

Title: Developing testing protocols to assure the quality of fertilizer materials for organic agriculture

Final Report

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Executive Summary

This study presents an approach and methodology for detecting, with high probability, adulteration of organic fertilizers and other amendments by synthetic fertilizer and other chemical nutrient sources. The low cost and relative simplicity of the protocol will ensure regulators and test laboratories can routinely and efficiently test commercially available organic fertilizers. We present a set of common methodologies that include analysis for ammonia and total carbon and nitrogen that are readily available to soil test labs and the California Department of Food and Agriculture (CDFA) Inspection Services Center for Analytical Chemistry. These minimum analyses are likely to flag greater than 90% of samples that could be adulterated with synthetic sources of nitrogen. Any organic farmer suspecting adulterated fertilizer could submit a sample of fertilizer to a commercial soil test lab or the CDFA to determine with high probability whether the fertilizer is authentic. Additional stable nitrogen isotope and spectroscopic analysis can likely confirm adulteration of fertilizer samples. Since these analyses are not routine for soil test labs, the California Department of Food and Agriculture Inspection Services Center for Analytical Chemistry may consider accepting these suspected samples for further analysis. Legitimate producers of fertilizers will benefit by having a defined set of testing protocols to ensure the quality of their products and manufacturers of adulterated organic fertilizers and amendments will face the appropriate scrutiny to ensure the authenticity of their products.

Project Justification and Background

Data from the last decade show the organic industry is on pace for a six fold increase from initial sales in the next five years having grown from \$6.1 billion (2000) to \$29 billion (2011) in sales (OTA, 2011). California is the national leader in organic farming with the highest number of organic farms, land under organic production and organic sales (Klonsky, 2010). Despite this industry accounting for only 3% of farm-gate sales in 2008, steady growth of this sector is anticipated and signified by year on year growth of 8%, compared to 1% for the entire food industry, in 2010 (OTA, 2011). Being an industry heavily based on trust in the “organic” brand, growth of the organic industry is partly threatened by any activity or factor resulting in

potential loss of grower and consumer confidence in the integrity of the brand. One such factor is the authenticity of the organic fertilizer used to grow organic food and oversight of this remains a major challenge.

Two incidents in the last five years pertaining to adulteration of organic fertilizers highlight this. In December 2008, the Sacramento Bee published an article entitled, “Organic farms unknowingly used a synthetic fertilizer” revealing an investigation by the California Department of Agriculture (CDFA) on the activities of a Salinas based company (Downing, 2008). The one-time supplier of organic fertilizer to approximately one-third of the state's organic farms was believed to have adulterated their organic fertilizer with ammonium sulfate. At about the same time, another Californian supplier was implicated in fraud charges, amounting to over \$40 million, arising from using cheaper inorganic compounds as substitutes in organic fertilizer made of fishmeal and bird guano. These unscrupulous practices increased concern about the authenticity and integrity of soil and crop amendments sold for use in organic production. The absence of simple verification methods or rigorous oversight hampered the agency's ability to analyze the tainted products and raised fears that such adulterated products could still be approved and labeled as suitable for organic agriculture.

California Assembly Bill (AB) 856 (Chapter 257, Statutes of 2009), was passed to substantially increase the penalties for violation of organic fertilizer standards, require registration of all organic fertilizers sold in the state and give regulators greater authority to monitor and review organic fertilizer labels (CDFA, 2012). However, one of the challenges faced by regulators to administering this law has been the absence of a systematic protocol for regulators and product end users testing the authenticity of the organic products sold. Depending on the degree of adulteration, basic laboratory tests often fail to identify a problem. For example, analysis of nitrogen content may confirm a product label, but will not indicate the source of nitrogen (organic or inorganic).

This research provides insight into analyses that can be used to provide quality control in the production and testing of organic fertilizers and amendments. The major objectives of this study are to: 1) construct a database of materials used in organic and synthetic fertilizers through a detailed review of the literature and, also via, chemical and physical analyses of these materials; 2) establish parameters which represent the natural ranges for the specific chemical properties (i.e., NH_4^+ , $\delta^{15}\text{N}$, and C:N) of these materials that can be used to distinguish between

pure, or unadulterated, and adulterated materials; and 3) develop a stepwise protocol that laboratories and regulatory agencies can follow to identify fertilizers that may have been adulterated by synthetic fertilizers.

Objectives

The major new product generated by this project is a method of detecting with high probability adulteration of organic fertilizers and other amendments by synthetic fertilizer and other chemical nutrient sources.

The following objectives guided this research project.

1. Construct a database of materials used in organic and synthetic fertilizers and their quantifiable properties through thorough search of the literature and additional chemical and physical analyses of such materials.
2. Establish natural ranges for the chosen properties of these materials that can be used to distinguish between pure, or unadulterated, and adulterated materials.
3. Develop a stepwise protocol test that labs and regulatory agencies can follow to identify organic fertilizers that have likely been adulterated by synthetic fertilizers.
4. Carry out blind tests with collaborating test labs to evaluate the robustness of the above protocol.
5. Disseminate the results and products of the project to potential users, such as organic fertilizer test labs and regulatory agencies.

Summary of task completion

The activities for the project were broken into tasks and a brief description and results is presented for each.

Task 1. Conduct a literature review of materials that may end up as part of organic fertilizer production, including marketed products and the raw materials used to develop these products.

Task 2. Collect and analyze solid and liquid organic fertilizers samples using wet bench chemical (stable isotope, ammonia, total C and N and nutrient content) and Fourier transform infrared (FTIR) spectroscopy methods.

Task 3. Produce a database including literature data and detailed laboratory characterization of expected or “natural” range of values for each of properties of interest for different organic fertilizer groups.

Task 4. Develop a systematic protocol for use in investigating potential adulteration of organic fertilizers

Task 5. Refine the protocol to a form usable by national test laboratories or other interested party.

Task 6. Evaluation: Measuring success

Task 7. Outreach.

Task 1: Conduct a literature review on the materials used in organic and inorganic fertilizer materials.

The initial task was to conduct a literature review of materials that may end up as part of organic production, including marketed products and the raw materials used to develop these products. Synthetic products, as potential adulterants, were also an important part of this review. The literature review provided a basic framework with which to construct the experimental dataset, as well as to provide for comparison with experimental results. Relatively large amounts of useful data were obtained from the literature review particularly for ecologically relevant materials (such as raw fish and guano) and agronomic materials (compost and soybean meal). Only a small amount of literature was found which had as its main objective the analysis of organic or synthetic fertilizers for data reporting.

Task 2 and 3. Analyze the collected materials for stable isotope and nutrient content and build a database.

For ease of comparison and analysis, the organic fertilizers were classified into categories based on feedstock components as used by Organic Materials Review Institute (OMRI) and signify the most popular products currently available in organic agriculture (Table 1).

Blends of varied composition that contained more than two components e.g. kelp/seaweed extract, humic acid, molasses, vinegar, compost and alfalfa tea; liquid compost / humate and molasses; and 4-2-3, fish emulsions, seaweed extract, humic acid and molasses were combined into one category called “Other blends”. A few comparison synthetic fertilizers were also analyzed: urea, ammonium sulfate or phosphates, ammonium nitrate, aqueous ammonia, nitrates and synthetic blends.

Table 1: Categories of Organic fertilizers used for comparison and analysis

UNPROCESSED FISH	(PROCESSED) GRAIN
LIQUID FISH PRODUCTS	HUMATES
SOLID FISH PRODUCTS	CHILE NITRATE
BLOOD MEAL	FISH / GUANO
COMPOST and MANURE	FISH / GRAIN
BAT GUANO	FISH / SEAWEED
SEABIRD GUANO	GRAIN / FEATHER
FEATHER MEAL	OTHER BLENDS*
SOY MEAL	UREA
COTTONSEED MEAL	AMINO ACIDS
BONE MEAL	URIC ACID
SEAWEED PRODUCTS	ALGAE

* contained more than two components e.g. kelp/seaweed extract, humic acid, molasses vinegar, compost and alfalfa tea

Synthetic and organic samples (solid and liquid) were obtained from commercial fertilizer suppliers for analysis (n = 180). Prior to analysis, all non-homogenous liquid samples, such as raw fish, were homogenized by mechanically shaking the sample with glass beads or steel balls. Solid samples were homogenized by grinding with a mortar and pestle, or in the case of very fibrous samples, by mechanically shaking in a steel

ball mill. The following properties were measured, when appropriate: carbon content, nitrogen content, phosphorus content, carbon-13/carbon-12 isotope ratio, nitrogen-15/nitrogen-14 isotope ratio, solid content, density, dissolved organic carbon, dissolved organic nitrogen, ammonium content, and nitrate content. Ammonium content, carbon to nitrogen ratio (C/N), and nitrogen isotope ratio ($\delta^{15}\text{N}$) were identified as most useful for initial inspection of the database and evaluation of fertilizers and the methodologies for these analyses is presented below.

