2) | Lake Champlain Sao Thome | 1/30/07 | 17.853 | 0.129 | 15.714 | 0.114 | 37.933 | 0.117 | 85.23 | 1.0470 | 81.40 |
| T 679(2) | Lake Champlain Grenada | 1/30/07 | 18.397 | 0.012 | 15.619 | 0.013 | 38.291 | 0.012 | 34.51 | 1.0416 | 33.13 |
| T 680(2) | Lake Champlain Venezuela | 1/30/07 | 18.275 | 0.038 | 15.621 | 0.038 | 38.300 | 0.039 | 19.70 | 1.0154 | 19.41 |
| T 681(2) | Lake Champlain African Blend | 1/30/07 | 18.366 | 0.009 | 15.649 | 0.010 | 38.355 | 0.010 | 18.80 | 1.0741 | 17.51 |
| T 1180(2) | Target Venezuela 49% | 1/31/07 | 18.099 | 0.009 | 15.607 | 0.009 | 37.948 | 0.009 | 15.59 | 1.0053 | 15.51 |
| T 1181(2) | Target Ghana 58% | 1/31/07 | 18.674 | 0.337 | 15.772 | 0.325 | 38.577 | 0.333 | 10.44 | 1.0425 | 10.02 |
| T 1182(2) | Target Costa Rica 64% | 2/1/07 | 17.829 | 0.018 | 15.605 | 0.018 | 37.735 | 0.017 | 19.38 | 1.0016 | 19.35 |
| T 1183(2) | Target Peru 64% | 2/1/07 | 18.354 | 0.012 | 15.644 | 0.012 | 38.543 | 0.012 | 19.48 | 1.0034 | 19.41 |
| T 1184(2) | Target Ecuador 71% | 2/28/07 | 18.724 | 0.013 | 15.697 | 0.013 | 42.629 | 0.013 | 16.63 | 1.0018 | 16.60 |
| T 1185(4) | Navitas cocoa beans Peru | 2/2/07 | 18.624 | 0.115 | 15.598 | 0.117 | 38.337 | 0.117 | 1.638 | 1.0123 | 1.62 |
| T 1220(2) | Chocovic Ocumare Venezuela | 2/28/07 | 18.221 | 0.006 | 15.637 | 0.005 | 38.248 | 0.005 | 55.15 | 1.0334 | 53.37 |
| T 1221(2) | Chocovic Guaranda Ecuador | 3/1/07 | 18.552 | 0.006 | 15.666 | 0.006 | 38.614 | 0.006 | 56.20 | 1.0035 | 56.01 |
| T 1222 | Trader Joe's, Sao Tome | 3/1/07 | 18.144 | 0.005 | 15.630 | 0.006 | 38.149 | 0.005 | 36.15 | 1.0717 | 33.73 |
| T 1223 | 365 Organic Swiss, Dominican Republic | 3/1/07 | 18.493 | 0.007 | 15.669 | 0.007 | 38.754 | 0.008 |  | 1.1851 | did not run |
| T 1224 | Newman's Own #2, Costa Rica/Panama | 3/3/07 Daly sector | 18.631 | 0.023 | 15.665 | 0.026 | 38.427 | 0.026 | 25.90 | 1.0250 | 25.27 |
| T 1245 | Guittard - Ecuador | 3/5/07 | 18.695 | 0.007 | 15.660 | 0.007 | 38.569 | 0.007 | 55.29 | 1.0615 | 52.08 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T 1246 | Valrhona Manjari Madagascar | 3/5/07 | 18.270 | 0.008 | 15.659 | 0.008 | 38.346 | 0.008 | 13.47 | 1.0559 | 12.76 |
| T 1247 | Valrhona Guanaja Honduras Caribbean | 3/5/07 | 18.348 | 0.007 | 15.628 | 0.008 | 38.194 | 0.008 | 18.46 | 1.0161 | 18.16 |
| T 1248 | El Rey Carenero Venezuela (Gran Saman) | 3/5/07 | 18.477 | 0.004 | 15.626 | 0.004 | 38.348 | 0.004 | 117.7 | 1.0223 | 115.1 |
| T 1249 | Guittard - Madagascar Criollo | 3/6/07 | 18.327 | 0.014 | 15.637 | 0.014 | 38.148 | 0.014 | 9.717 | 1.0445 | 9.303 |
| T 1273 | Dagoba Pacuare Costa Rica | 3/6/07 | 18.459 | 0.005 | 15.645 | 0.006 | 38.435 | 0.006 | 32.05 | 1.0055 | 31.88 |
| T 1274 | Dagoba Los Rios Ecuador | 3/6/07 | 18.338 | 0.003 | 15.590 | 0.003 | 38.181 | 0.003 | 201.6 | 1.1038 | 182.7 |
| T 1275 | Dagoba Milagros Peru | 4/3/07 | 18.426 | 0.007 | 15.616 | 0.007 | 38.360 | 0.007 | 156.9 | 1.2568 | 124.8 |
