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## Special Issue: Reducing global mercury pollution...



## Editorial

WHY a special issue of *TOS Forum* on reducing global mercury pollution? The answer is two-fold: i) the general topic addressed in this issue was unfortunately not covered at WCSB9 (there is a minor, but critical sampling issue involved), and ii) the *timing* of the issue treated in the two first articles is *critical*. At the risk of being thought of as alarmists, the Editor and the Publisher have decided to sound a warning—by offering their help in bringing the World's attention to the first two articles published in here.

- Reducing global mercury pollution with simultaneous gold recovery from small-scale mining tailings

- Barefoot sampling in San Juan de Limay, Nicaragua: remediation of mercury pollution from small scale gold mining tailings

In the first article, senior researcher Peter Appel tells the story of the preceding decade of his work contributing towards turning around what is a rapidly developing global toxicological crisis—the global mercury pollution crisis. This is a *de facto* analogue to Rachel Carson's famous alarm published in her book *The Silent Spring*. The increasing footprints from the world's growing population has one particularly dangerous impact in the amount of mercury released to the environment, stemming from the rapidly increasing number of small-scale gold miners in Asia, Africa, Central and South America, who presently provide food on the table for 10s of millions of very poor households. Small-scale artisanal gold miners use vast amounts of mercury to capture the gold, and much of this mercury is released directly to the environment. A large part evaporates to the atmosphere and the rest is transported downstream in rivers, ending up in the oceans. The amount of mercury released by small-scale gold miners alone is phenomenal: an estimated 3000 tons of mercury annually, a staggering 37% of the total world mercury emissions. A vast proportion enters the food chain in fish and

sea mammals, as well as in rice polluted by spillage waters which enter irrigation pathways. Human consumption of polluted fish and/or rice already has a severe impact on human health, and this will have even greater consequences if the current situation is not changed radically and rapidly.

The second article deals with the challenging tasks involved in sampling for gold and mercury analysis under severe economic, technology and logistic constraints because of extremely low analyte concentrations and the resultant overwhelming distributional heterogeneities. This combination of highly adverse conditions is pretty much unique, but, here, it is shown how to succeed even so.

As a didactic addendum, the last two articles also treat the issue of optimal sampling of *hidden heterogeneity*, exemplified by lots composed by animal feed and biomass. These articles form a topical complement to the two major features, and we do not ask forgiveness for having published them before; every added momentum with which better to present and illustrate the first two features is important.

This Special Issue forms a dedicated vehicle directed at a technology-poor application arena, which is of equal interest as the much more prominent large-scale (or super-scale) major commodities mining and minerals extraction sectors in general. These are characterised by abundant up-to-date high-technology and a positively over-abundance of economic means in comparison (iron, base metals, precious metals). "Interestingly", the sampling *principles* involved in the very different industrial application sectors in this Special Issue are in fact *identical*, a point well worth emphasising for the readers of *TOS Forum*.

The Mercury Crisis is another "silent crisis" mostly unknown to the global population, even though a much larger fraction is actually falling victim to it. PLEASE READ THE TWO MAIN ARTICLES MOST CAREFULLY!



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# Reducing global mercury pollution with simultaneous gold recovery from small-scale mining tailings

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The increasing population on planet Earth has many impacts—one is a strong influence on the amount of mercury released to the environment. The worst influence stems from the rapidly increasing number of small-scale gold miners in Asia, Africa, Central and South America, who presently provide food on the table for tens of millions of households. Small-scale gold miners use vast amounts of mercury to capture the gold, and much of this mercury is released directly to the environment. A large part evaporates to the atmosphere and the rest is transported downstream in rivers ending up in the oceans. The amount of mercury released is phenomenal: an estimated 3000 tons of mercury is released annually by small-scale gold miners alone, a staggering 37 % of global mercury pollution. A vast proportion enters the food chain in fish and sea mammals, as well as in rice polluted by spillage waters which enters irrigation pathways. Human consumption of polluted fish and/or rice already today has a crippling impact on human health in some countries, and this will have even more severe consequences if the current situation is not changed radically and rapidly. It is of particular concern if mercury-intoxicated women become pregnant, because the foetus extracts mercury from the mother. The human foetus is much more sensitive to mercury intoxication and thus has a high risk of being born with brain damage as well as physical disabilities. Over just one generation this will cause reduced intelligence for exposed children. Through such organisations as the United Nations Environment Programme (UNEP), the World community has become acutely aware of the rapidly increasing global mercury pollution. The treaty designed to protect human health and nature, the “Minamata Convention” has today been signed by the majority of world countries. Signatory countries are hereby obliged to start initiatives to reduce and even stop mercury use. This grim outlook has prompted a group of international concerned researchers and small-scale gold miners from Philippines to start teaching small-scale gold miners to work without the use of mercury and simultaneously to find ways to clean mercury-polluted gold mining tailings, which are one of the main polluting agents. This latter will have an immediate positive economic effect for the communities involved, which should be a significant motivation to change to non-mercury recovery processes. We here describe the specific technological drive to be able to go mercury free.

**Keywords:** mercury flour, mercury pollution, gold loss, cleaning tailings, small-scale mining, artisanal mining, state batteries

## Introduction

Mercury pollution has become a serious problem for life on planet Earth. Through such organisations as the United Nations Environment Programme (UNEP), the World community has become acutely aware of the rapidly increasing global mercury pollution. The treaty designed to protect human health and nature, the “Minamata Convention” has today been signed by the majority of world countries. Signatory countries are hereby obliged to start initiatives to reduce and even stop mercury use. Small-scale gold mining accounts for 37 % of global mercury pollution. Millions of poor people resort to this type of mining as the only way of sustaining their families. A large part of the mercury used in the final step of gold extraction ends up as mm-sized droplets in dumps (tailings) from which mercury slowly evaporates to the atmosphere. These droplets make up what is referred to as *mercury flour*, which is a main contributor to the global mercury pollution. The flour also

contains large amounts of gold. This paper describes a road map to clean up mercury from tailings with dual benefit: environmental and economic. The gold in the mercury flour, will cover most, if not all, cleaning-up costs and may even provide a handsome profit in addition. Possible ways of safe long-time storage of the recovered mercury are also outlined below.

## Background

Global mercury pollution is a fundamental problem for life on Planet Earth since, in addition to causing other serious health problems, it affects foetal brain development.<sup>1</sup> This creates major learning problems for generations to come all over the World. The global mercury pollution affects millions of poor people in Southeast Asia, Africa, Central and South America who, in order to provide a livelihood, resort to gold mining using primitive equipment and low tech approaches. The final step in the gold extraction process is relying on mercury to

capture the numerous small gold grains in pulverised hard rock or river sediments. Carried out for hundreds of years in the past, this type of local gold mining earlier only caused relatively minor mercury pollution to the planet. However, the dramatic population increase during the last century has caused a massive increase of this pollution. While we cannot easily provide immediate alternative sources of income for millions of small-scale gold miners, we **can** influence the prevalent way of thinking about how to extract gold in an equally efficient, mercury-free approach and, furthermore, simultaneously be able to show an avenue to clean up the hundreds of thousands of heavily polluted mining dumps that litter Planet Earth.

## Small-scale gold mining

Small-scale gold mining, also termed artisanal gold mining,<sup>2</sup> is often caricatured as “three men and a wheel barrow”. In Africa, Southeast Asia, Central and South

America, it is driven by the abject poverty of millions of people, and there are inherent advantages as well as disadvantages. On the one hand, small-scale mining reduces urbanisation and provides food for millions of families in rural areas, but on the other, it creates massive mercury pollution with extremely serious consequences for humankind in generations to come.

Gold occurs in mineralised hard rock as  $\mu\text{m}$  to  $\text{mm}$ -sized grains, either as pure grains but more often enclosed in other minerals, and as free gold in river sediments, in *placer deposits*.<sup>3</sup>

Small-scale gold mining is carried out from pits, shafts or tunnels. The ore is crushed and further milled down to  $\text{mm}$ -sized powder in order to *liberate* the gold grains from their host minerals. This is a difficult process if the goal is for it to go to completion, but usually a certain fraction of liberated gold is acceptable enough at this minimum technology level. Depending on country, milling is performed in many different ways. In Southeast Asia and parts of Africa, metal drums filled with hard metal rods or balls are frequently used (Figure 1), whereas in Central America other methods are often in use. In Nicaragua, for example, four big stones slowly churn around in a cement cavity whereby the crushed ore is milled (Figure 2).

The next step is to *concentrate* the heavy minerals, among these, gold. The gravitational methods used vary greatly from simple to complex. The former, such as panning, are the most common, but more complex methods generally result in higher yields. The outcome is a mineral concentrate comprising a variety of heavy minerals including gold.

The following step is to *separate* gold from the other heavy minerals. This is more often than not done by adding mercury to the concentrate (Figure 3). Mercury has the capacity to *amalgamate* elements such as gold, silver and copper into an *alloy*. The key next step is to burn off the amalgam so that mercury evaporates and gold is left behind. This simple process does not require much investment in equipment, but it is extremely toxic because of a total lack of suitable protection technology; for artisanal mining there is absolutely not the economic ability to even contemplate introducing such protection. Increased awareness is one of the objectives for the drive described in this communication.

A slightly more advanced method consists in the miners adding mercury at the



**Figure 1.** Efficient artisanal processing plant (Sudan) with mill and gravitational processing of pulverised gold ore. The ore is milled in the drum in the background, after which the fine-grained ore is passed through a system of sluices, which concentrates the heavy minerals, including gold.



**Figure 2.** Low tech milling (rastra) with mercury addition (Nicaragua). The boulders mill around slowly for a couple of hours. When milling is complete, the cavity is cleaned of milled ore and mercury amalgamated with gold. The flour floats away.

milling stage. This saves time and work, since this also takes care of good mixing of mercury with the pulverising gold ore, thereby creating the desired gold amalgam very efficiently. When the amalgam is heated mercury evaporates, thus being released to and polluting the environment, leaving the gold behind (Figure 4).<sup>4</sup> The waste, called

tailings, is simply dumped. This procedure is used by millions of artisanal miners.

Besides the very serious atmospheric mercury pollution, from the point of view of the extraction technology itself there is also a serious disadvantage in the form of so-called “mercury flour”, a product of the mixing which is discussed more in the following section.

### Mercury flour

During milling and hand mixing, part of the mercury is transformed into  $\text{mm}$ -sized droplets referred to as mercury flour (Figure 5). This can float on water because the individual droplets are very small. Many of the droplets may float close together but they never coalesce, neither do they coalesce when dispersed in milled gold ore. Mercury flour disperses into the environment and so is lost for the miners. The remaining flour is scattered in the tailings and is therefore, likewise, unattainable to the miners.

Mercury flour is one of the main contributors to global mercury pollution. It constitutes one of the most severe threats to the environment and to the health of us all on Planet Earth. Mercury flour in the tailings gradually evaporates. Through wind, the vapour is actually incrementally spread all over Planet Earth. Rain brings the atmospheric mercury to the surface of the earth where it enters the drainage system. In the rivers and in the soil metallic mercury is changed into methylated mercury, which



Figure 3. Hand-mixing mercury with milled gold ore (Tanzania).



Figure 4. Gold has been concentrated and smelted to a small bead.

enters the food chain. The mercury is thus not only a health risk in the countries where small scale gold miners release it to the atmosphere, but it very quickly creates a global problem.