Analysis methods

Ammonia. A subsample of the solid samples, approximately 200 mg, was shaken with 100 mL of water for about an hour. The solids were removed, either by centrifugation or filtration, and the remaining solution diluted as required for colorimetric ammonia determination (Doane and Horwath, 2003; Verdouw et al., 1978). For liquid samples, aliquots were taken and

transferred to a volumetric flask and concentrations of ammonia determined as above. Data from the analyzed and from literature are shown in Fig. 1. All data are shown together, including possibly adulterated products, resulting in a large spread of data in some categories.

All but five categories (i.e., liquid fish products, bat guano, seabird guano, and fish/seaweed blends) of the fertilizers had < 1% NH₄-N with the greatest variability in the NH₄-N content in

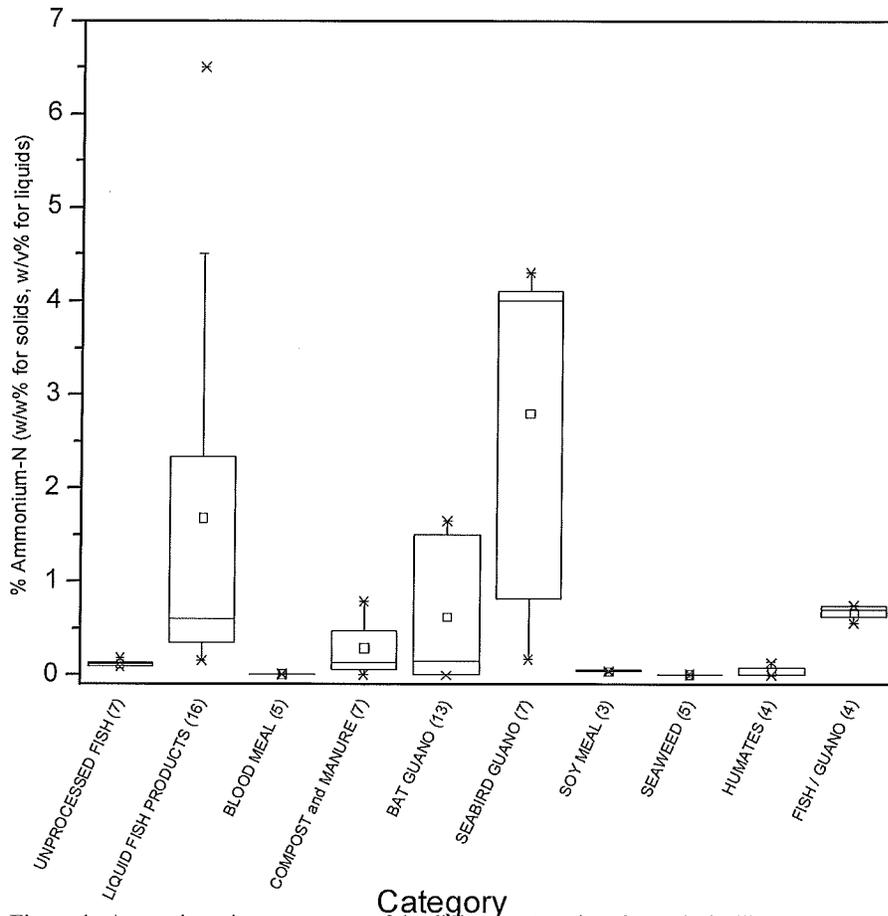


Figure 1. Ammonium nitrogen content of the different categories of organic fertilizers. Percentages were calculated weight to weight (w/w) for solids and weight to volume (w/v) for liquids. The lines of the boxplot represent the median, 25th and 75th quartile values, and the whiskers represent the maximum and minimum values used in the calculation. The number of samples is shown in parentheses.

the liquid fish fertilizers (Fig. 1). Naturally, categories such as seaweed, bloodmeal, compost and feathermeal do not contain much ammonium and thus this measurement would be an effective determinant of potential adulteration for fertilizers from these particular categories. However, for fish-derived and guano fertilizers which naturally contain ammonium and can contain

elevated NH₄-N concentrations (through industrial processes such as heating and enzymatic hydrolysis) this measurement is less effective as a determinant of potential adulteration for these fertilizers. Furthermore, the decomposition of fish tissue and products can also naturally result in increased ammonia concentrations (Spotte, 1970).

Carbon and Nitrogen. For total nitrogen and carbon by combustion, an appropriate amount of sample (2-5 mg) was dispensed into standard tin capsules used in elemental analysis. The method for total nitrogen by digestion was adapted from a protocol by Lindner (1944). A subsample, typically 700 mg, was dispensed into a 100 mL volumetric flask and the weight of the sample recorded. Five milliliters of concentrated sulfuric acid were added, and the samples were gently heated, (to approximately 150 °C) until all of the moisture was driven off. The samples were heated strongly (400-500 °C) until they were clear and colorless or almost colorless. The samples were made to volume with water (18.2 MΩ-cm) and the NH₄⁺ concentration, and therefore total N concentration, determined. For liquid samples, a small piece of glass fiber filter was placed inside the tin capsule to absorb the sample. The samples were analyzed by combustion-gas chromatography (Elementar Vario MicroCube), and results for all samples are expressed as percent by weight of nitrogen or carbon. The carbon to nitrogen ratio (wt:wt) was calculated from these data.

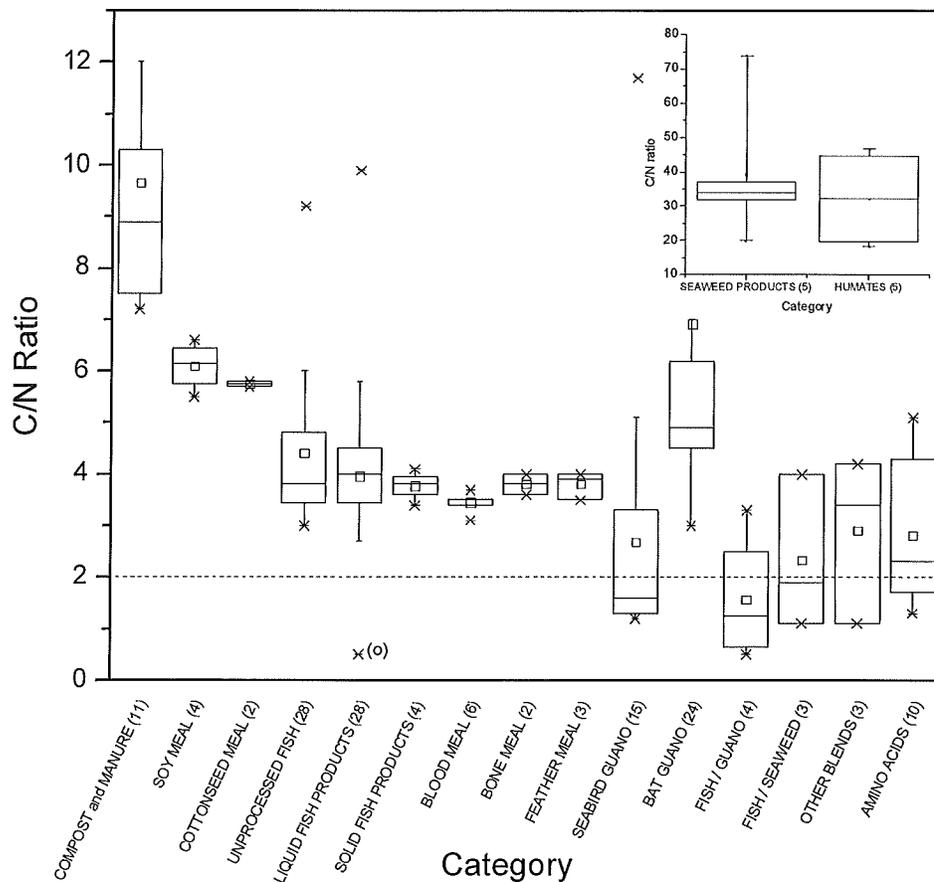


Figure 2. C:N ratios of the different categories of organic fertilizers. The dashed line denotes the threshold value, based on typical protein C:N ratios; organic fertilizers with C:N ratios lower than the threshold might warrant investigation for potential adulteration. Based on 99% confidence intervals, guano and guano blend fertilizers are a possible exception. The lines of the boxplot represent the median, 25th and 75th quartile values, and the whiskers represent the maximum and minimum values used in the calculation. The number of samples is shown in parentheses.

The literature values of C/N, used as potential reference levels for both organic and inorganic fertilizers, were variable and mostly > 2 (Fig. 2). The only exceptions were the seabird guano derived fertilizers, urea, proteins and amino acids, and uric acid. Several of the analyzed samples showed C/N of < 2 (liquid fish products, bat guano, seabird guano, fish/guano blends and fish/seaweed blends) which suggests possibly adulteration of samples. This is consistent with addition of N from a chemical source with small amounts or without C, such as urea and ammonia, which would lower the C/N ratio. This may be the explanation for the outlier sample (o) observed in the liquid fish products which has a much lower value than the rest of the category and even guano fertilizers (Fig. 2).