| T 1276 | Guittard Chucuri Colombia | 3/6/07 | 18.602 | 0.011 | 15.650 | 0.011 | 38.429 | 0.010 | 19.87 | 1.0645 | 18.67 |
| T 1277 | Guittard Quevedo Ecuador | 3/6/07 | 18.582 | 0.006 | 15.642 | 0.007 | 38.448 | 0.006 | 42.25 | 1.1402 | 37.06 |
| T 1278 | Guittard Ambanja Madagascar | 3/9/07 | 18.324 | 0.007 | 15.622 | 0.007 | 38.186 | 0.008 | 16.46 | 1.0237 | 16.08 |
| T 1279 | Guittard Sur del Lago Venezuela | 3/9/07 | 18.421 | 0.009 | 15.617 | 0.008 | 38.209 | 0.009 | 30.26 | 1.0226 | 29.59 |
| T 1280 | El Rey Apamate Carenero Venezuela | 3/9/07 | 18.417 | 0.008 | 15.623 | 0.008 | 38.308 | 0.008 | 40.92 | 1.0318 | 39.66 |
| T 1281 | Hacienda Bucare Criollo Venezuela | 3/9/07 | 17.578 | 0.010 | 15.555 | 0.009 | 37.382 | 0.012 | 64.48 | 1.0211 | 63.14 |
| T 1288 | Dagoba Pacuare #2 Costa Rica | 3/9/07 | 18.402 | 0.008 | 15.611 | 0.008 | 38.276 | 0.009 | 40.67 | 1.0085 | 40.33 |
| T 1289 | Dagoba Los Rios #2 Ecuador | 3/10/07 | 17.796 | 0.004 | 15.561 | 0.004 | 37.591 | 0.004 | 150.9 | 1.0410 | 145.0 |
| T 1290 | Dagoba Milagros #2 Peru | 3/12/07 ID only | no data | | | | | | | 1.0749 | approx 150 |
| T 1291 | Dagoba Sambirano Madagascar Trinitario | 4/3/07 | 18.359 | 0.008 | 15.602 | 0.009 | 38.271 | 0.008 | 63.02 | 1.0356 | 60.85 |
| T 672(3) | Nirvana Santo Domingo | 4/5/07 | 18.400 | 0.023 | 15.623 | 0.022 | 38.222 | 0.023 | 28.46 | 1.0111 | 28.15 |
| T 677(3) | Nirvana Java | 4/6/07 | 17.999 | 0.013 | 15.606 | 0.012 | 37.911 | 0.012 | 8.852 | 1.0245 | 8.64 |
| T 678(3) | Lake Champlain Sao Thome | 4/6/07 | 18.281 | 0.010 | 15.633 | 0.010 | 38.297 | 0.009 | 26.96 | 1.0812 | 24.93 |
| T 1181(3) | Target Ghana 58% | 4/9/07 | 18.669 | 0.010 | 15.682 | 0.010 | 38.211 | 0.010 | 14.58 | 1.0810 | 13.49 |
| T 1224(2) | Newman's Own #2, Costa Rica/Panama | 4/9/07 | 18.533 | 0.009 | 15.634 | 0.009 | 38.266 | 0.010 | 22.47 | 1.0119 | 22.21 |
| T 069(3) | Newman's Own #1, Costa Rica/Panama | 4/4/07 | 18.690 | 0.008 | 15.648 | 0.008 | 38.384 | 0.008 | 32.16 | 1.1127 | 28.90 |

| **Reproducibility: JF series samples from 2004** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **MIT #** | **Sample Name** | **Type of Sample** | **206/204** | **% SE** | **207/204** | **% SE** | **208/204** | **% SE** |
| T 1220(2) | Chocovic Ocumare Venezuela | chocolate | 18.221 | 0.006 | 15.637 | 0.005 | 38.248 | 0.005 |
| **JF 2(2)** | Chocovic Ocumare Venezuela | chocolate | 18.180 | 0.007 | 15.608 | 0.007 | 38.101 | 0.007 |
| | | | | | | | | |
| T 1221(2) | Chocovic Guaranda Ecuador | chocolate | 18.552 | 0.006 | 15.666 | 0.006 | 38.614 | 0.006 |
| **JF 3(2)** | Chocovic Guaranda Ecuador | chocolate | 18.541 | 0.007 | 15.648 | 0.008 | 38.601 | 0.007 |
| | | | | | | | | |
| T 1222 | Trader Joe's, Sao Tome | chocolate | 18.144 | 0.005 | 15.630 | 0.006 | 38.149 | 0.005 |
| **JF 4(2)** | Trader Joe's, Sao Tome | chocolate | 18.163 | 0.005 | 15.623 | 0.005 | 38.149 | 0.005 |
| | | | | | | | | |