“Interestingly” (see further below) this is not the only issue associated with mercury flour. Another, a potentially positive issue, is that it contains large amounts of gold. Such gold, if realised, has such a high value that this could provide quite a substantial lift to the miners’ livelihood. Reaping this gold amounts to a win-win achievement for all.

A prime question is why droplets of mercury flour formed during gold extraction do not coalesce as they otherwise normally do? Per Møller, professor in

metallurgy at the Danish Technical University in Copenhagen, through a series of dedicated tests, has shown that flour droplets are coated with an oxide film during milling and stirring. It is this thin coating that inhibits droplet coalescence. Furthermore, it was also found that by adding small amounts of a certain non-toxic organic compound, the droplets lose their mercury oxide coating and so again coalesce more easily.<sup>5</sup> This organic compound is aptly named “DETOX”; Professor Møller’s process is currently being patented.

### Capturing mercury flour

At first sight, it would seem an insurmountable task to recover the immense number



Figure 5. Mercury flour (droplets) in a spoonful of tailings (Philippines).

of very fine droplets scattered throughout all the innumerable local artisanal tailings from small-scale gold mining, on several continents; the logistics appear completely overwhelming. There is a way, however, ...

The first attempt at this was carried out in 1894 by the Australian Government during the major gold rush in Western Australia.<sup>6</sup> The Australians termed the new facilities “State Batteries”, but they apparently soon went out of use. The next attempt was in 2011 where a research group supported by the Benguet Federation of small-scale miners in the Philippines, the Sumitomo Foundation (Japan) and the Geological Survey of Denmark and Greenland (Denmark) improved the working processes inherent in the State Batteries.<sup>7</sup> The resultant facility is now known under the name “Peter Plates”, a name coined by the Benguet Federation of small-scale gold miners.

### “Peter Plates”

“Peter Plates” consist of a number of copper plates stacked at an inclined angle, one plate on top of the next in a continuous flow train (Figure 6). Before use, the plates are thoroughly cleaned with nitric acid, after which they are treated with metallic mercury, which forms a thin coating of copper amalgam. Tailings with mercury flour are now slowly flushed down the plates. On contact with the copper amalgam, the flour sticks to the plate and is so *captured*. If the first plate does not retain all droplets, subsequent plates come into play in a classic cascade process. When the plates are at capacity, the amalgam is scraped off and the process can easily be repeated.

After processing, the amalgam is heated and the vapour captured in a cold trap. Testing carried out in the Philippines in 2010 and 2011 proved that this method can extract up to 60% mercury from tailings.<sup>7</sup> Although this is promising by itself,



**Figure 6.** Prototype of “Peter Plates” in action (Philippines). Tailing slurry from the tub is passed over the plates in succession.

reflecting a capacity of only about 100kg tailings processed per hour, when considering the millions of tons of polluted dumps that today wait to be cleaned, a long-term viable solution still would appear far away.

### Large-scale recovery

Thus, the efficiency of “Peter Plates” to capture mercury is promising, but their capacity is currently not at a level to make a significant quantitative contribution to the clean-up that is needed in order to reduce the many tons of tailings in existence already.

As a signatory of the Minamata convention, the Danish Government feel obliged to make an effort to contribute to reducing the global mercury pollution. Consequently, in 2015 and 2016 the Ministry of Environment and Food awarded a group funding for two tests to explore if more efficient ways to clean-up mercury-polluted tailings from small-scale gold mining. A consortium—composed of two Danish companies, Elplatek A/S and AppelGlobal, together with Oro Industries, California—obtained funding for a two-year feasibility project aimed at *increasing the capacity* of mercury recovery from tailings. The first work was carried out in Northern Nicaragua in 2015 and 2016.<sup>5</sup> Further work, but on a smaller scale, carried out in Peru was financed by the US Department of State. Future test work on a larger scale is planned for cleaning the River Naboc in Mindanao, Philippines. This river has drained a rich gold occurrence (Diwalwal), which has been mined by small-scale miners using mercury for well over 50 years.



**Figure 7.** Mercury Recovery Plant (MRP) being loaded by tailings (Nicaragua).

### A mobile, high-capacity processing station

In 2013, the Californian company Oro Industries invented a Mercury Recovery Plant (MRP; Figure 7). It is a large mobile machine on wheels, towable by truck and thus suitable for reaching tailing dumps spread across large geographical areas. It processes heavy mineral concentrates through a series of cyclones with the concentrate from each cyclone directed on to the next. The concentrates from the two first cyclones are directed into a centrifuge, and the concentrate here from is finally directed into the last cyclone. The process is described in detail in Reference 5. One MRP unit has a capacity of 15–20 tons per hour. Based on this, each plant produces a concentrate in the order of 10–20kg heavy minerals per hour, including mercury and gold. A combination of MRP and Peter Plates increases efficiency significantly; the latter hooked on the MRP outlet, extracts mercury flour and gold from the heavy mineral concentrate as shown in (Figure 8).

The capacity of the combined MRP and Peter Plates can extract auriferous mercury from 20 tons per hour, 24/7. A rough estimate of the total tonnage of current tailings produced per day will require in the order of 5000 processing plants to travel Africa, South and Central America and South-east Asia to just to keep up with the daily production. It will thus require many more processing stations if the target is to clean the tailings produced previously. However, the thrust of the present communication is

that the necessary dual-purpose technology is at hand, and that the still impressive clean-up intensity, can in fact be tackled—it is simply a matter of scaling-by-numbers of the combined MRP-Peter Plates units.

### Sampling—a critical success factor

In order to benchmark the combined MRP–Peter Plates process, it is necessary to assess the efficiency, and how much mercury and gold in total can be extracted from a set of selected test tailings by the developed processing system. For this it



**Figure 8.** Mercury Recovery Plant (MRP) hooked up with Peter Plates (Nicaragua).

is necessary to get a reliable assessment of the overall mercury and gold content before processing. The specific sampling issues involved are far from standard. How does one obtain a reliable figure for mercury and gold content in a typical, say, 10-ton tailings stock, in which both elements are very irregularly distributed? In fact the average tailing concentration is at the extreme low end of trace levels for both elements. Due to this extreme heterogeneity, there are fewer more challenging sampling scenarios, when almost all levels of sampling technology and equipment is virtually absent. “Barefoot sampling” was what was needed,<sup>8</sup> but with the exact same stringent objective—obtaining a *reliable* estimate of the concentration levels present in the seven test piles selected. Tailings for this test were collected from a number of different small-scale mining sites where the gold ore varied significantly both w.r.t. ore type, mineralogy and contents; thus, a wide span of target lots could be expected.

Under such difficult field conditions the best way to achieve this sampling

goal is by so-called incremental *composite sampling*, a technique developed at research institutes and private companies over many years. The specific approach used during the phases of this project, carefully crafted to comply 100 % with the demands of the Theory of Sampling (TOS). The critical primary sampling procedure is comprised of ~2000 increments (each ~100 g) from each test tailing (ranging from 4 ton to 21 ton in weight), which, when aggregated, resulted in primary composite samples of the order of 200 kg. After these documented representative samples were collected in the field, they were subsequently further mass-reduced both in the field (field site, Nicaragua) as well as in the laboratory (GEUS, Denmark), in order to arrive at reasonably sized aliquots for analysis for mercury and gold, which was subsequently carried out in a commercial laboratory (Actlabs Canada). The full “from-lot-to-aliquot” sampling pathway is described in detail by Esbensen and Appel,<sup>8</sup> together with a complete reference curriculum.

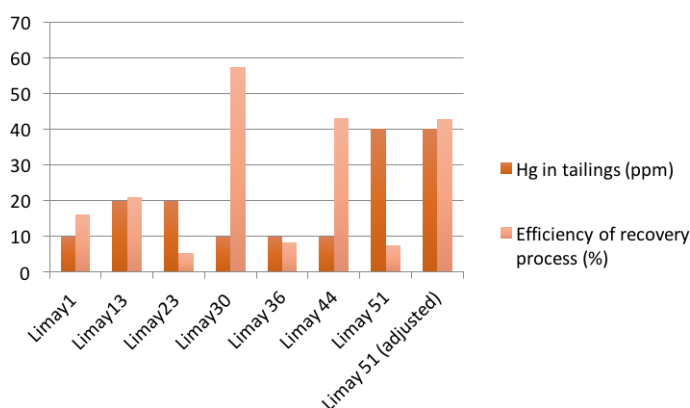
### MRP-Peter Plates efficiency testing

Seven test tailings were sampled in full TOS-compliance from lot-to-aliquot, and the resulting analytical results are presented in Tables 1 and 2. The overall average content of mercury ranged from 0ppm per ton to 40ppm per ton, and for gold the range was from 1.15g per ton to 13g per ton. These tailings were subsequently processed by the local experimental MRP-Peter Plates setup in a feasibility study.<sup>8</sup> The recovered amalgam and mercury (Figure 9) was scraped off the plates and weighed. The amalgam and mercury were distilled, and the condensed mercury weighed together with the gold. This allowed estimation of the efficiency of the MRP + Peter Plates combination, which is listed in Table 1.

Gold recovery varies widely from one tailings pile to the next, with no immediate observable pattern. A likely reason is that part of the gold is free gold in some samples, and so is easy to recover by the Peter Plates, whereas in other samples gold is still found embedded in other minerals.

Table 1. First feasibility results: mercury recovery efficiency ranges from 5% to 57 %.

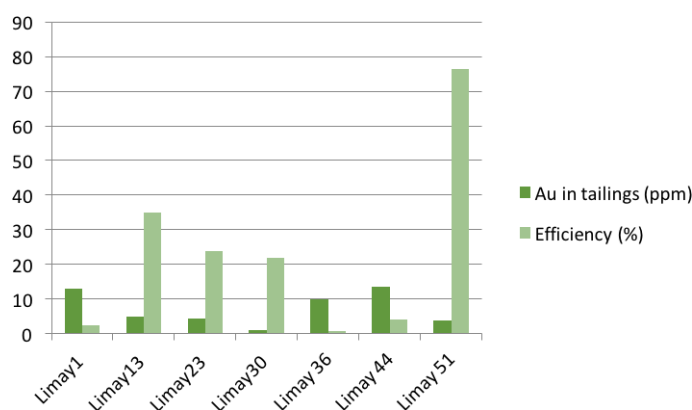
	Municipality	Location	Hg in tailings (g/tonne)	Hg recovery (g/tonne)	Efficiency (%)
Limay 1	San Fransisco	El Nancital Rastra #1	10	1.6	16
Limay 13	San Fransisco	Rastra las Agua	20	4.23	21.1
Limay 23	San Juan De Limay	El Portillo	20	1.05	5.2
Limay 30	San Juan De Limay	El Morcillo	10	5.74	57.4
Limay 36	San Fransisco	El Nacital Rastra #2	10	0.84	8.4
Limay 44	San Fransisco	El Nacital Rastra #3	10	4.317	43.2
Limay 51	San Fransisco	Rastra las Agua	40	2.99	7.5
Limay 51 (adjusted) <sup>a</sup>	San Fransisco	Rastra las Agua	40	16.99	42.8



<sup>a</sup>It proved difficult to purchase DETOX in Nicaragua at short notice. Therefore, enough for one test was shipped to Managua from Copenhagen. The heavy mineral concentrate from sample Limay 51, which previously had passed over the Peter Plates, was soaked overnight in a solution of the organic compound. When passed over the Peter Plates again, the results were very promising; the efficiency of mercury recovery increased from 7.5% to 42.8% (see Table 1). This demonstrates the potential of adding DETOX to future operations.