Nitrogen Isotope Ratio. The relative abundance of $\delta^{15}\text{N}$ was determined with an isotope ratio mass spectrometer (PDZ Europa 20-20 IRMS, UC Davis Stable Isotope Facility). For liquid samples that were difficult to homogenize adequately, the $\delta^{15}\text{N}$ content was also determined by diffusion of the ammonium in the sulfuric acid digest (Sørensen and Jensen, 1991). This allowed a larger subsample to be accounted for, compared to the low mass used for combustion analysis.

Most of the organic fertilizers, had $\delta^{15}\text{N}$ values > 5 (Fig. 3). The exceptions were soybean, feathermeal and seaweed derived fertilizers. Leguminous plants, such as soybeans, certain seaweeds and algae are capable of fixing atmospheric nitrogen ($\delta^{15}\text{N}$ of zero) resulting in very low $\delta^{15}\text{N}$ values. The blends (fish/ guano, fish/ grain, fish/ seaweed and grain/ feather), like the majority of synthetic fertilizers (urea, ammonium sulfate, ammonium nitrate and nitrates) had values $\delta^{15}\text{N} < 5$ as well as less variability. Although not marked, this difference between organic and inorganic fertilizers enabled determination of threshold values for determining potential adulteration. The setting of these values is however complicated by the low values for some organic fertilizers such as seaweed and humate products. A similar study of the $\delta^{15}\text{N}$ isotopic ratios of organic fertilizers by Verenitch and Mazumder (2012) observed ranges and magnitudes of $\delta^{15}\text{N}$ data consistent with that observed in this study.

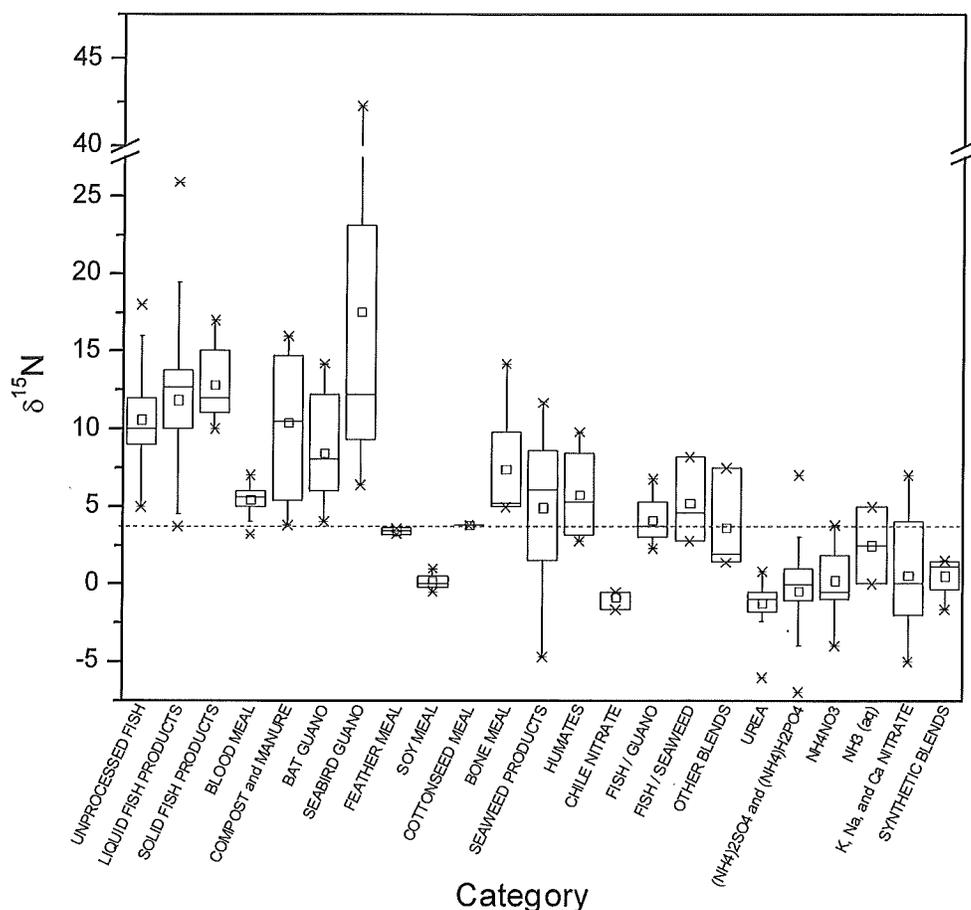


Figure 3. Nitrogen isotope ratios. The dashed line denotes the threshold value, based on the natural isotopic abundance of different materials; an organic fertilizer with a ratio below the line may warrant investigation for adulteration. Based on 99% confidence intervals, seaweed, algae, Chilean nitrate and soybean fertilizers and their blends are possible exceptions. The lines of the boxplot represent the median, 25th and 75th quartile values, and the whiskers represent the maximum and minimum values used in the calculation. The number of samples is shown in parentheses.

Spectroscopy Spectroscopic analysis (single bounce attenuated total reflectance [ATR] Fourier transform infrared [FTIR] spectroscopy and Fourier-transform [FT] Raman) of the organic fertilizers was also performed. ATR-FTIR spectra were collected on a Thermo Nicolet 6700 spectrophotometer (Madison, WI), with 128 scans per sample and a resolution of 4 cm^{-1} . FT Raman spectra were collected on a Bruker RFS 100/s FT Raman spectrometer with a Nd:YAG laser operating at 1064 nm. The spectra were collected at a power level of 100 mW, resolution of 4 cm^{-1} and approximately 256 scans per sample which were averaged.

ATR-FTIR spectra of organic fertilizers and several synthetic fertilizers were combined to create a database of IR spectra. Clear trends based on fertilizer category are evident making this an important point of reference for future spectral comparison (Figs. 4-6).

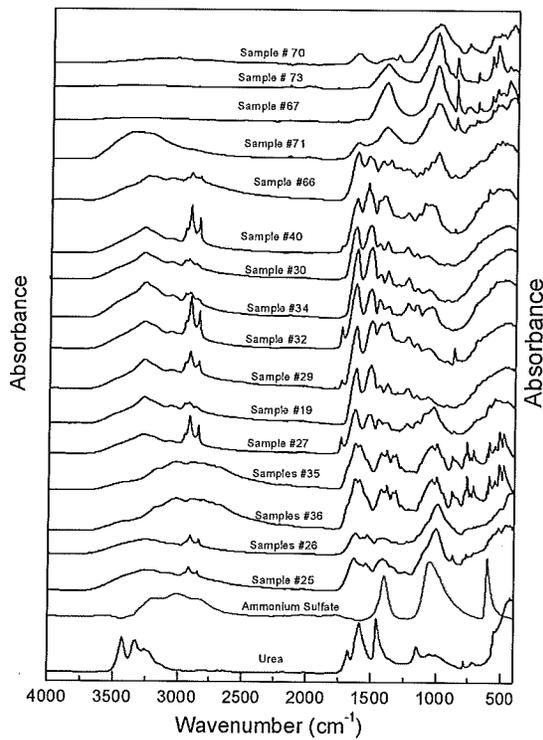


Fig 4. ATR_FTIR spectra of selected bat guano fertilizers

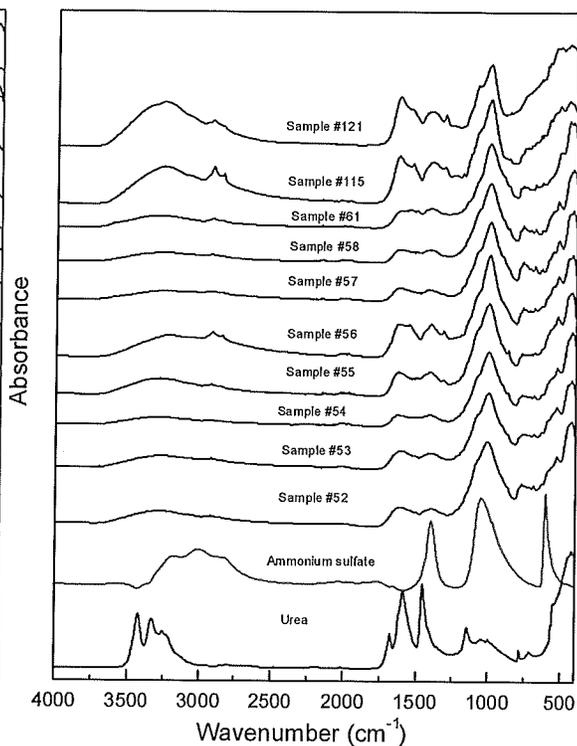


Fig 5. ATR_FTIR spectra of organic compost fertilizers.