| T 1223 | 365 Organic Swiss, Dominican Republic | chocolate | 18.493 | 0.007 | 15.669 | 0.007 | 38.754 | 0.008 |
| **JF 7** | 365 Organic Swiss, Dominican Republic | chocolate | 18.481 | 0.006 | 15.648 | 0.006 | 38.394 | 0.006 |
| | | | | | | | | |
| T 1224 | Newman's Own #2, Costa Rica/Panama | chocolate | 18.631 | 0.023 | 15.665 | 0.026 | 38.427 | 0.026 |
| **JF 8** | Newman's Own #2, Costa Rica/Panama | chocolate | 18.583 | 0.005 | 15.627 | 0.005 | 38.282 | 0.005 |
| **JF 1(2)** | Newman's Own #1, Costa Rica/Panama | chocolate | 18.706 | 0.012 | 15.660 | 0.013 | 38.421 | 0.013 |

| MIT # | Sample Name | Date of Analysis | Type | 206/204 | % SE | 207/204 | % SE | 208/204 | % SE | Pb (ng) (3) | Sample Wt. (g) | Pb (ppb) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | |
| T 1283 EtOH | Paria soil, surface | 5/3/07 | soil | 18.715 | 1.740 | 15.651 | 1.760 | 38.769 | 1.820 | 87.85 | 0.3317 | 264.9 |
| T 1283 HCl | Paria soil, surface | 5/3/07 | soil | 18.727 | 0.006 | 15.677 | 0.007 | 38.640 | 0.006 | 527.7 | 0.3317 | 1591 |
| T 1283 AR | Paria soil, surface | 5/3/07 | soil | 18.871 | 0.003 | 15.689 | 0.003 | 38.801 | 0.003 | 6068 | 0.3317 | 18290 |
| T 1283 HF | Paria soil, surface | 5/9/07 | soil | 19.182 | 0.011 | 15.723 | 0.010 | 38.939 | 0.011 | 248.7 | 0.3317 | 749.7 |
| | | | | | | | | | | | | |
| T 1283(2) EtOH | Paria soil, surface | 5/8/07 | soil | 18.899 | 0.319 | 15.810 | 0.333 | 39.144 | 0.332 | 16.16 | 0.3100 | 52.11 |
| T 1283(2) HCl | Paria soil, surface | 5/6/07 | soil | 18.703 | 0.004 | 15.676 | 0.004 | 38.614 | 0.004 | 479.4 | 0.3100 | 1546 |
| T 1283(2) AR | Paria soil, surface | 5/6/07 | soil | 18.880 | 0.005 | 15.693 | 0.006 | 38.816 | 0.006 | 2878 | 0.3100 | 9285 |
| T 1283(2) HF | Paria soil, surface | 5/9/07 | soil | 19.119 | 0.008 | 15.712 | 0.009 | 39.049 | 0.008 | 194.9 | 0.3100 | 628.6 |
| | | | | | | | | | | | | |
| T 1282 EtOH | Paria soil, 30 cm | 5/6/07 | soil | 18.962 | 0.017 | 15.699 | 0.017 | 38.899 | 0.017 | 136.1 | 0.3461 | 393.2 |
| T 1282 HCl | Paria soil, 30 cm | 5/6/07 | soil | 18.862 | 0.006 | 15.692 | 0.006 | 38.799 | 0.006 | 340.1 | 0.3461 | 982.7 |
| T 1282 AR | Paria soil, 30 cm | 5/6/07 | soil | 18.981 | 0.006 | 15.704 | 0.006 | 38.942 | 0.007 | 5285 | 0.3461 | 15270 |
| T 1282 HF | Paria soil, 30 cm | 5/9/07 | soil | 19.565 | 0.011 | 15.741 | 0.011 | 39.304 | 0.011 | 112.3 | 0.3461 | 324.3 |
| | | | | | | | | | | | | |
| T 1282(2) EtOH | Paria soil, 30 cm | 5/8/07 | soil | 18.895 | 0.069 | 15.699 | 0.070 | 38.821 | 0.069 | 134.1 | 0.3724 | 360.0 |
| T 1282(2) HCl | Paria soil, 30 cm | 5/6/07 | soil | 18.554 | 0.007 | 15.674 | 0.008 | 38.474 | 0.008 | 508.8 | 0.3724 | 1366 |
| T 1282(2) AR | Paria soil, 30 cm | 5/9/07 | soil | 18.913 | 0.005 | 15.708 | 0.007 | 38.895 | 0.008 | 5560 | 0.3724 | 14930 |
| T 1282(2) HF | Paria soil, 30 cm | 5/9/07 | soil | 19.260 | 0.010 | 15.712 | 0.010 | 38.963 | 0.009 | 218.1 | 0.3724 | 585.7 |

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