Table 2. First feasibility results: gold recovery efficiency ranges from 1% to 76%.

	Municipality	Location	Au in tailings (g/tonne)	Au recovery (g/tonne)	Efficiency (%)
Limay1	San Fransisco	El Nancital Rastra #1	13	0.32	2.4
Limay13	San Fransisco	Rastra las Agua	4.99	1.75	35.1
Limay23	San Juan De Limay	El Portillo	4.41	0.36	23.8
Limay30	San Juan De Limay	El Morcillo	1.15	0.25	21.9
Limay 36	San Fransisco	El Nacital Rastra #2	9.87	0.09	0.9
Limay 44	San Fransisco	El Nacital Rastra #3	13.5	0.57	4.2
Limay 51	San Fransisco	Rastra las Agua	3.95	3.02	76.5



Such gold is unrecoverable although it features in the chemical analyses of the tailings, undoubtedly contributing significantly to the very wide recovery efficiency range observed. At present the relevant mineralogical information is not available for the first test tailings made available for the present project. As always when dealing with gold ore mining and processing, there is a critical need for detailed mineralogical characterisation of the samples and sub-samples in addition to the *in toto* chemical and amalgam-capturing data.<sup>9</sup> While lamentable, this uncertainty was unavoidable with the kind of funding and budgets available for the present pre-feasibility survey. However, the layout for a fully comprehensive feasibility study is now available. This is the other important take-home message from these scanty, but very promising first results.

### Fate of recovered mercury

When the combination MRP + Peter Plates goes into production across three continents, the amount of mercury recovered will reach many tons per year. This raises the important question about the destiny of this mercury. Fortunately, there are several research groups currently working on this problem, which is not only pertinent with respect to gold mine tailings but also for

cleaning up other sites with large mercury spills. Two of these are:

- i) Nomura Kohsan Co. of Japan ([www.nkcl.jp](http://www.nkcl.jp)) which has constructed a solidification system which provides safe, long-term storage of mercury. The company has expressed interest in constructing a portable processing plant that can follow the MRP + Peter Plates activities.
- ii) Batrec Group in Switzerland ([www.batrec.ch](http://www.batrec.ch)) has to date solidified more than 600 tons of metallic mercury into the naturally occurring cinnabar (HgS). The cinnabar is stored in German salt mines.

### Conclusions

These first foray studies have shown that the combination of MRP + Peter Plates is able to recover substantial amounts of mercury from the numerous tailings from small-scale gold mining that litter Southeast Asia, Africa, Central and South America; the present results point to that crushing and milling efficiency is a critical success factor for increasing the fraction of gold ultimately recoverable. As MRP-Peter Plates units go into production, re-processing of tailings will increase recovery substantially. Minor technical improvements may likely be added as well in the future.

It is clear that local adjustments will be needed in order to be able to characterise

local tailing compositions more comprehensively to be able to compensate for differences in mineral composition of the tailings from one area to the next, especially regarding the degree of liberation of the most prominent amounts of gold. It would be highly advantageous to be able to use fast “barefoot” mineralogical assessment methods to assess gold particle liberation, i.e. allowing artisanal miners definite information as to whether the tailing gold has been sufficiently crushed to allow complete liberation. While the gold liberation issue has been the target of an enormous R&D effort in the mining industry for numerous decades, an easy approach has not yet emerged. Should not the gold mining industry be able to divert just a minute fraction of its enormous revenues to this low-tech challenge, and thereby help millions of starkly impoverished artisanal mining communities in addition to contributing towards the Minamata convention goals as well? It will also likely be important to observe and compensate appropriately for the characteristics of local climatic conditions regarding whether the climate is humid or dry.

### Fully comprehensive feasibility study

The major remaining question concerns why some tailings are more amenable to





**Figure 9.** Large amount of mercury captured on Peter Plate (Nicaragua). The plate is about 20 cm wide.

mercury extraction than others? First generation mineralogical investigations have not provided a clear answer,<sup>4</sup> but to date it has not yet been possible to carry out more comprehensive studies due to lack of appropriate funding. The specific comminution/crushing/milling approach further developed, and attendant problem-dependent processing times, will likely also play an important role in increasing the degree of recovery.

It is hoped the present report and its companion<sup>8</sup> will provide a fully satisfactory foundation for future funding. What is clear already is that the amount of gold recovered together with mercury will more than likely cover the costs of the clean-up operations.

Legal problems are also likely to arise. As of now, ownership of old and highly polluted tailings is not particularly obvious, and there is understandably a certain lack of willingness to claim ownership. One expectation is that when the MRP + Peter Plates clean-up system begins to take effect, and so demonstrates an efficient way of recovering gold and thus to make money from what was before a hopeless prospect, the situation will likely change with declarations

from many potential owners. There may, therefore, paradoxically turn out to be significant potentially troublesome legal issues surrounding this aspect.

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# Barefoot sampling in San Juan de Limay, Nicaragua: remediation of mercury pollution from small scale gold mining tailings

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The increasing population on planet Earth has many impacts—one is a strong influence on the amount of mercury released to the environment. Burning of coal in power plants, particularly in China, has tripled several times during the last century and so has the use of coal for cooking for the ever-increasing population of India and elsewhere. These sources account for the second largest release of mercury to the environment. But the worst sinner is the rapidly increasing number of small-scale gold miners in Asia, Africa, Central and South America, who presently provide food on the table for 10s of millions of households. Small-scale gold miners use vast amounts of mercury for capturing the gold and much of this mercury is released directly to the environment. A large part evaporates to the atmosphere and the rest is transported downstream in rivers ending up in the oceans. The amount of mercury released is phenomenal, an estimated 3000 tons of mercury is released annually by small-scale gold miners alone. A vast proportion enters the food chain in fish and sea mammals, as well as in rice polluted by spillage waters which enters irrigation pathways. Human consumption of polluted fish and/or rice already today has a very severe impact on human health, and this will have even more severe consequences if the current situation is not changed radically. It is of particular concern if mercury-intoxicated women become pregnant because the foetus extracts mercury from the mother. The human foetus is much more sensitive to mercury intoxication and thus has a high risk of being born with brain damage as well as physical disabilities. Over time this will cause reduced intelligence for exposed children in the next generation. This grim outlook has prompted a group of concerned researchers to teach small-scale gold miners to work without the use of mercury and simultaneously to find ways to clean mercury-polluted gold mining tailings, which are one of the main polluting agents. Here we report on one specific part of this endeavour where the Theory of Sampling (TOS) was needed in order to secure reliable estimates of gold and mercury contents in dispersed mining tailings.

## Background

Mercury pollution constitutes an environmental time bomb of potentially alarming proportions. The two main sources of global mercury pollution are small-scale gold mining (SSGM) and coal burning in power plants, as well as domestic cooking in developing countries.<sup>1</sup> The massive release of mercury to our environment will cause a serious global health issue for generations to come; the possibly worst scenario is that humanity will experience a dramatic decrease in intelligence in future generations. Small-scale gold mining is a low-technology, poverty-driven way for many tens of millions of people to provide for their daily needs.

Small scale miners crush and mill gold ore together with mercury. The mercury captures the gold by forming an *amalgam*. This is subsequently heated in open vessels whereby mercury evaporates and the gold is left behind for economic recovery. This is a technologically simple and very easy processing method that does

not require any noticeable investment in equipment and in this way “nothing goes to waste” of the precious gold. Unfortunately, it is the key process element of milling gold ore together with mercury that creates the serious health problem described. During milling a large part of the mercury is ground to small drops called *mercury flour*.<sup>2,3</sup> Mercury flour cannot coalesce and can therefore not be recovered by the miners, but ends up in tailings (waste dumps from SSGM operations). This mercury loss is doubly unfortunate—both for the miners and for society. Not only is mercury flour harmful to the environment, but it also constitutes a financial problem since it still contains appreciable amounts of gold that cannot be recovered with the simple methods employed and thus reduces the economic viability of mercury-based SSGM. Over time mercury flour in SSGM tailings will *evaporate* or gradually be *washed* into the drainage system, ultimately ending up in the world’s oceans from where the evaporated mercury will be distributed over the entire

planet. The part of mercury that ends up in rivers, lakes and oceans will be transformed to the compound *methylated mercury*, or “organic mercury”, which readily enters the food chain(s), where it will be bio-magnified, resulting in high concentrations of toxic mercury compounds in top-level fish and sea mammals. Polluted fish are unfortunately consumed by humans—this is the root cause of the very serious health problems that have been called the impending global mercury disaster.<sup>4</sup>

One principal way to *mitigate* this mercury disaster is to clean the hundreds of thousands of SSGM tailings containing mercury flour which are littering large parts of South-east Asia, Africa, Central and South America. If an efficient, inexpensive low-tech method can be found, it will benefit not only the global environment and health status of millions, but will at the same time also produce considerable amounts of gold in quantities that may well cover the costs of modified processing methods, and eventually result in more profitability for the SSGM communities.

## Barefoot sampling in San Juan de Limay, Nicaragua

Experiments to extract mercury flour from polluted tailings by alternative, mercury-free approaches have been carried out in the Philippines<sup>2</sup> and are presently being tested in Nicaragua. In Nicaragua, tailings from a number of different SSGM processing sites were subjected to the most advanced alternative recovery process currently available. The experimental tailing lots varied from 4 tons to 15 tons. The first critical step in these experiments was to obtain *reliable* estimates for the average mercury and gold contents in the tailing heaps available for this experimental campaign; these concentrations are known to be of the order of 2–15 ppm. This is no small challenge in a setting where tailings typically are of the order of ~10 tons, and everything has to be carried out *manually*.

It is critical to follow the tailing mercury and gold throughout the full multi-stage recovery process and to be able to carry out a complete metallurgical accounting. For this the original Au and Hg concentrations in the primary lots are the key information needed, as are the sampling processes employed for dealing with these very low abundances. We here report on *barefoot sampling* in which application of the principles of the Theory of Sampling (TOS) was *de rigueur*, but with only DS 3077 and willing, able hands available. For environmental and individual miner health reasons, it is even more important to keep track of the Hg concentrations at all sampling stages as well, which poses its own specific problems.