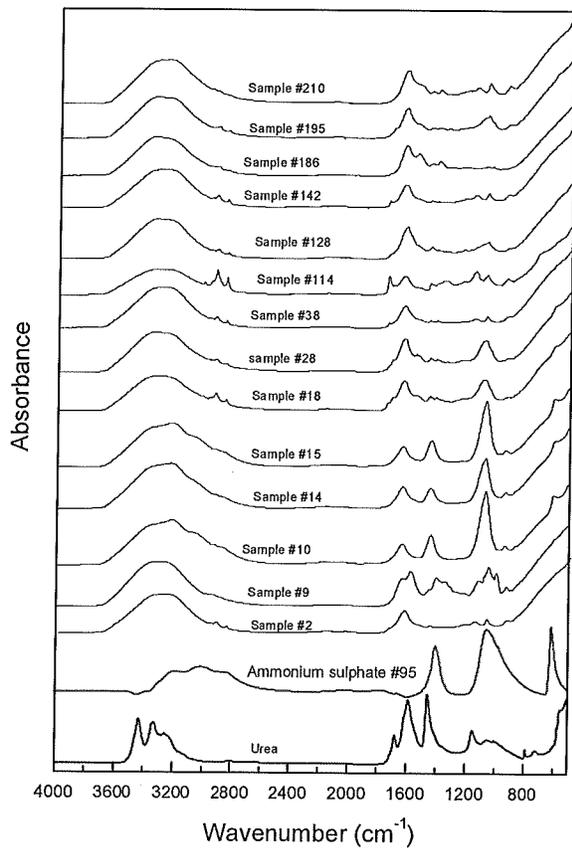


Fig 6. ATR_FTIR spectra of fish organic fertilizers. Includes processed liquid and solid fish,

Selected fertilizer samples were doped with ammonia sulfate and urea (potential adulterants) to test the robustness of ATR-FTIR in detecting their presence.

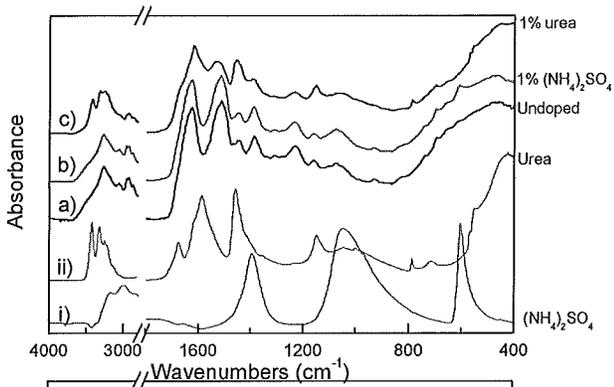


Fig 7. ATR FTIR spectra of i) ammonium sulphate; ii) urea and bloodmeal fertilizer a) undoped; b) doped with 1% ammonium sulfate and c) doped with 1% urea.

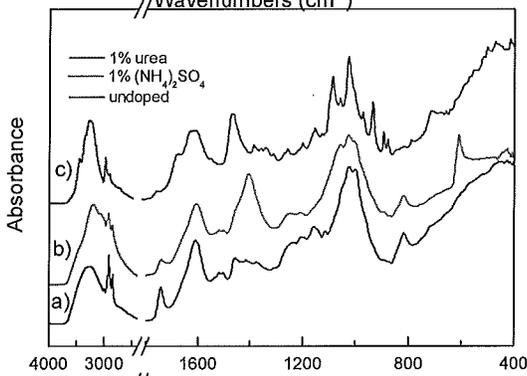


Fig 8. ATR-FTIR spectra of seaweed a) undoped; b) doped with 1% ammonium sulfate and c) doped with 1% urea.

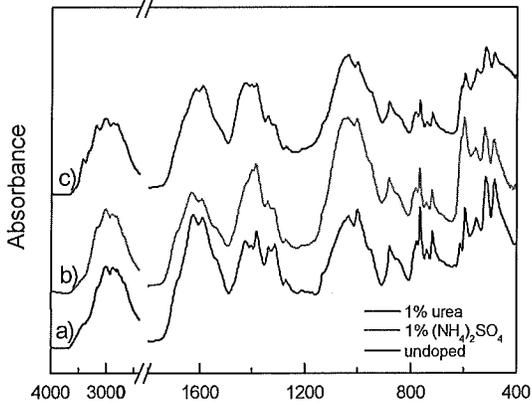


Fig 9. ATR-FTIR spectra of seabird guano a) undoped; b) doped with 1% ammonium sulfate and c) doped with 1% urea.

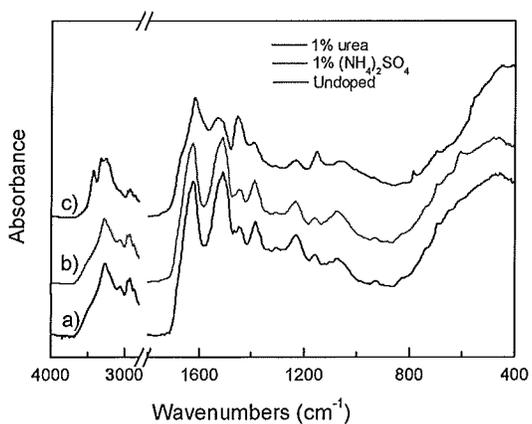


Fig 10. ATR-FTIR spectra of compost a) undoped; b) doped with 1% ammonium sulfate and c) doped with 1% urea.

Spectra of the doped samples showed it was possible to detect the presence of the adulterants (Fig7-10). Using Fig 7 for example, the effect of the addition of urea (trace *ii*) can be seen in the top trace *c* (1 % urea) compared to the undoped sample (trace *iii*). The peaks at approximately 3450 cm^{-1} (N-H vibrational bond stretch); 1450 cm^{-1} (urea N-C-N vibrational bond stretch); and 1600 cm^{-1} (urea C=O vibrational bond stretch) in the doped sample (trace *c*) show the presence of urea. Also post processing of the data by subtraction of the undoped spectra from the doped spectra (example not shown), will give a more clear indication of the presence of the adulterant.

A similar analysis of the ammonium sulfate doped sample (Fig 7 trace *b*) can also be performed and ammonium sulfate (trace *i*) peaks detected (at approximately 1400 cm^{-1} [ammonium sulfate N-H bond deformation] and 600 cm^{-1} [ammonium sulfate SO_4^{2-} bending mode]).

FT-Raman analysis of the organic fertilizer samples also revealed similarities in the spectra of organic fertilizers from the same category (Figs 11-14). As done for ATR-FTIR analysis, selected organic fertilizer samples were doped with the adulterants (Fig 11 traces *b* and *c*) and FT-Raman proved to be more straight forward than ATR-FTIR at detecting the presence of the adulterants with minimal post processing of the spectra required. The presence of ammonium sulfate (Fig 11 trace *i*) and urea (Fig 11 trace *ii*) can be observed by significant peaks at

approximate
ly 980 cm^{-1}
(ammonium
sulfate SO_4^{2-}
stretching
mode) and
 1012 cm^{-1}
(urea N-C-N
bond
stretch).

However,
due to the
complex
sample
matrix that
caused
scattering of

the Raman

signal resulting in a large background noise signal, detection of adulterants was somewhat more challenging in spectra of the compost samples (Fig. 14). One drawback to this technique is that

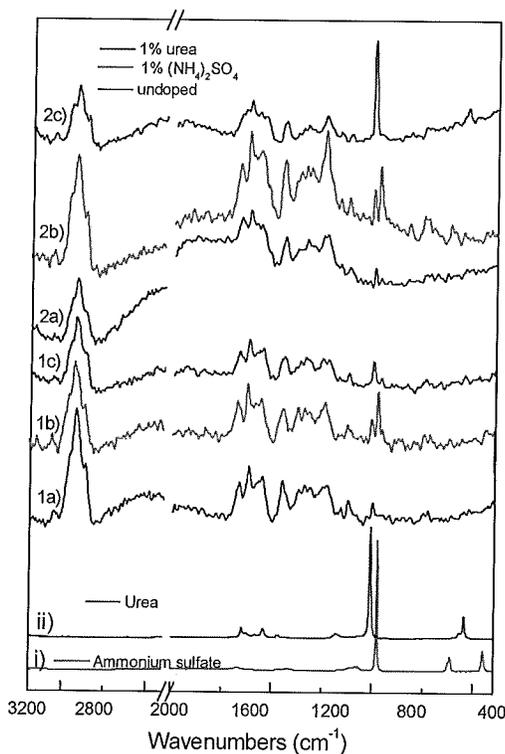


Fig 11. FT-Raman spectra of i) ammonium sulphate; ii) urea and two bloodmeal fertilizer a) undoped; b) doped with 1% ammonium sulfate and c) doped with 1% urea.