### 2015 Nicaragua field experiment—prospects

During spring and autumn 2015, feasibility tests were out carried in Nicaragua to estimate the efficiency of extracting mercury flour from SSGM tailings. To the degree this is feasible, and to the degree it can be successfully recovered at a sufficiently high recovery rate (~75%), this will be a significant driver to allow SSGM collectives to accept the alternative process.<sup>†</sup>

Investment capital for this type of local mining reclaiming is available a.o. from developed nations' development funds, and there is also direct commercial potential. The alternative process will be profitable, at *assumed* recovery rates larger than 75%, as long as tailing concentrations are above 3 ppm. The same process will

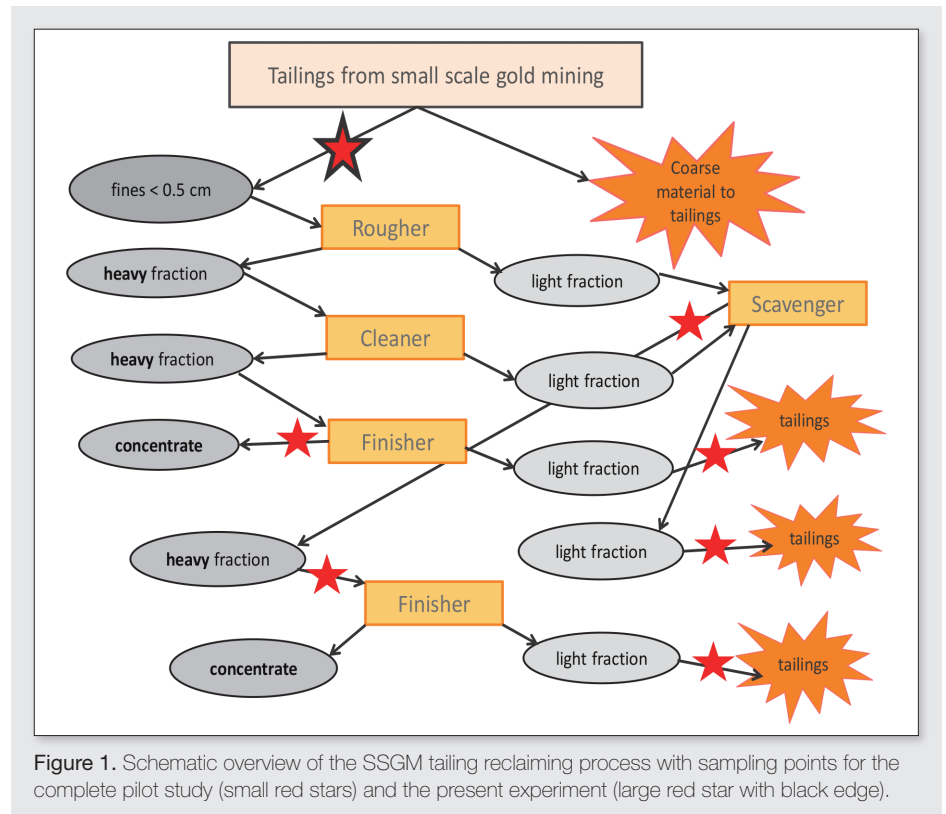


Figure 1. Schematic overview of the SSGM tailing reclaiming process with sampling points for the complete pilot study (small red stars) and the present experiment (large red star with black edge).

remedy the otherwise continuing SSGM tailings pollution—a double whopper—and all essentially with barefoot technology!

### Field experiment design and sampling requirements (TOS)

Tailings for the experiments were gathered from five different SSGM processing sites. The tailings were selected to represent different types of gold mineralisations and thus different general compositions with presumably differing *processabilities*, and milling efficiency was indeed observed to vary widely locally. The overall mercury contents would therefore be expected to vary significantly between different tailings (corroborated by the analytical results, see Table 1). Tailing lot masses varied from 4 tons to 21 tons with an average of ~10 tons.

The crucial first step is to establish the *average* mercury content of each tailing lot with absolutely no primary sampling bias allowed because of the ultra-low grade levels present. For this reason, sampling expertise in the form of the TOS was called upon. It is equally important to be vigilant with respect to the representativity of all subsequent field and laboratory mass-reduction steps. From original tailing size to analytical mass, sampling rates are of the order of 1:10<sup>7</sup>. The principles of TOS

have to be upheld scrupulously along the **entire** lot-to-analysis pathway. While this is trivial in most scientific, technological and industrial contexts in the developed world, the present project poses a highly challenging twist: almost everything must be carried out *manually*—which calls for *barefoot field sampling* (for the first two stages), but in the subsequent laboratory mass-reduction stages some innovative approaches were also called upon, as described below.

We welcome this challenge—how better to contribute to helping tens of millions of SSGM families with a life-threatening mercury danger?

### SSGM tailing recovery process—a brief

Tailings were *scooped* into a drum, which selects and discard >0.5 mm material. The resulting fines are directed into a train of three spiral concentrators, which separate heavy from light minerals. These are termed “Rougher”, “Cleaner” and “Finisher”, respectively, in Figure 1. The heavy fraction from each spiral is directed to the next spiral. The light fraction from “Rougher” and “Cleaner” is directed to a centrifuge, termed “Scavenger”. The light material from here is directed to tailings while the heavy fraction is directed to the



Figure 2. First stage in the SSGM tailing reclaiming process feasibility project, initial particle size screening.



Figure 3. Halfway through the intensive task of moving a complete original lot one shovel at the time, taking great care to extract an increment from each, as detailed in Figure 4.

“Finisher”. The heavy fraction from the “Finisher” is directed to a stack of copper amalgamated plates “Peter plates”,<sup>2</sup> which finally capture the mercury flour and free gold particles to be reclaimed. Figure 2 shows the first stage of the full feasibility study (drum loading for initial particle size screening). Below we are exclusively interested the critical primary sampling from the original tailings: how to get a documentable representative analytical estimate of the average gold grade?

### Primary lot sampling—the crucial stage

Manipulating lots of the size of 5–15 tons is usually not a problem when the appropriate industrial equipment is at hand, e.g. front-loaders, bobcats or the like—of which there most emphatically are **none** available to very poor artesian mining collectives. But able hands, picks and shovels are in abundance. It was decided to follow the principle: “move the original lot 10m to the right” and perform process sampling along this 1-D transportation stage. Thus each tailing dump was transported manually, one shovel-full after another in order to facilitate sampling, Figure 3.

Figure 4 shows this primary composite sampling *in extremis*: the material in each shovel-blade (approx. 5kg) is intercepted by a scoop of approximately weight 100g. For an average 10-ton primary lot size, this translates into 2000 increments (each of ~100g), by all standards an overwhelming coverage of each original lot with a solid guarantee for compliance with the

Fundamental Sampling Principle (FSP). The resulting composite primary sample weighs ~200kg. This material was subjected to forceful mixing before further sub-sampling, based on the abundant man-power available.

Figure 5 shows how the next sub-sampling stage was executed: *each* 200kg primary sample was passed through a riffle splitter, in a series of 50/50 split sessions until the sub-sample mass had been reduced to ~1–2kg, which was the sample size subsequently transported to GEUS, Denmark for further processing and preparation for analysis.

### Slurry sub-sampling in the laboratory

After processing all primary tailings in the manner illustrated, quantitative analysis was carried out on a selected set of seven primary samples (project financing was at the time of the analysis also at a decidedly “barefoot” level). These samples were not easy to process, however, as they were all *slurries* and with very different Au and Hg contents. Slurry sampling is not easy under any circumstance, but especially not when stringent counter-volatility demands are to be upheld. Also, sub-sampling, although here carried out in a well-equipped



Figure 4. Incremental sampling from each shovel used to transport all original lots, see Figure 3.



**Figure 5.** Loading the project riffle splitter (kindly provided by GEUS). Sub-sampling is made effective by the fact that the sample to be split does not need to be split all in one, but can be subjected to riffle-splitting in an intermittent loading process.<sup>7</sup>

laboratory (GEUS, Copenhagen), had to be performed with procedures that potentially can be carried out under the relevant ambient field conditions in Nicaragua.

Due to the severe risk of segregation (free Au particles, mercury flour), handling the slurry samples became a critical issue, not easily tackled with the standard riffle splitters at hand. It is critical to *counteract* any-and-all segregation effects present in the sub-sampling procedure employed (while these effects *may* be small, intermediate or large, they are never absent and the only responsible approach is to assume such effects are always significantly present). For this reason, conventional riffle splitting could not be used. A better way was required, illustrated in Figure 6.

A novel twist had to be devised: after vigorous and extensive mixing, the entire 1–2 kg slurry samples, which came in tightly sealed but otherwise conventional plastic bottles or bags, were stored in a freezer (–16°C) for 24 h, sufficient for the entire content to freeze solid. The “splitting” was then effectuated as a two- or three-step *longitudinal* sectioning of the solid bottle or bag content, see Figures 7 and 8. In this way **any** residual segregation affecting the *vertical* container contents in their slurry state was sampled in a fully representative fashion, while maintaining quite effective



**Figure 6.** Two types of slurry sample containers as received from Nicaragua, plastic bottles and bags. The photo shows the frozen versions after 24 h in a freezer at –16°C, ready for sub-sampling, see Figures 7–8.

sampling rates of the order of 1 : 10 at this stage. This sub-sampling technique is only dependent on the cohesiveness of the frozen solid w.r.t. the thickness of the slice cut with the diamond saw (and subsequently with a hobby knife).

From the primary sampling stage all the way through the penultimate sample shipped off to the analytical lab, the critical success factor was counteracting segregation. Even the commercial, accredited ACTlab analytical laboratory was directed to document the in-house sub-sampling employed with reference to DS 3077.<sup>8,†</sup>

All 34 final sub-samples were of analytical mass ~12 g when shipped off for multi-element analysis (standard economic geology element suite *plus* Au and Hg) at Actlabs Labs, Canada.

### Sampling perspective

The original lots were on average of a size corresponding to 10 tons. The average assumed Au-grade in the mine tailings was of the order of 2–15 ppm, based on the best local mining experience available. The lot material, earlier mining tailings, is crushed to an average grain size diameter of 50 µm, but the tailings have never been subjected to mixing or blending to any extent. It follows that such very low-grade gold concentration carriers (flakes, particles) must be present in an exceedingly irregular spatially heterogeneous

distribution pattern, i.e. for the present study the lot distributional heterogeneity is extreme. It would be close to a miracle if standard haphazard scooping of a small primary sample from 10 tons, archetype grab sampling, could ever be representative.<sup>8</sup> Only a very thorough composite sampling can be accepted. In order to meet these hard demands, the “field-to-analysis” pathway consisted of i) primary sampling (extremely effective composite sampling with a sampling rate of 1 : 10<sup>4+</sup>); ii) field mass-reduction (riffle splitting, sampling rate ~1 : 100); longitudinal diamond saw cutting of pre-mixed vertical slices (1 : 10); in-house aliquoting sub-sampling (~1 : 10). The complete sampling pathway mass reduction thus spanned seven orders of magnitude (mass/mass). All stages were carefully designed and scrupulously performed in accordance with TOS’ every principle for representative sampling to a degree only rarely deployed within the geosciences, while at the same time **exclusively** only relying on manual processes.<sup>7,9</sup> The term “barefoot sampling” appears apt.

### Project results—preliminary findings

Table 1 shows the analytical results from the primary samples representing the seven mining tailings addressed in the sampling feasibility pilot study.



**Figure 7.** Two-stage mass reduction of frozen sample bottles. First cutting is a 50/50 split, followed by a further slice of one of the randomly selected half cores produced, resulting in a 10–15% final sub-sample mass, which is guaranteed to be representative of the original container content in the vertical dimension irrespective of the degree of possible residual segregation present.



**Figure 8.** Three-stage mass reduction of frozen sample bag content. All cuts are vertical slices again resulting in a 10–15% final sub-sample mass, which is guaranteed to be representative of the original container content in the vertical dimension irrespective of the degree of residual segregation present.

## Discussion

In industrial contexts, an average gold grade of above ~5ppm is considered profitable; in the SSGM community, 3ppm (and above) is eminently interesting. In this view the values revealed in Table 1 show the inefficiency of traditional gold extraction using mercury. There is therefore an important incentive to address these types of tailings. There are two options:

- 1) extraction of gold from original deposits using the alternative mercury-free procedure and/or
- 2) re-process SSGM tailings, also based on the alternative approach.

Re. 1) Teaching small-scale miners mercury-free extraction procedures constitutes

an important objective—which will result in an increased profitability of at least 50%, while simultaneously contributing significantly to solving the global mercury poisoning threat.<sup>2</sup>

Re. 2) This will directly reduce the global mercury pollution—while at the same time being able to score economically from a source never mined optimally before, SSGM tailings. Some operators have run these tailings through a cyanide leaching process, which has its own, severe environmental problems of course.

## Conclusions

This work originated as part of a global mercury pollution reduction endeavour to which

SSGM is the major contributor. The mercury is hosted in tailings as tiny droplets, which are difficult/impossible to recover. The present feasibility project, financed by the Danish Ministry of Environment and Food, focuses on methods for mercury cleaning with the aim of two potential bonuses for the SSGM community, and for the world, one environmental, the other economic.

In this context, a need for careful primary sampling was identified. Global SSGM tailings, destined for an improved, non-toxic reclaiming process, need to be characterised with extraordinary focus on reliable estimates of average grades for Au and Hg.

For this purpose, the TOS was invoked which had to be applied subject to stringent

Table 1

Analyte	Au	Ag	Hg	Cu	Pb	Zn	As	Se	Sb
Unit	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Detection limit	0.03	0.5	10	0.03	0.03	0.3	0.05	0.05	0.05
Analysis method	FA-GRA	ICP-MS	ICP-OES	ICP-MS	ICP-MS	ICP-MS	ICP-MS	ICP-MS	ICP-MS
Limay1	13	7.1	10	45.1	54.2	50.2	10.4	4.89	4.78
Limay13	4.99	121	20	332	592	88.2	96.1	9.37	4.2
Limay23	4.41	28.2	20	317	241	55.7	8.03	7.12	2.62
Limay30	1.15	7	10	151	148	165	58	6.15	2.86
Limay36	9.87	8.9	10	46.9	65	61.6	6.74	6.14	4.13
Limay44	13.5	10.5	10	34.1	67.7	51.9	7.58	5.88	3.44
Limay51	3.95	222	40	176	709	197	224	7.71	7.36

“barefoot” technology requirements. Amongst others, use was made of extraordinarily intensive composite primary sampling in full compliance with the FSP. The project also developed a “freeze-drying” technique for sampling “difficult” slurry samples with severe gold segregation and mercury integrity issues. As described and illustrated, these TOS tasks were satisfactorily resolved. There is likely a carrying-over potential for the freeze-dried sub-sampling procedure to other similar types of slurry material.

## Appendix 1

“These samples originate from a study of low (to very low) Au and Hg concentration in mine tailings and tailing dumps (estimated 2–15 ppm), implying a highly irregular distribution of elemental micro-Au flakes/fragments in the 15 ton original tailing dump. The project has invested a considerable effort in arriving at the seven sample flasks supplied (masses ~30g) with outmost care in using Theory of Sampling compliant primary and secondary sub-sampling throughout, as documented in DS 2077 (2013). It is critical that also the final mass-reduction needed for ACTLABS to extract the precise analytical aliquot mass/volume are fully representative, i.e. extracting the aliquot mass from the sample flasks supplied by spatula is unacceptable. We ask ACTLABS to follow one of the recommended procedures in Petersen et al. (2004), Esbensen & Julius-Petersen (2009). Because this project is a method-development feasibility study in which sampling, handling and analysis are

of equal importance, we ask ACTLABS to supply a complete documentation of the in-house sub-sampling employed.”

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# Representative sampling in biomass studies—not so fast!

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Proper execution of representative sampling and laboratory mass reduction procedures are critical for the validity and reliability of chemical analyses of highly heterogeneous biomass fuels. In the study reported by Thy *et al.*,<sup>1</sup> it was demonstrated that faulty sampling had resulted in apparent ash compositions that differed from the true compositions by factors of two to three for many major oxides. Analytical results based on non-representative samples may thus not be representative for the specific fuel and processes being studied. Despite the general acceptance that accurate and representative compositions is a critical prerequisite for understanding reactions and elemental fractionation, the biomass energy community appears largely to have ignored the critical issues surrounding representative primary sampling. This can have resulted in misleading or faulty conclusions and may have restricted reliable predictive modelling.

## Background

Knowledge of representative compositions of biomass fuels and their derivatives is critical for understanding reactions and elemental fractionation during thermal conversion such as fuel combustion. Achieving proper knowledge for highly heterogeneous biomass fuels is not a straightforward matter, but calls for careful considerations of the primary sampling procedures. Although the literature outside the biomass realm contains a wealth of established sampling principles, drying and ashing used as mass-reduction measures in fuel and combustion studies introduce further complexities. This mandates careful considerations also of laboratory procedures such as mass reduction techniques for secondary sampling of biomass byproducts in addition to the analytical procedures themselves. Despite the general knowledge in other fields that sampling errors can attain magnitudes of 20–50 times the analytical errors alone, in biomass studies the focus is all too often mainly on the precision of the analytical procedures alone, which is usually gauged by repeating the analytical procedure. Thus the quality of chemical analysis is typically evaluated by analysing as unknowns, well-characterised and compositionally similar standards. This approach only furthers the total analytical uncertainty for controlled samples, however (certified standards or in-house standards). But highly precise chemical analyses are of very limited blessing if the materials analysed are based on faulty or poorly documented and little understood sampling and mass reduction procedures. The main guarantee for accuracy of the analytical results rests with the documented representativeness of the entire sampling pathway.<sup>2</sup>

The biomass and energy community has unfortunately largely ignored or underestimated the effects of these problems. This can have impeded the ability to perform accurate predictive modelling, either experimentally or theoretically, of phase equilibria, elemental mobility and fractionation, and physical behaviour of residual silicate systems from thermal conversion of biomass materials.

This short note refers to a case study of the possible detrimental effect of non-representative chemical analyses on predicting relative element mobility during combustion of common wood fuel published by Thy *et al.*<sup>1</sup>

## Wood fuel case story

This study reported attempts to characterise the inorganic part of a mixed conifer wood (mixed white fir and ponderosa pine), which was obtained from an operating power plant in California. The average grain-size of the fuel chips was inch-size (2–3 cm) and composed principally of solid wood with only minor bark, branches and foliage (Figure 1). The fuel was treated using standard methods of drying. The total air-dried mass of about 150 kg was stored in a closed master bin.

Three samples were taken from the master bin over the years of the duration of the study, two 1 kg primary samples (from which were produced 100 g of ashes for each). The analytical results in the present studies were elemental analyses of the ash fraction. These two samples were analysed twice by the same established commercial laboratory following accepted ASTM standard protocols. A larger primary fuel sample (25 kg) was also

extracted from the same bin, which was milled to a finer 3-mm grain-size before being ashed in a similar fashion. This latter ash (~2500 g) was sampled after manual homogenisation, the analytical mass was 3 g and analysed by X-ray fluorescence techniques. The same ash was similarly sampled and analysed by the earlier used commercial laboratory mentioned following the same ASTM standard protocols previously used. Thus there is a basis for comparison of the analytical results based on this small experimental sampling design.

The four analyses summarised in Table 1 were all obtained with the purpose of representing the ash fraction of the same wood fuel. Since the particular purpose of the study was to evaluate alkali metal volatilisation as a function of temperature, see Reference 1 for details, an accurate knowledge of the ash composition was critical. NIST fly ash reference material was analysed concurrently with the unknown wood ashes and the results are also listed in Table 1 together with their recommended standard values.

Comparison of the results in Table 1 reveals very large discrepancies between the individual analyses. The content of the three main components varied unexpectedly by factors of two to three for the major constituents SiO<sub>2</sub>, CaO and K<sub>2</sub>O. The repeated results on the standard fly ash (last two columns) clearly show that analytical procedures were not the cause of these highly significant deviations, despite the two different analytical techniques used. Although the fly ash standard does not compare closely in composition to the wood ash, one would be hard pressed



to attribute the highly diverse analytical results to analytical problems only. In this context it is particularly revealing that when the two different laboratories analyse the same ash, relatively consistent results were obtained.

These results forced us to reconsider the entire sampling–handling–subsampling–analysis pathway as implemented in the biomass energy community.

## Implications

The study in the original 2009 paper in *Biomass & Bioenergy*<sup>1</sup> was motivated by a failed attempt to mass balance a set of high temperature, partial melting wood ash experiments.<sup>3</sup> The results led to the unexpected indication that appreciable amounts of silica were apparently lost during heating to temperatures of well above 1500°C. Because silica is known to be immobile at atmospheric pressure to very high temperatures, and indeed perhaps *only* volatile at conditions believed to have prevailed during formation of the primitive solar nebula, a second look at the data was warranted. This reconsideration clearly showed that the erratic results were caused by chemical analytical results that were not representative of the biomass investigated. We were able to rule out, using different analytical methods, the possibility that large analytical biases and errors were responsible (Table 1). The conclusion was inescapable: unwittingly large sampling errors were committed by basing our initial analysis on the results from a non-representative primary sampling process.

Because of the heterogeneous nature of the biomass, a grab sample, as is routinely used in this realm, is highly unlikely to be representative for the bulk fuel composition. When we later re-analysed the actual ash used in the experiments and used this composition in new mass balance calculations, we obtained reasonable results that indeed suggested that only the alkali metals were mobile at high temperatures simulating combustion as indeed reported by Thy et al.<sup>3</sup>

This experience prompted us to take a new look into available standard procedures and common practices in related and/or similar studies published in the scientific fuel and biomass literature. A brief survey of papers published in *Biomass & Bioenergy* between 1991 and 2009 showed that very few combustion studies have indeed made the effort to document, far less to ensure,



**Figure 1.** Air dried wood chips used in the original study. Largest shards are approximately 1 inch (3 cm) in length. Although seemingly of uniform composition, the fuel actually consists of a mix of white fir and ponderosa pine. Grab-sampling of the pristine material will obviously give rise to severe sampling errors (FSE + GSE) if not guided by proper TOS-compliant principles, possibly aggravated by using significantly too small sample masses.

that the biomass material being studied was representative with respect to a particular geographic region or specific location, plant species or the actual power plant fuel intake. Fuel material used in scientific studies is often obtained in limited quantity (~100 kg or less) from forest and agricultural harvest locations or from feedstock intake stations of commercial power plants. Such feedstock samples for forest materials

are very unlikely to be representative and to be sufficiently well documented in all relevant aspects. Forest wood fuel is highly heterogeneous (segregated, stratified and contaminated) (Figures 2 and 3) typically composed of components such as pure wood chips, branch and root fragments, bark, foliage, as well as adhering soil. It is neither a simple practical nor an easy intellectual task, if not impossible, to

**Table 1.** Duplicated analyses of ash fraction of wood fuel (normalised to 100%).

	AN 2002	AN 2005	AN 2006	AU 2002	NIST 1633a	Recom.
Sample size	100 g	100 g	25 kg	25 kg		
SiO <sub>2</sub>	33.95	19.89	12.98	14.01	48.61	48.78
TiO <sub>2</sub>	0.13	0.33	0.12	0.19	1.37	1.33
Al <sub>2</sub> O <sub>3</sub>	6.21	9.38	4.11	4.68	27.04	27.02
Fe <sub>2</sub> O <sub>3</sub>	2.43	3.60	1.40	1.71	13.63	13.44
MnO	2.01	1.99	2.66	2.64	0.02	0.02
MgO	4.33	10.05	7.02	7.39	0.79	0.75
CaO	35.67	23.92	47.40	48.04	1.56	1.55
Na <sub>2</sub> O	0.58	0.60	0.63	0.58	0.21	0.23
K <sub>2</sub> O	11.36	20.08	18.42	16.06	2.23	2.26
P <sub>2</sub> O <sub>5</sub>	3.33	10.18	5.25	4.69	0.38	0.38
Sum	100.00	100.00	100.00	100.00	95.84	95.76

Recommended composition of NIST 1633a (coal fly ash) are from GeoReM (2006) (<http://georem.mpch-mainz.gwdg.de>). Other analyses are from Thy et al.<sup>1</sup>

aim for the proverbial statistically sound “random and representative” sample from such materials. This would require that the probability of all individual “elements” being sampled is exactly identical, irrespective of size, shape and their constituting elements (wood chips, bark, leaves, roots). In fact, the heterogeneity of biomass feedstock easily ranks among some of the most difficult materials to sample (Figures 2 and 3). In such a context, the unwitting quest for an intuitive and simple sampling procedure will always be on the agenda. This may have been a major scientific hindrance wherever reliable analytical results were essential for achieving a specific goal.