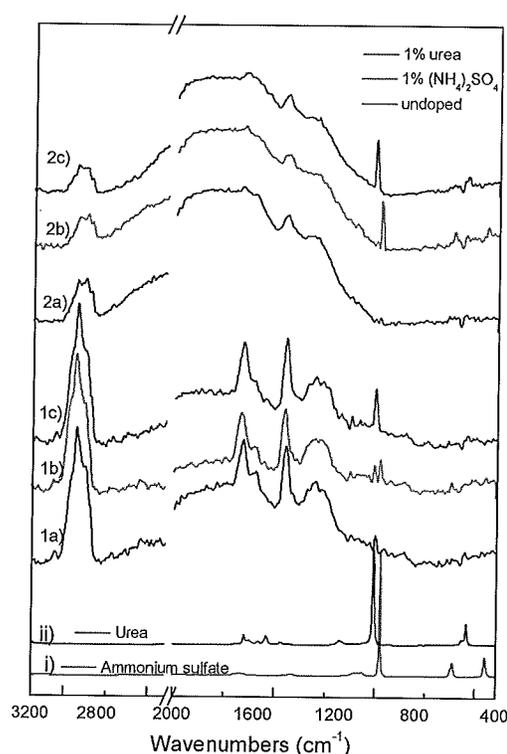


Fig 12. FT-Raman spectra of i) ammonium sulphate; ii) urea and two feathermeal fertilizers a) undoped; b) doped with 1% ammonium sulfate and c) doped with 1% urea.

the greater signal to noise (S/N) ratio of this technique resulted in longer analysis times (8 min per sample) per sample compared to ATR-FTIR (4 min per sample).

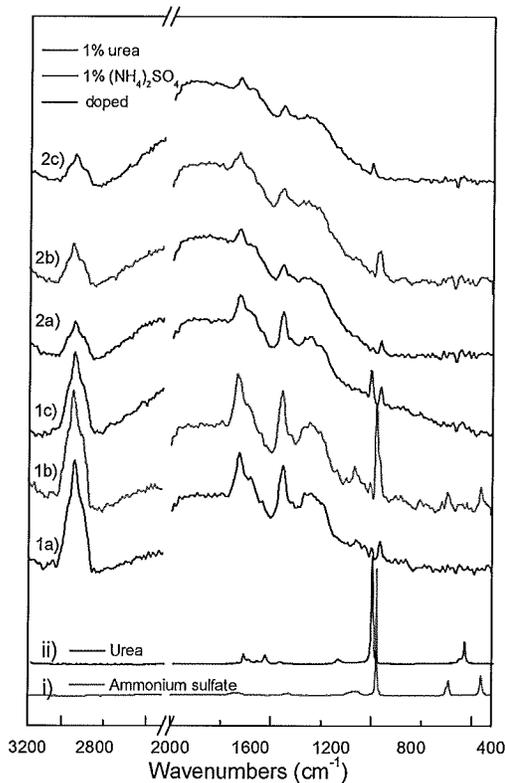


Fig 13. FT-Raman spectra of i) ammonium sulphate; ii) urea and two liquid fish fertilizers a) undoped; b) doped with 1% ammonium sulfate and c) doped with 1% urea.

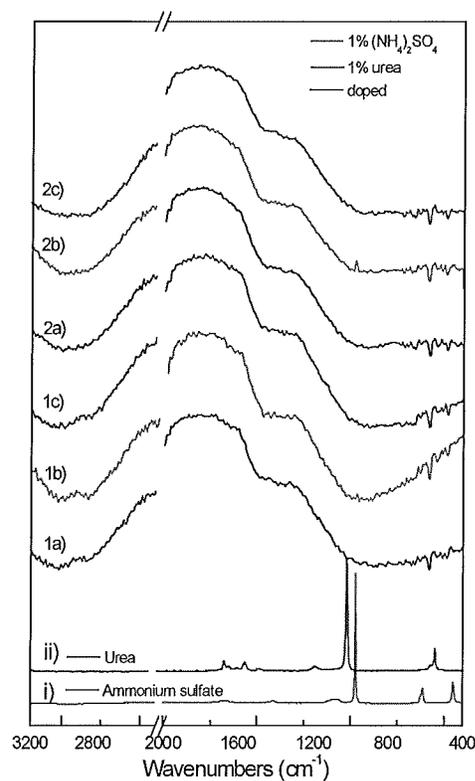


Fig 14. FT Raman spectra of i) ammonium sulphate; ii) urea and two compost fertilizers a) undoped; b) doped with 1% ammonium sulfate and c) doped with 1% urea.

These spectra, combined with those from several synthetic fertilizers have been combined to create a spectral database of 157 spectra. The spectral database currently consists of 41 fish (liquid, solid and unprocessed), 28 guano, 14 blends, 13 compost, 11 seaweed, 8 ammonia, 7 bloodmeal, and 5 feathermeal fertilizers. The remaining spectra are from less popular categories including soy meal, urea and Chile nitrate.

Near Infra-red spectroscopy is already widely used in the agricultural sector for testing the quality of forage and feed making its potential use for organic fertilizer quality determination beneficial. NIRS is dependent on calibrations derived from correlations of spectral properties to chemical information of the test materials and thus to develop powerful and robust calibrations, analysis of multiple samples will be required.

To test the efficacy of this technique, spectra from samples of representative categories of the organic fertilizers were collected using a FOSS InfraXact NIRS spectrometer (scanning range

570-1850 nm). ISIScan software was used to analyze the spectra and develop potential calibration curves for the different categories of organic fertilizers. Although results from several sample categories show trends in the spectra e.g. liquid fish (Fig. 15) and bat guano (Fig. 16), attempts to perform chemometric analysis of the to determine calibration equations for the different categories did not yield meaningful equations. Analysis of more categories of samples and over a wider range of wavelength (> 1850 nm) will likely be more successful and should be considered in the future.

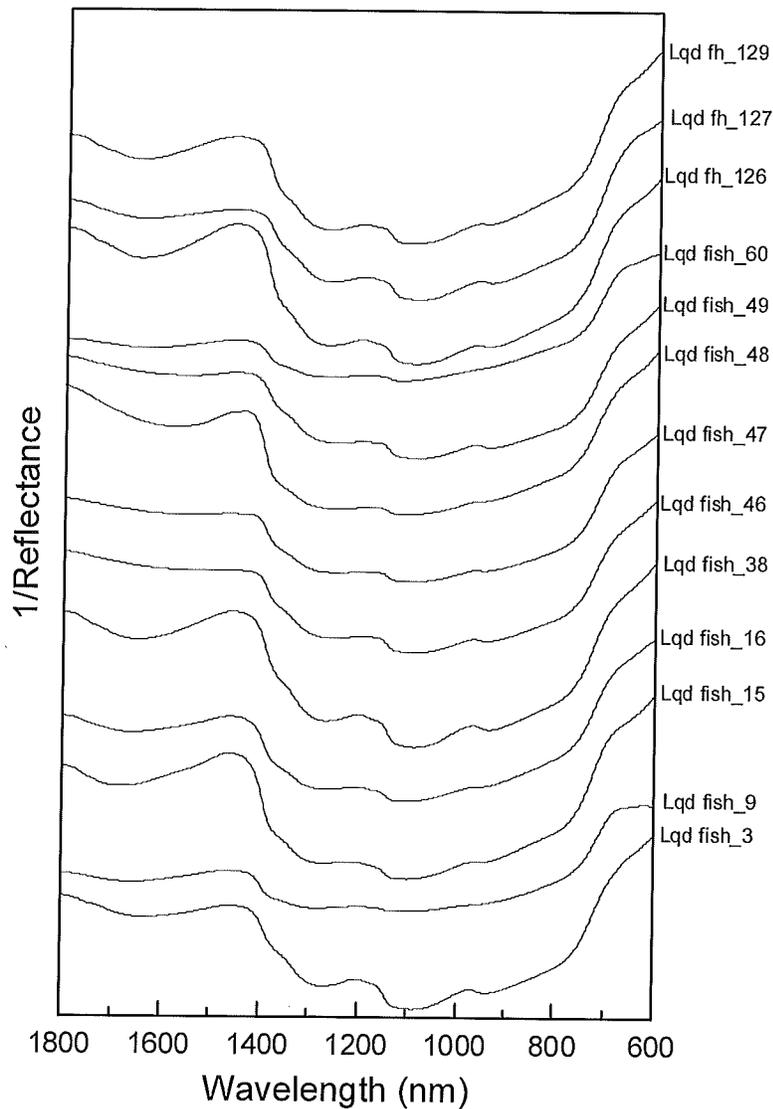


Figure 15. NIRS spectra of several samples of liquid fish organic fertilizer.