Without knowledge and respect for proper sampling principles, selection of supposedly representative samples all too often boils down to a personal intuitive judgement tied to the purpose of the particular study at hand, and this is almost invariably carried out by grab-sampling. If it is intended that the primary sample will represent the specific part of a forest, or a specific tree species, it may perhaps be possible to design a spatially random sampling strategy based on statistical knowledge from forest biomass surveys. Most likely it is more often desired that a sample should represent a specific biomass type and/or a seasonal average intake at a power plant (such as spring white pine wood). It is often possible to get sampling access to the feedstock at either an intake station at a

power plant or from a conveyor belt prior to being admitted to the boiler. But to conduct representative sampling at such locations is still considered a daunting task for which most investigators often do not have the knowledge, patience or means to succeed. Because few fuel laboratories possess the required facilities for storing, preparing, ashing and sampling large fuel volumes for study and analysis, there is little doubt that truly representative samples are considered merely an ideal and unobtainable dream for many combustion studies of biomass fuels, whether originating from agricultural or urban sources.

Many investigators likely proceeded as was done in the original study: with the kind help of a plant fuel intake manager, we obtained a few, large plastic containers with wood chip feedstock claimed to be “as received” at the plant from a typical supplier. The information obtained in our case was that it represented mixed conifers (white fir and ponderosa pine) harvested from the north-eastern slopes of Mount Shasta, California. There is an almost unavoidable tendency to trust such claims regarding the provenance of primary samples, if not experienced regarding proper sampling principles, but this is most often a fatal trust. The fuel in our case was a high quality, whole-tree fuel composed of relatively clean wood chips with limited bark, branch and foliage parts. We proceeded to process about 100 kg of this fuel, which was the maximum

that could reasonably be handled with the available facilities. We ashed a rather large proportion (25% of the primary sample mass) and were reasonably confident that the resultant ash after homogenisation and the sub-samples subsequently taken, represented the fuel, i.e. the secondary and tertiary sampling/mass-reduction steps were reasonably in control. There is no knowledge, however, of the degree to which the fuel truly represented the harvest biomass, the fuel received at the plant, the fuel conveyed to the boiler or combinations thereof. The primary sampling accuracy and hence the *representativity* may thus literally have been lost in the woods.

## Discussion

The critical question obviously is whether the biomass field can live with this kind of uncertainty. Most of the scientific endeavours are designed toward understanding combustion and gasification processes and not toward obtaining absolute and truthful values representing the original feedstock fuel. The interest is most often to understand how certain elements behave during thermal conversion. The answers that we are seeking are thus typically relative to specific processes and not absolute with respect to original lot materials. Often secondary sampling from a primary sample (which may be more-or-less representative with respect to the primary lot) appears to provide an acceptable basis for this kind of specific studies, allowing us



**Figure 2.** Typical fresh wood chips characterised by significant proportion of bark and foliage. Grab-sampling of this type of material will give rise to severe sampling errors (FSE + GSE) if not guided by proper TOS-compliant principles. Add hereto Incorrect Sampling Errors (ISE) if not considered.



**Figure 3.** Typical wood shard bio-fuel at a power plant intake. At this plant, routine primary sampling (for moisture determination), takes place following fully TOS-compliant procedures, see Reference 4 for details. An incremental primary increment sampler is shown (centre) just before being inserted into the lot (truckload); the sampler is closed when inserted. Three increments are used for composite sampling, i.e. from the top, middle and bottom level, respectively, with random sampling location in the X-Y plane.

to gain insight into the central processes as long as fuel and products are sampled and handled in a sensible and identical representative manner from the secondary sampling stage onwards.

The seasoned experimentalists may advise that instead of trying to understand the behaviour of heterogeneous fuel systems, one may gain a better understanding of compositional variables by studying the individual components before embarking on the daunting task of examining experimentally the full complexity of realistic multi-component fuel systems. This way one can build an understanding of the complex system from knowledge of the behaviour of the individual components (bottom-up approach). Such an approach will also reduce the problem of obtaining representative components as long as we can sort and purify the material into its separate constituents. This bottom-up principle has been highly successful in phase equilibria studies of silicate systems, either simple or complex, and many other types of material science studies. However, at the end of this endeavour we are still left with the challenge of accounting for the total system in industrial use. In the understanding of biomass combustion, as well as in most other areas of science, the summation of all parts is often likely to be considerably more complex than a mere aggregation of partial results.

Thus, irrespective of method, scope and goal, it is critical for future biomass studies relying on analyses of experimental products that these be sampled only in a representative manner. This involves representativity in sampling of the primary lot, as well as for subsequent splitting of potentially large volumes (secondary sampling), milling of sub-samples to workable particle sizes and homogenising before the ultimate analytical aliquots are taken. In some cases it may be advantageous that sampling is done on ash fractions despite potential loss of elements during ashing, because smaller volumes and finer grain-sizes are easier to handle and ashes results in lower analytical detection limits. But relatively small grab samples of raw biomass or ash are, as shown also by our own experiences, prone, indeed likely, to be *non-representative* and may thus exhibit strongly diverse compositional variations. This is particularly true for the elemental composition of an ash fraction that only constitutes a minute proportion of the total sample (the ash fraction of clear wood is typically below 0.2). An increase in primary

sample volume is often the only variable known that is believed to bring down these compositional sample-to-sample variations, but in fact this will only be true for samples approaching the total volume. A scientifically founded and improved sampling must counteract every feature of the complex lot heterogeneity, e.g. as per the principles presented in DS 3077.<sup>2</sup>

It is an essential, key insight in particular for all significantly heterogeneous materials, which cannot be freely mixed before the primary sampling stage (either too large and/or too heterogeneous lots), that composite sampling is the *only way forward*. A particularly relevant example is provided by Møller and Esbensen<sup>4</sup> for the primary characterisation of intake wood chips at a Danish power plant (Figure 3).

### Conclusions and recommendations

Studies of biomass combustion processes are critically dependent on whether analyses of primary fuel (and ashes and slag) are conducted on samples that are demonstrably representative for the processes and materials being studied. The inherent problems in conducting traditional “statistically and sound sampling” of highly heterogeneous and stratified biomass critically restrict our ability to design valid and meaningful experiments of combustion processes. It is sometimes suggested, as a first alternative, that studies are conducted on the individual fuel components before multi-component fuel systems are being investigated, but

this approach only dodges the ultimate purpose and will not necessarily address the full problem at power plant or incinerator plant scales. Consideration of proper mass reduction procedures (secondary sampling and sampling preparation) is still a prerequisite for the success of all biomass related studies. For this demand, as well as for primary sampling issues, a consistent theory of sampling is critically needed. There is an overwhelming TOS literature available to everybody's needs, a judiciously selected part of which can be found referred to in DS 3077.<sup>2</sup>

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The authors (Peter Thy, left): “WHOA—factors 2–3× wrong due to inappropriate sampling”.

# Sampling for mycotoxins in feed—heterogeneity characterisation

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The presence of mycotoxins, in particular aflatoxin B1, can cause significant health problems as well as severe societal economic losses, and is, therefore, regulated with respect to maximum acceptable concentration in various feed- and foodstuffs. International regulatory authorities have begun to recognise the importance of representative sampling, but sampling guidelines are only partly in compliance with the Theory of Sampling. In particular, practical guidance regarding sampling, including correct design and operation of sampling devices, including explanations on how to develop sufficient sampling protocols are lacking in current guidelines. These are critical practicalities of major importance, especially when dealing with trace concentrations and/or concentrations that are irregularly distributed—as is the case for mycotoxins. Furthermore, heterogeneity characterisation, which is a necessary requirement to be able to develop valid sampling protocols or validation assessments of existing sampling operations, is currently not mentioned in the existing guidelines. The present paper focuses on heterogeneity characterisation with respect to sampling of mycotoxins for 1-D and 3-D feed lots (a full analysis of all critical practicalities in sampling mycotoxins is published elsewhere). Structural guidelines for correctly designing experimental heterogeneity characterisations are presented, allowing evaluation of sampling representativeness and determination of optimal number of increments per composite sample.

## Background

Mycotoxins are toxic secondary metabolites of moulds, which can occur during plant growth and during storage and processing. Among various mycotoxin types, aflatoxins are of major concern due to their potential impact on human and animal health. The food and feed industry has set a special focus on aflatoxin B1, which occurs most frequently and is the most toxic aflatoxin, since it has been directly correlated with adverse health affects.<sup>1</sup> Mycotoxins can occur within a concentration range of  $\mu\text{g kg}^{-1}$  to  $\text{mg kg}^{-1}$ . The Food and Agriculture Organization of the United Nations (FAO) has estimated that approximately 25 % of the world's agricultural production is contaminated with mycotoxins, resulting in significant economic loss due to their impact on human health, trade and animal productivity.<sup>2</sup> Due to the fact that the presence of mycotoxins in food- and feedstuffs cannot be avoided, valid testing is demanded and, therefore, sampling methods for raw and processed materials are a critical necessity. The US Department of Agriculture (USDA) and its Grain Inspection, Packers & Stockyards Administration (GIPSA) has estimated that non-representative sampling accounts for nearly 90 % of the error associated with aflatoxin detection,<sup>3</sup> mainly due to non-random spatial distribution throughout materials when occurring in the trace concentration range ( $\text{mg kg}^{-1}$  or  $\mu\text{g kg}^{-1}$ ).

Below, critical practicalities with a focus on the heterogeneity characterisation required for developing sampling protocols for determining mycotoxins in feed (equally applicable to food) are presented. Results are substantiated with data from field trials. The real-world data used here have been redacted and serve specifically to strengthen the general arguments and not to represent specific results of the studied field trials, which are proprietary.

## Critical sampling practicalities

The reason for all sampling errors is *lot heterogeneity*, causing material to vary irregularly throughout the lot on spatial but also on compositional dimensions and scales. Increasing the number of correctly extracted increments in a composite sample is the most effective way to decrease primary sampling errors, and will lead to results that are closer to the true lot value. The difficulty is to determine the “optimal number of increments”, since this depends on heterogeneity, the analyte concentration level, and the size and lot geometry. In practice, sampling is often a compromise between the desired levels of accuracy/precision and labour/cost deemed necessary. The only criterion that must never be up for negotiation is representativity, which needs to be based on sampling correctness. In particular when dealing with trace concentrations or highly heterogeneous distributions, as is the case for mycotoxins, the sampling variance is by far the

dominating source of uncertainty, due to the characteristically skewed, polymodal, highly irregular “distribution” of these analytes.<sup>4,5</sup>

Below, tools for determining optimal number of increments and minimising errors at each sampling and mass reduction step are presented. Examples are based on a real-world field trial performed on various materials used as animal feedstuff for determining aflatoxin B1 levels within each feed component, as well as within the total feed mixture (also termed “total mixed ration”, TMR).

## Sampling stages

In the present field trials, all total mixed ration components are stored in piles and could only be sampled once unloaded (3-D sampling situation). The feed components are mixed in a predetermined ratio to form the total mixed ration (TMR), which is spread out in elongated feed bunks (1-D sampling situation). For each feed component, as well as the TMR, an individual sampling strategy determining the optimal number of increments has been developed, based on preceding material heterogeneity characterisations. All individual feed components have been analysed for aflatoxin B1 including pre-set control variables (protein, fibre and moisture). Samples collected from the feed mixture (TMR) have also been analysed for the same analytes, allowing a comparison of the TMR results with the analytical results of the individual TMR components.

To develop an appropriate aflatoxin sampling plan, the following steps have been undertaken:

- Assessment of optimal sampling location (preferentially sampling in a 1-D sampling situation)
- Selection of appropriate sampling devices and mass reduction procedures for each material and lot type
- Design of experiments for characterising material heterogeneity
- Determination of optimal sampling frequency based on empirical experimental outcomes

As stated above, only the total mixed ration can be considered as a 1-D sampling situation, while all individual feed components are piled up in 3-D lots, which were regrettably not able to be sampled during unloading.



Figure 1. TMR “sampling box” covering entire depth and width of target material, which is spread out in the longitudinal (horizontal) direction.

### Primary sampling

Before presenting the experimental design for the required material heterogeneity characterisations, the sampling tools used for the elongated TMR, the individual TMR components, as well as applied mass reduction procedures are presented.

The total mixed ration is pre-mixed and spread out in elongated feed bunks. Such a sampling situation (one-dimensional lot) allows extraction of increments covering the entire depth and width of the material, while a fully comprehensive spatial distribution of the increments is covered in the longitudinal direction of the lot (distance in-between increments as well as total number of increment is based on experimental design). In order to correctly delineate and

extract the increments, a “sampling box” has been designed, suitable for the relevant lot dimensions and material characteristics, as depicted in Figure 1.

For individual TMR components (three-dimensional lots), the use of sampling spears is claimed to allow the best accessibility for all lot dimensions. Various types of sampling spears exist in the market; but they are seldom designed in compliance with the Theory of Sampling (TOS). The most important aspects with respect to sampling spear design are its length, width, aperture positions and opening width, as well as the closing mechanism. In the optimal case the length of the sampling spear should cover the entire depth of the lot, which allows

insertion of the sampling spear vertically at every position within the lot (as indicated by the arrows in Figure 2, left side). However, due to the fact that some of the TMR component piles exceeded the maximum available length of sampling spears, positioning and inserting direction were carefully considered. On the right-hand side of Figure 2, a pile is depicted that exceeds the length of the sampling spear. In order to cover all lot dimensions, i.e. also the lower and bottom parts of the lot at its highest level (row 3), the sampling spear was inserted horizontally in row 2 at the lowest accessible inserting point. It is emphasised that this spear sampling procedure is a result of a compromise based on the actual situation

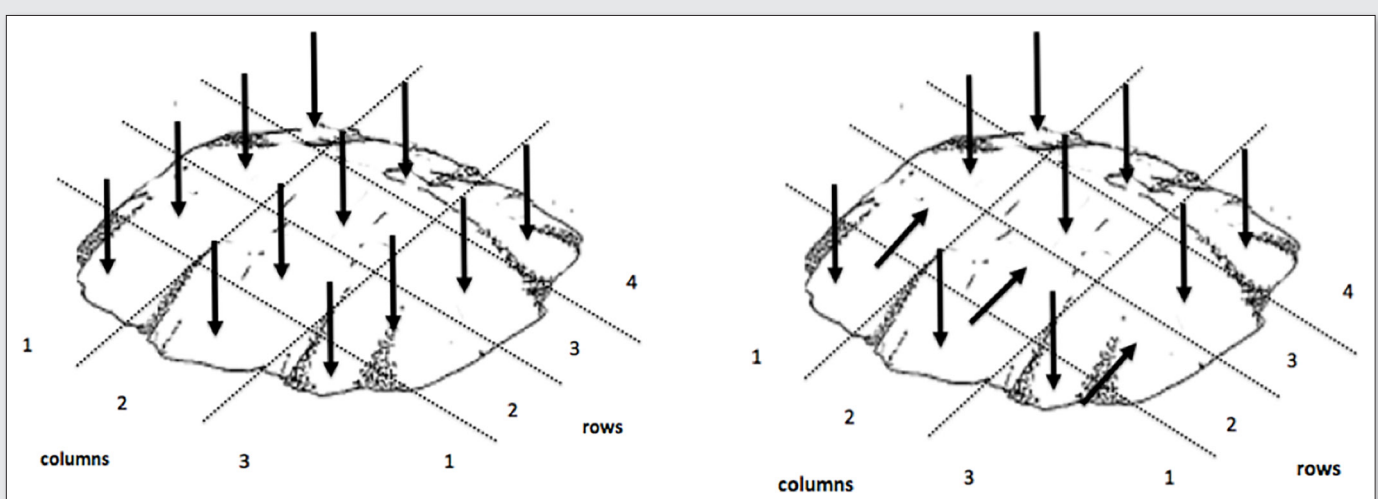


Figure 2. Illustrating stratified composite sampling of non-equal height 3-D storage piles. Sampling spear length versus pile height—spear inserting directions.

that the individual TMR components could not be sampled during unloading (1-D sampling situation). Muzzio *et al.* have published a particularly illuminating exposé of the deficiencies in spear sampling for powders and granular mixtures.<sup>6</sup>

### Mass reduction

Correct mass reduction procedures need to be applied or sampling errors will adversely impact the secondary, tertiary etc. sampling stages and inflate the total measurement uncertainty.<sup>7</sup> Petersen *et al.* have performed an extensive study of various available mass reduction procedures and have rated them according to their representativeness, with the conclusion that only riffle splitters and rotational splitters allow correct mass reduction.<sup>8</sup> For the majority of the TMR components, riffle splitters with appropriate chute opening widths have been used, while for some fibrous, very light and wet materials the primary samples have been mass reduced using a circular cutting device, dividing the primary sample in eight equal sectorial cuts (increments). Four of the eight cuts have been used in the secondary sampling stage, while the other four cuts were discarded. All primary samples were mass reduced and further processed in the laboratory, including comminution and mass reduction to analytical sample size. Also in the final analytical mass reduction stage riffle splitters and bed-blending technique have been used to avoid sampling errors, especially important since dealing with a trace concentration range of aflatoxins, *ibid.*

### Design of experiments for characterising material heterogeneity

Following the proposed outline for developing an appropriate aflatoxin sampling protocol, the steps are (1) assessment and decision on optimal sampling location (3D vs 1D), (2) selection of appropriate sampling devices and mass reduction procedures, (3) the design of experiments for material heterogeneity characterisation in order to determine (4) the optimal sampling frequency for each material.

Depending on the lot type, the sampling variance associated with the final sampling protocol and the heterogeneity distribution of the targeted analyte (e.g. aflatoxin B1) can be quantified using two different procedures: the replication experiment (stationary 3-D decision units) and variographic

analysis (dynamic or stationary 1-D decision units). These assessment methods can also be applied to incorrect sampling procedures, for which the result would reflect the material heterogeneity plus the significantly inflated sampling errors. For the present field trials, sampling errors have been minimised by selection of appropriate increment sampling location and procedures allowing the sampling variability of the heterogeneity of the target analyte in the lot to be characterised; based on that the optimal number of increments for the final composite sample has been determined.

The replication experiment was applied to all TMR components (3-D sampling situations), while a variographic experiment was applied to the sampling variance for the TMR in a 1-D sampling situation.

For the replication experiments, ten primary samples were collected from each TMR component, each time repeating the full lot-to-test portion sampling pathway in completely identical fashion, DS 3077 (2013). Each primary sample consists of 30–40 increments depending on the lot dimensions. The minimum requirement is that the entire spatial geometry of the target material is fully covered by the sampling tool and the selected number of increments. It is important that all sampling operations, particularly at the primary sampling stage, are fully realistic during the replication experiment, meaning for example that the replicates should not be extracted at the exact same locations. In the described experimental field trial, different sampling operators collected the replicate primary samples in order to reflect all possible variation also that caused by individual differences regarding operating the sampling and mass reduction devices. For each replication experiment, the “relative sampling variation (RSV)” and the statistical relative “coefficient of variation (CV<sub>%</sub>)” were calculated, giving a measure of the specific heterogeneity of the target material (e.g. aflatoxin distribution), as expressed by the specific sampling procedure applied.

Heterogeneity characterisation of the TMR is based on a variographic experiment, for which 60 equally spaced increments have been extracted from the feeding lane using the described TMR sampling procedure. The main objective of the variographic experiment is similar to the replication experiment, meaning to determine the RSV (here called RSV<sub>1-dim</sub>). Additionally, the influence of different sampling rates (i.e.

distance between extracted increments) has been evaluated, allowing determination of the optimal sampling frequency or the optimal sampling interval.

### Results and discussion of heterogeneity characterisations

The following section explains how results gained from heterogeneity characterisation experiments have been interpreted to correctly determine aflatoxin levels in feed. The results have been redacted, rather serving to explain general features and interpretation possibilities than to present the actual values of the studied field trial, which are proprietary.

#### Results of individual TMR components

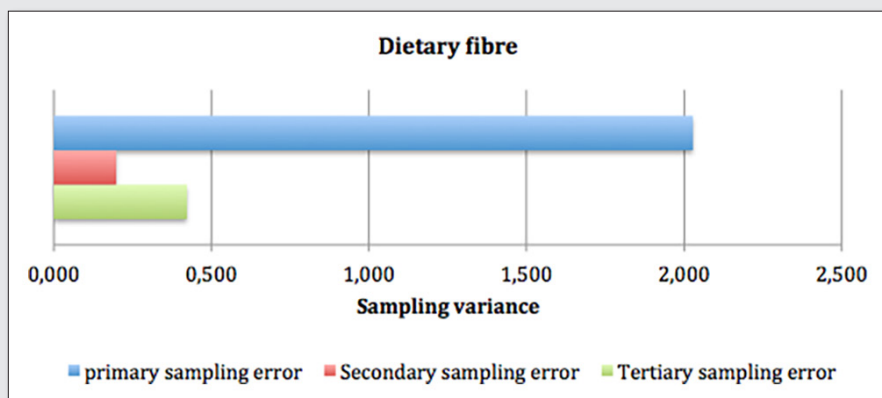
In addition to aflatoxin B1, all materials were also analysed for protein, dietary fibre and moisture content, which serve as control variables to evaluate the applied sampling methods. For TMR components containing no detectable aflatoxin, protein, dietary fibre and moisture are used as control variables to determine required sampling frequency for reflecting inherent material heterogeneity.