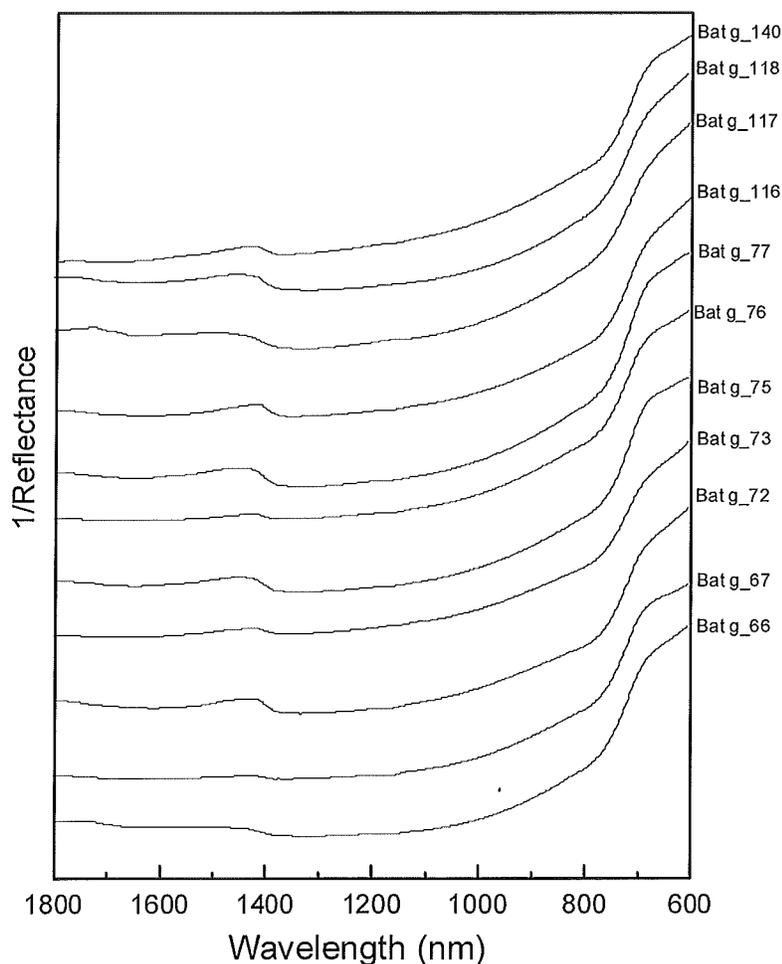


Figure 16. NIRS spectra of several samples of bat guano organic fertilizer.

Trace metal analysis. Trace metal composition is another proposed proxy investigated as a means of detecting potential synthetic adulterants added to organic fertilizers. Concentrations of trace and rare earth metals vary amongst the organic fertilizer categories and can readily be related to composition. Some synthetic nitrogen fertilizer sources potentially have elevated amounts of certain trace metals e.g. arsenic in the hydrogen gas generated from coal burning (the hydrogen is subsequently used to manufacture ammonia through the Haber process).

According to an established digestion method, the fertilizers (0.5 g) were placed in digestion tubes and treated with concentrated nitric acid (5 ml) and hydrogen peroxide (1 ml). The tubes were placed in a block digester and heated to 120 °C. Digestion was considered complete when no solid particles were present and a clear or yellow liquid remained. The samples were filtered and appropriately diluted before analysis using inductively coupled plasma mass spectroscopy (ICP-MS). The samples were analyzed for 17 trace metals and 15 rare earth

elements. Results show concentrations comparable to those observed in studies of similar organic fertilizers as well as expected elevated concentration of certain trace metals in particular organic fertilizer categories e.g. iron (Fe) content in the bloodmeal and compost fertilizers (Fig. 17) and copper (Cu) and manganese (Mn) (Fig. 18 and 19 respectively) in compost fertilizers.

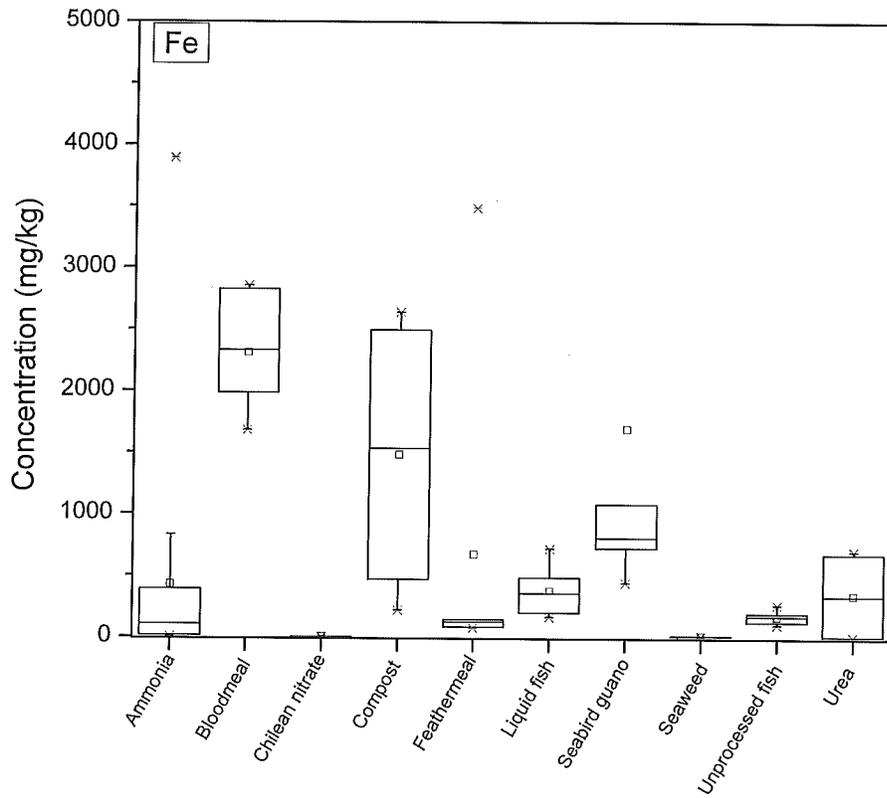


Figure 17. Concentration of iron (Fe) in nitric acid and hydrogen peroxide extracts of organic fertilizers.

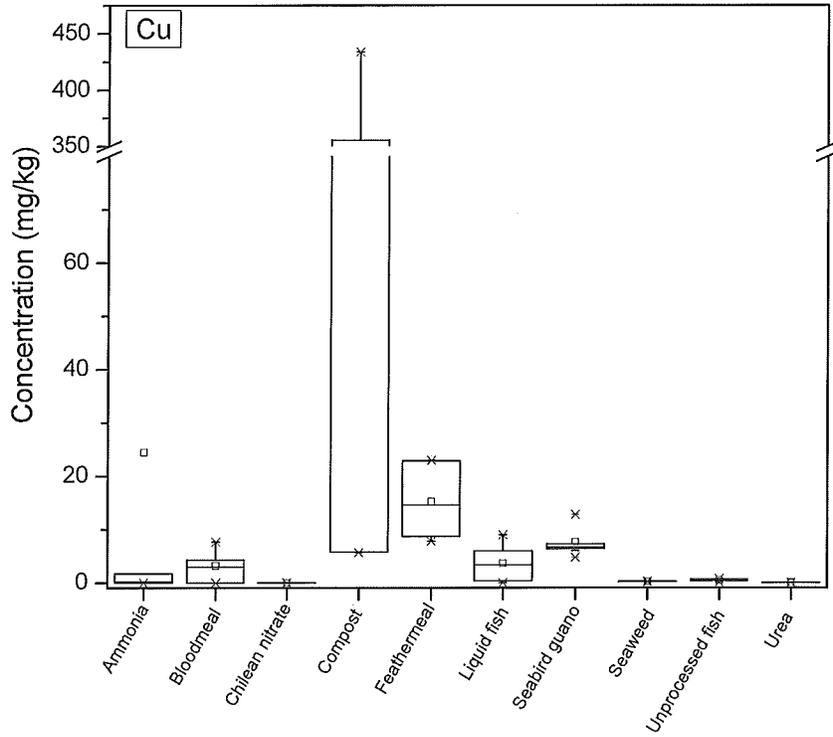


Figure 18. Concentration of copper (Cu) in nitric acid and hydrogen peroxide extracts of organic fertilizers.

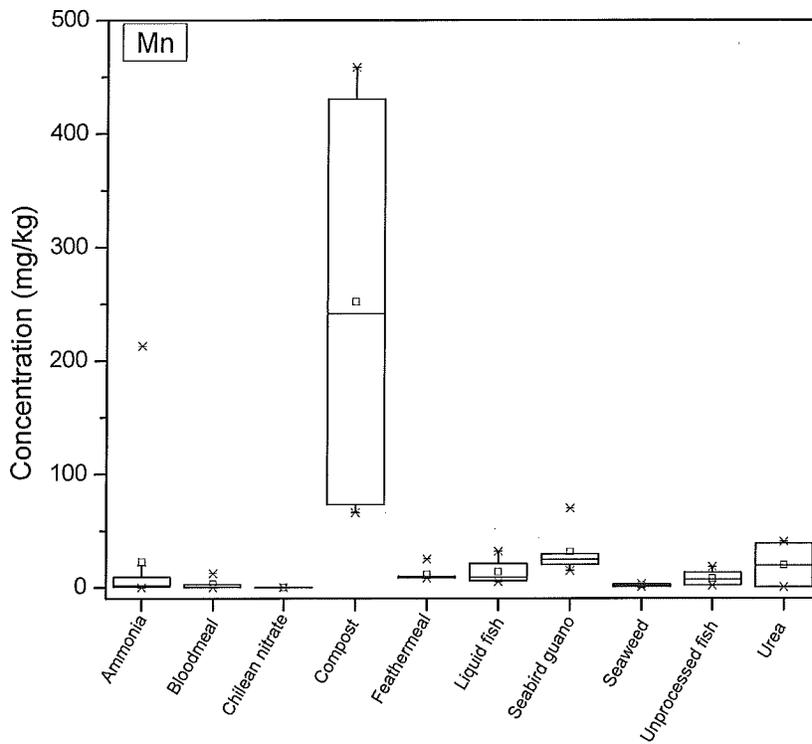


Figure 19. Concentration of manganese (Mn) in nitric acid and hydrogen peroxide extracts of organic fertilizers.