The replication experiments used for characterising 3-D lots also allow comparison of the sampling variances originating at different sampling stages (i.e. primary sampling, secondary sampling, tertiary sampling etc.). Figure 3 shows a result of the sampling variances in the different sampling stages for one of the TMR components, protein content. For nearly all materials and analytes in the study, similar results established the primary sampling variance as completely dominating over the secondary and tertiary sampling variance. This also confirmed the correctness of the mass reduction procedures used.

In contrast to Figure 3, Figure 4 shows the sampling variance of dietary fibre for a different TMR component (proprietary), revealing that the sampling variance decreases from primary to secondary sampling stage, but actually increases in magnitude in the tertiary sampling stage. This latter is a clear indication that an incorrect sampling procedure was used at this stage. This example demonstrates how a replication experiment allows detection of “hidden” sampling errors. In this particular case, it was discovered that grab samples were extracted to gain the final test portion (despite the pre-designed, correct mass reduction steps),



**Figure 3.** Typical example of comparison of sampling variances from different sampling stages. Dominance of primary sampling variance over secondary and tertiary sampling variance is the typical case.



**Figure 4.** Typical example of comparison of sampling variances from different sampling stages, revealing an incorrect mass reduction procedure in the tertiary sampling stage (see text for details).

disobeying the TOS' principles of sampling correctness. After correction of this incorrect procedure (replacement by a bed-blending technique), the sampling variance of the tertiary sampling stage decreased to a level below the secondary sampling variance, confirming reduction, or elimination of the incorrect mass reduction procedure.

The replication experiments of the field trial have also been used to quantify the heterogeneity of each TMR component, in particular with respect to the aflatoxin concentration. For all TMR components containing aflatoxin, the pertinent distributions are significantly skewed to the right; a characteristic of aflatoxin which has also been confirmed by various other studies.<sup>9,10</sup> The relative sampling variation (RSV) confirms this observation, ranging from around 50% to above 300% for the analysed materials. Since sampling errors have been minimised by means of the experimental design, the determined RSV values measure the total empirical sampling variance influenced by the aflatoxin heterogeneity of the target

material. The RSV values for the control variables for all TMR components ranges between 2% and 15%, confirming that the comparatively high RSV values for materials containing aflatoxin is dominantly caused by the irregular, non-normal distribution of aflatoxin, rather than by incorrect sampling procedures. In order to lower the sampling variance for aflatoxin (if required by quality specifications), the number of increments per composite sample would need to be increased.

### Results of TMR mixture

The total mixed ration (mixture of all individual feed components) is the last point at which aflatoxins can be detected before being fed to the animals and potentially causing dangerous health effects. The high RSV values determined for the various TMR components with respect to aflatoxin B1 indicate that despite elimination of potential incorrect sampling errors, the overall uncertainty on aflatoxin concentration is still uncomfortably high. For the field trials,

a specific uncertainty level on aflatoxin level in the TMR was pre-set, requiring that the sampling method and sampling frequency guarantee this uncertainty level. A variographic analysis also allows determining the influence of different sampling rates on the overall uncertainty, which has also been assessed for the present field trial.

Figure 5 shows the variographic results of the control variables for the TMR, comparing the number of increments used for final composite sample with the corresponding relative uncertainty incurred. The exact numerical values of the corresponding uncertainty are again not shown here due to confidentiality reasons.

Adding the variographic results for aflatoxin B1 to the same graph (see Figure 6), it is obvious that the corresponding uncertainty for aflatoxin is dramatically higher (~10x higher) compared to the control analytes, as also concluded from the assessment of the RSV values of the individual TMR components. The steepest decrease of uncertainty can be observed increasing the number of increments from one to two and from six to ten for the final composite sample. For this field trial the pre-set acceptable uncertainty level has been reached combining 10 increments to a final composite sample. In case a lower uncertainty level is required in the future, the appropriate number of increments can be selected directly from these variographic results, allowing full detection and uncertainty control of the aflatoxin concentration present in the TMR.

### Conclusions

Critical practicalities in feed sampling for mycotoxins have been presented, which are currently not considered in the relevant sampling guidelines. The main problem for detection of mycotoxins, and especially aflatoxin in feed, is their decidedly irregular, non-normal distribution in the target feed/food materials. "Hot spot" characteristics and low trace concentration ranges and distributions make representative sampling critical for valid mycotoxins concentration control. Assessment of optimal sampling locations as well as selection of the appropriate sampling and mass reduction devices forms the basis for representative sampling. A primary consideration is to determine the optimal number of increments, since practical sampling is a trade-off between labour/economic efforts and sample quality. When the empirical effect from increasing the number of increments is known, an

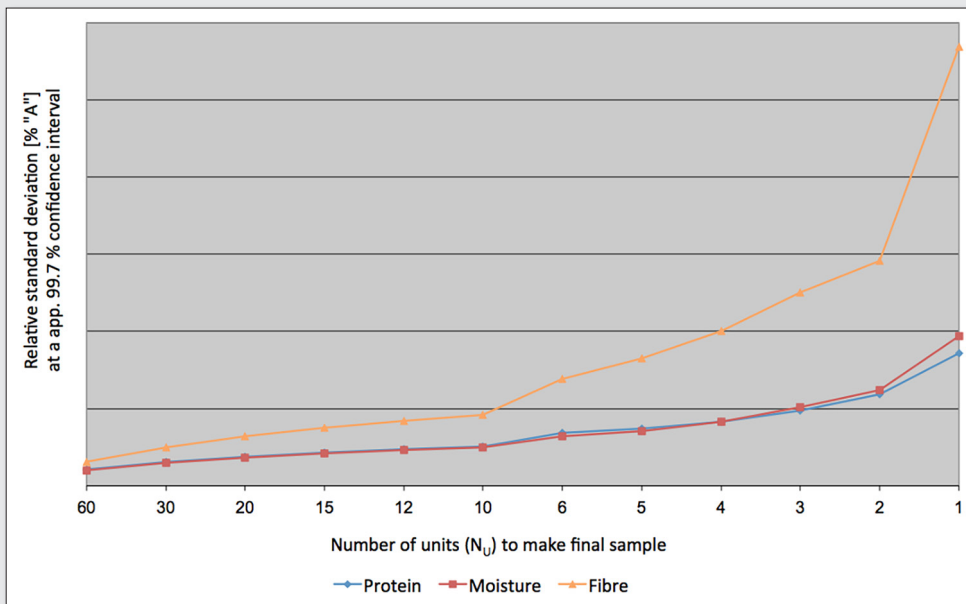


Figure 5. Variographic result for TMR (excluding aflatoxin) for a varying number of increments in a composite sample and the corresponding rel. total sampling-plus-analysis uncertainty. Values of the y-axis have been removed due to confidentiality reasons without any loss of generality. Results are calculated for a systematic sampling mode.

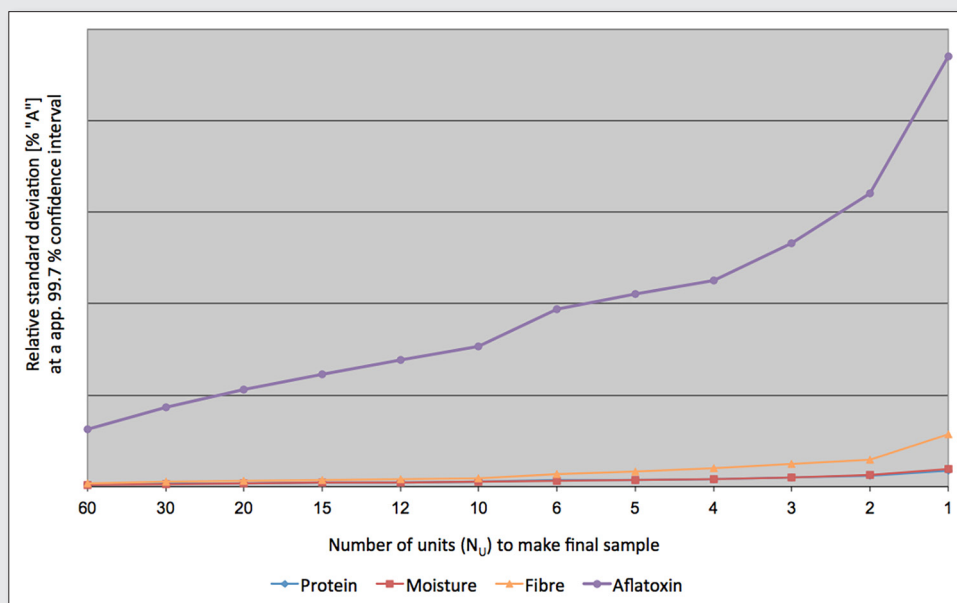


Figure 6. Variographic result for TMR (including aflatoxin) for a number of increments in a composite sample and their corresponding rel. uncertainty. Values of the y-axis have been removed due to confidentiality without any loss of generality. Results are calculated for systematic sampling mode.

educated decision can be made. Replication experiments for 3-D decision units and variographic analysis for 1-D decision units serve as a basis for the mandatory initial material heterogeneity characterisation; and can be used to derive an optimal number of increments. Examples of an industrial field trial were presented including heterogeneity characterisations for various total mixed ration components, as well as for mixed feed itself. Interpretation guidelines were

given on how to assess applied sampling methods on the basis of these experimental designs and how to determine an optimal increment number and location. It was highlighted how variography can be used to compare various sampling strategies based on their corresponding total uncertainty levels. The developed criteria regarding sampling practicalities can be transferred to many other feed- and foodstuffs and other commodities with similar characteristics

regarding trace concentrations or concentrations which are irregularly distributed throughout the target material.

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# Introduction to the Theory and Practice of Sampling

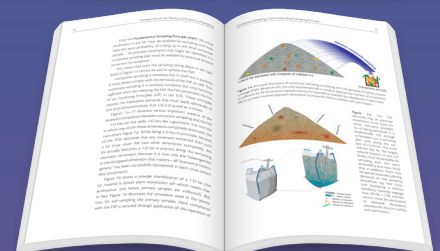
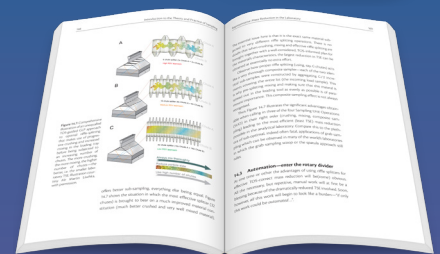
Kim H. Esbensen

with contributions from Claas Wagner, Pentti Minkkinen, Claudia Paoletti, Karin Engström, Martin Lischka and Jørgen Riis Pedersen

Sampling is an important, but sometimes hidden, part of everyday life in science, technology, industry, society and commerce where decisions are made based on analytical results, which must be based on reliable samples. However, there is a very long and complex pathway from heterogeneous materials in “lots” such as satchels, bags, drums, vessels, truck loads, railroad cars, shiploads, stockpiles (in the kg–ton range) to the miniscule laboratory aliquot (in the g–µg range), which is what is actually analysed.

This book presents the Theory and Practice of Sampling (TOS) starting from level zero in a novel didactic framework without excessive mathematics and statistics. The book covers sampling from stationary lots, from moving, dynamic lots (process sampling) and has a vital focus on sampling in the analytical laboratory.

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