Though informative, the trace metal dataset did not afford significant different results between the synthetic and non-synthetic fertilizers to warrant inclusion in the protocol.

Task 4. Evaluate and summarize the dataset.

The data were evaluate and analyzed according to appropriate statistical methods meant for publishing in scientifically peer reviewed journals. Upper and lower quartile values of the analytes were calculated and utilized to show ranges of values per category.

Task 5. Construct usable guidelines to be used by test labs and regulatory agencies.

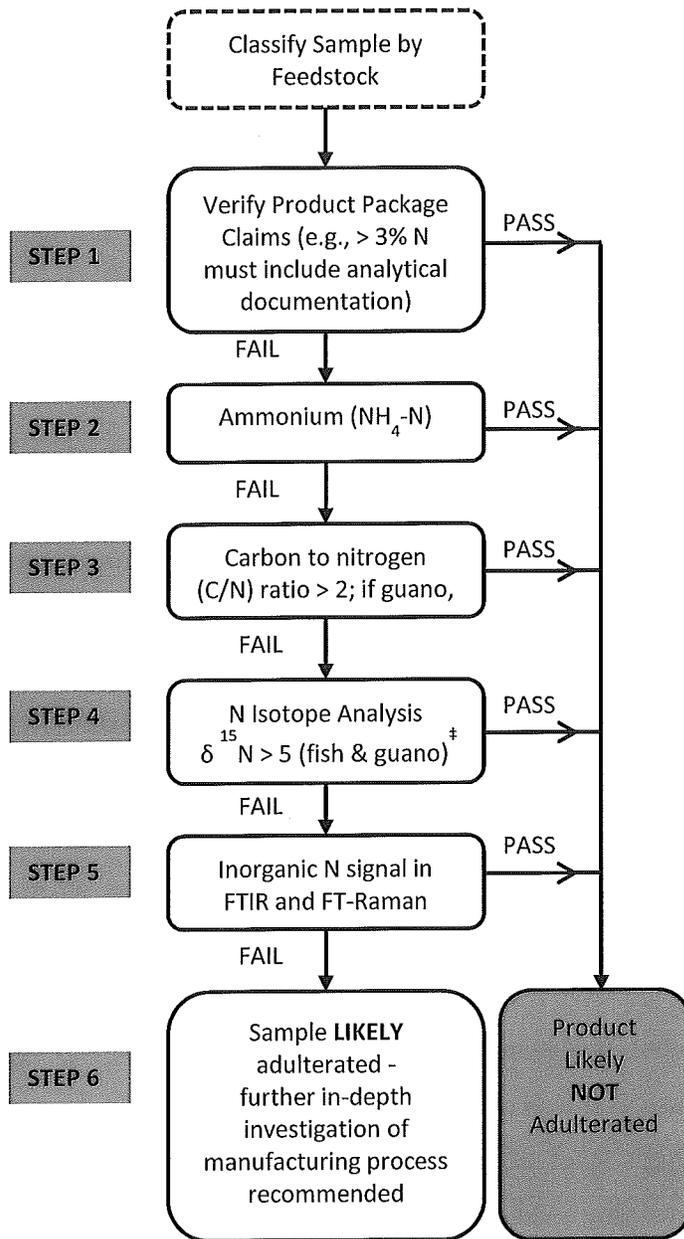
Through integration of literature and laboratory information, a systematic protocol for the detection of potential adulterants in organic fertilizer is presented. This is the first such protocol and provides a rapid and simple methodology for use by test laboratories and regulators of organic fertilizers. This protocol will assist in attaining the objectives of the California Department of Food and Agriculture Fertilizer Research and Education Program (FREP), a program within the industry funded Fertilizing Materials Inspection Program, which include checking the truth of labeling claims and testing the compliance of the guaranteed analyses (CDFA, 2012).

A comprehensive database of $\delta^{15}\text{N}$, C/N ratio and $\text{NH}_4\text{-N}$ has been built up for the different categories of organic fertilizers with the data showing good agreement between the literature and analyzed values. This database will provide a readily accessible resource for comparison of fertilizer samples and provide a cheap and rapid mechanism of flagging fertilizer samples for more comprehensive analysis. The results from the database compilation have also facilitated the setting of thresholds of expected values in ammonium content, C/N ratios and $\delta^{15}\text{N}$ (Fig. 20).

The rationale for the protocol is based on the most likely adulterants of products marketed as organic fertilizers being various forms of ammonia (e.g., aqua ammonia or ammonium sulfate), or possibly urea, which is itself converted to ammonium carbonate and ultimately ammonia in the presence of urease (Volk, 1959). These adulterants are favored primarily due to their low cost and high nitrogen content. Since nitrogen isotope ratio, carbon to nitrogen ratio, and ammonium content most effectively separate different classes of organic and synthetic materials, they best help indicate the presence of adulterants. Furthermore, they provide the greatest opportunity to compare with literature data, and are relatively easy to measure (and therefore most useful to a testing lab). Although not particularly investigated, adulteration

through the addition of nitrate salts is also possible but this protocol would still be able to detect this through the C/N ratios and $\delta^{15}\text{N}$ values.

The protocol shows a six-step systematic flow-chart to follow when investigating the potential adulteration of an organic fertilizer selected in order of increasing effort and expense.



† Chile nitrate is an exception.

‡ Seaweed, algae, and soybeans are exceptions, requires FTIR or FT-Raman analysis.

Figure 20. Flow chart of the organic fertilizer verification protocol proposed for use by test labs and regulatory agencies.

This protocol minimizes the potential of incorrectly flagging a fertilizer as potentially adulterated. No single metric alone is a sufficient determinant to classify a sample as adulterated or unadulterated (Verenitch and Mazumder, 2012).

Initially, identifying the category to which a sample belongs and also knowledge of the components constituting the fertilizer is necessary in order to interpret the results of analysis and use the protocol effectively, since values which are suspect for one kind of sample may not be suspect for another kind.

Step 1. Prior to any laboratory analysis, attention is directed toward the label and/or price of a product as a simple way to identify where to begin analytical efforts. One of the most important metrics to focus on is the nitrogen content. As

stipulated by the USDA, organic fertilizers labeled as containing $> 3\%$ N must be evaluated through a material evaluation program (USDA, 2009). This program requires oversight from third party evaluators capable of verifying compliance of the component inputs (including processing and handling of the product) independent of the crop producer and fertilizer manufacturer. The suppliers of such products should have this data for their products.

Step 2. As a first analytical step to evaluating a product, the ammonia (ammonium) content may be estimated in the field. For common, well-characterized categories of products such non fish or guano based fertilizers, this is an easy preliminary step toward selecting samples for further investigation. Any product in these categories found to contain more than 1% nitrogen as ammonium (10000 mg L^{-1}) should be retained for further analysis. Setting a threshold for samples that naturally have ammonia (e.g. fish products) is more challenging and potential adulteration of these samples can be detected by other tests in the protocol.

Step 3. The ratio of carbon to nitrogen (C:N; w/w) in any material is a good indication of how “organic” a material is. It is not necessary to check the ammonium concentration if C:N is determined. The nitrogen in organic materials is derived primarily from protein, for which the C:N does not fall below 1. The same is true of guano, although guano may contain much of its nitrogen in the form of uric acid rather than protein. For the C:N threshold the average confidence interval (CI) of the 5 lowest materials (i.e., seabird guano; fish guano; fish/seaweed; amino acids and other blends) is 1.28 ($P < 0.01$). For guano alone the CI at the same significance level is 1.09. Any materials that show C:N ratios below these values probably (99%) contain inorganic N. However, if values are higher than these thresholds it is impossible to say whether a given material has organic N only. However, while theoretically possible, these are conservative values, since it is rare that any protein would have a C:N of less than about 2, hence a threshold value of 2 has been selected for this protocol. For guano fertilizers, a reasonable threshold, based on literature values and the current database, is a C:N of 1. An obvious exception is Chile nitrate, an approved product with a naturally high level of nitrogen relative to carbon. Due to the potentially low C:N ratios of blends containing guano and Chile nitrate, questionable samples may warrant further investigated using Step 4.

Step 4. The ratio of nitrogen-15 to nitrogen-14 (expressed as $\delta^{15}\text{N}$) is another parameter which rarely falls below a certain threshold value in natural material, with few exceptions. Fish tissue and guano, for example, do not have a $\delta^{15}\text{N}$ value of less than 5, and values are typically greater.

A threshold value of 2.3 was calculated from the CI for all non-organic sources using 0.01 as the significance level and accounting for variations in sample size (number of values used in the calculation of each product's CI). The CI is a range of values where the probability of obtaining a sample mean similar to that of the non-organic sources is low ($P < 0.01$). Any products that go beyond this threshold are almost certainly not adulterated. It is important to note, however, that plants that rely on symbiotic N uptake (e.g. legumes) can have $\delta^{15}\text{N}$ values as depleted as non-organic N sources. So in fertilizers where biomass from N-fixing plants has been added it may be difficult to distinguish their signature from that of non-organic sources.

Step 5. The two spectroscopic techniques provide additional tools for investigating the authenticity of the organic fertilizers. Detection of adulterants by FTIR can be performed by 1) comparing sample spectra with spectra of samples from a similar feedstock; 2) comparing the sample spectra with that of urea or ammonia sulfate and looking for characteristic peaks for ammonia or urea and 3) intentionally doping the sample with urea or ammonium sulfate and analyzing for increased magnitude in peaks characteristic to the adulterants as in Figs 7-10. For FT Raman, similar methods of analysis can be used. The spectral interpretation of FT Raman is much simpler with clear peaks associated with potential adulterants being evident (Figs 11-14). Both techniques require no sample preparation and very little sample set-up resulting in high throughput of samples. The cost of the instrumentation may be prohibitive hence the use of these techniques is suggested after all other less expensive options of verification are exhausted. The success of the spectroscopic techniques bodes well for similar analysis of solid fertilizers using near-infrared reflectance spectroscopy (NIRS), which is routinely used in plant/ forage and feed tissue analysis to determine components such as crude protein content. The liquid nature of most organic fertilizers does present a challenge for NIRS due to water being a strong absorber of NIR light (Stuth et al., 2003).

Step 6. When a sample clearly fails all or some of the tests, adulteration is likely and warrants further investigation of the manufacturer and process of production.

Task 6 Evaluation: Measuring Success

To investigate the robustness of the protocol, selected samples of adulterated and unadulterated organic fertilizer were sent to independent laboratories. A total of four samples were sent, with one of the samples intentionally doped. All the laboratories were successful in identifying the

adulterated fertilizer sample (Sample 4 Table 2) despite the differences in the values of the different parameters, particularly the ammonia content.

Table 2: Table of results from wet chemistry analyses performed by laboratories.

	Sample #	Total Carbon (%)	Total Nitrogen (%)	C:N Ratio	NH ₄ -N (%)
Lab A	1	15.2	4.8	3.2	0.67
	2	14.0	5.2	2.7	1.99
	3	10.9	2.9	3.7	0.14
	4	2.6	4.4	0.6	3.31
Lab B	1	15.4	4.5	3.5	0.04
	2	14.6	5.0	2.9	0.12
	3	10.7	2.8	3.8	0.02
	4	5.3	6.1	0.9	0.55
Lab C	1	17.1	4.9	3.5	0.27
	2	16.3	5.5	3.0	0.83
	3	11.2	2.6	4.3	0.12
	4	3.5	6.6	0.5	4.35
Our Lab	1	15.2	4.0	3.2	0.34
	2	14.2	4.8	2.9	1.31
	3	10.7	2.4	4.5	0.41
	4	2.8	5.9	0.5	6.50

Continued refinement of the protocol is anticipated and we are already working with the CDFR to test out the protocol on samples of interest.

Task 7. Outreach

1) Oral presentation: *The authenticity of organic fertilizers*, Dr. William Horwath
Russell Ranch Sustainable Agriculture Field Day, May 31, 2012; Davis CA

2) Oral presentation: *The use of ATR-FTIR and FT-Raman in the investigation of potential organic fertilizer adulteration*, Dr. Fungai Mukome, Western Soil Science Society Meeting, June 26, 2012; Davis CA

3) Oral presentation: *Developing testing protocols to ensure the authenticity of fertilizers for organic agriculture*, Dr. William Horwath, 2012 Western Soil Science Society Meeting, June 26, 2012; Davis CA

4) Oral presentation: *Developing testing protocols to ensure the authenticity of fertilizers for organic agriculture*, Dr. Fungai Mukome, 20th Annual Fertilizer Research Conference, October 30-31, 2012; Modesto CA

5) Published Manuscript: Mukome, F. N. D., Doane, T. A., Silva, L. C. R., Parikh, S. J., and Horwath, W. R. (2013). *Developing testing protocols to assure the quality of fertilizer materials for organic agriculture*. California Agriculture 67(4), 210-216.

6) Oral presentation: Mukome, F. N. D., Doane, T. A., Silva, L. C. R., Parikh, S. J., and Horwath, W. R. (2013) Developing Testing Protocols to Investigate Potential Adulteration of Fertilizer Materials Used in Organic Agriculture. ASA, CSSA, and SSSA International Annual Meeting, Tampa FL. 303-3

Potential Impacts:

There is a pressing demand for methodology to validate claims of fertilizers labeled “suitable for organic production” in order to confirm their authenticity. A standard and vigorous protocol to address this urgent need will bring transparency and authentication to the array of organic fertilizer products on the market. The systematic protocol developed through this research has the potential to be used by test laboratories and regulatory agencies to detect adulteration of organic fertilizers and soil amendments adulterated with synthetic nitrogen sources. Given the current growth of the organic industry, the importance of having a framework for detecting adulteration of fertilizer inputs cannot be understated and may be pivotal in the monitoring of the industry.

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Project Title: Developing testing protocols to assure the quality of fertilizer materials for organic agriculture

Grant Agreement Number (13-0223)

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Start Year/End Year

July 1, 2013 to June 30, 2014

Location

The research was performed at the University of California, Davis in the Department of Land, Air and Water Resources facilities.

County

The research involved procuring samples of fertilizers from multiple counties in California, multiple states in the US and multiple countries.

Highlights

1. The project developed an extensive database of organic and synthetic fertilizers that established the natural ranges for the chosen properties of these materials that can be used to distinguish between pure, or unadulterated, and adulterated materials.
2. Developed a stepwise protocol test that commercial labs and regulatory agencies can follow to identify organic fertilizers that have likely been adulterated by synthetic fertilizers.
3. The stepwise protocol was validated in a blind test with commercial labs to identify adulterated organic fertilizers with 100% accuracy.

Introduction

The organic industry is on pace for a six fold increase in sales during the next five years having grown from \$6.1 billion (2000) to \$29 billion (2011) in sales. California is the national leader in organic farming with the highest number of organic farms, land under organic production and organic sales. Being an industry heavily based on trust in the “organic” brand, growth of the organic industry is partly threatened by any activity or factor resulting in potential loss of grower and consumer confidence in the integrity of the brand. One such factor is the authenticity of the organic fertilizer used to grow organic food and oversight of this remains a major challenge. Several incidence of fertilizer alteration have been documented recently. This research provided insight into analyses that can be used to provide quality control in the production and testing of organic fertilizers and amendments. The major objectives of this study are to: 1) construct a database physical and chemical properties of materials; 2) establish chemical properties (i.e., NH_4^+ , stable isotopes, and C:N) of existing marketed materials to determine purity and or adulteration; and 3) develop a stepwise protocol that laboratories and regulatory agencies can use identify fertilizers that may have been adulterated by synthetic fertilizers

Methods/Management

The following properties of fertilizer materials were measured: carbon, nitrogen, and phosphorus content, carbon isotope ratio, nitrogen-isotope ratio, solid content, density, dissolved organic carbon, dissolved organic nitrogen, ammonium content, and nitrate content. In addition to these chemical properties, spectroscopic analysis (single bounce attenuated total reflectance [ATR] Fourier transform infrared [FTIR] spectroscopy and Fourier-transform [FT] Raman) was also performed.

Findings

This study presents an approach and methodology for detecting, with high probability, adulteration of organic fertilizers and other amendments by synthetic fertilizer and other chemical nutrient sources. The low cost and relative simplicity of the protocol will ensure regulators and test laboratories can routinely and efficiently test commercially available organic fertilizers. A set of common methodologies includes analysis for ammonia and total carbon and nitrogen that are readily available to soil test labs and the California Department of Food and Agriculture (CDFA) Inspection Services Center for Analytical Chemistry. These minimum analyses are likely to flag greater than 90% of samples that could be adulterated with synthetic sources of nitrogen. Any organic farmer suspecting adulterated fertilizer could submit a sample of fertilizer to a commercial soil test lab or the CDFA to determine with high probability whether the fertilizer is authentic. Additional stable nitrogen isotope and spectroscopic analysis is likely to confirm adulteration of fertilizer samples. Since these analyses are not routine for soil test labs, the California Department of Food and Agriculture Inspection Services Center for Analytical Chemistry may consider accepting these suspected samples for further analysis. Legitimate producers of fertilizers will benefit by having a defined set of testing protocols to ensure the quality of their products and manufacturers of adulterated organic fertilizers and amendments will face the appropriate scrutiny to ensure the authenticity of their products.