

**COLLECTION AND PROCESSING OF PLANT, ANIMAL AND SOIL
SAMPLES FROM BIKINI, ENEWETAK AND RONGELAP ATOLLS**

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Table of Contents

List of Table and Figures.....	iv
Abstract.....	1
Sample Collection Strategy.....	1
Sample Gridding Method.....	1
Field Collection of Coconut Samples.....	2
Field Collections of Other Edible Plant and Animal Species.....	5
Field Collection of Water Samples.....	6
Well Pumping Procedure.....	6
Field Collection of Soil Samples.....	8
Field Log Book.....	10
Labeling.....	10
Shipment of Samples to LLNL.....	10
Agricultural Permits.....	11
Laboratory Preparation and Processing of Edible Plant Samples.....	11
Preparation.....	11
Processing.....	11
Laboratory Processing of Coconut Samples.....	11
Other Plant and Animal Samples.....	12
Freeze-Drying of Plant and Animal Samples.....	14
Northstar Freeze-Dryers.....	14
Manifold Freeze-Dry System.....	16
Mobile II Freeze-Drying System.....	16
Processing of Freeze-Dried Samples.....	17

Laboratory Preparation of Coarse Vegetation, Leaves and Grass	17
Laboratory Preparation of Liquid Samples.....	18
Coconut Juice Sample Processing Procedure.....	18
Ocean, Lagoon and Well Water Processing Procedure.....	20
Laboratory Preparation of Soil Samples.....	20
Purchasing and Shipping Field Equipment and Materials.....	21
Biannual Decontamination and Overhaul of Laboratories and Equipment.....	23
Acknowledgment	25
References.....	25
Appendix A:Soil and Vegetable Permit and Agreement.....	A-26

List of Table and Figures

Table 1. Laboratory dissected portions of fruit, nut and vegetable samples for analysis.....	12
Figure 1. Grid equipment field deployment.....	3
Figure 2. Cross-sections of LLNL coconut development categories.....	4
Figure 3. Well pumping array.....	7
Figure 4. Laboratory flow diagram of edible vegetation sample processing procedure.....	13
Figure 5. Northstar freeze-dryer.....	15
Figure 6. Coconut juice processing procedures flow diagram.....	19
Figure 7. Soil sample processing procedures flow diagram.....	22
Figure 8. Time line.....	25

Abstract

The United States used the Marshall Islands for its nuclear weapons program testing site from 1946 to 1958. The BRAVO test was detonated at Bikini Atoll on March 1, 1954. Due to shifting wind conditions at the time of the nuclear detonation, many of the surrounding atolls became contaminated with fallout (radionuclides carried by the wind currents). Lawrence Livermore National Laboratory's (LLNL) Marshall Islands Project has been responsible for the collecting, processing, and analyzing of food crops, vegetation, soil, water, animals, and marine species to characterize the radionuclides in the environment, and to estimate dose at atolls that may have been contaminated. Tropical agriculture experiments reducing the uptake of ^{137}Cs have been conducted on Bikini Atoll. The Marshall Islands field team and laboratory processing team play an important role in the overall scheme of the Marshall Islands Dose Assessment and Radioecology Project. This report gives a general description of the Marshall Islands field sampling and laboratory processing procedures currently used by our staff.

Sample Collection Strategy

The Marshall Island's field team is responsible for the environmental sampling required for characterization of radionuclides on Bikini, Enewetak, Rongelap and Utirik Atolls. To accomplish this task, a sampling scheme designed to cover each island as evenly as possible is necessary. Gridding the islands at 50 and 100 meter increments gives the best overall representation with the time and resources available. On islands where other environmental experiments have been done independent of the island characterization, the sampling schemes have been tailored to each individual experiment. On islands that had extensive sampling done prior to our present characterization assignment we chose to use the original sight numbering schemes to make efficient use of the existing data base.

Sample Gridding Method

Where practical, a grid is the preferred method. It gives even coverage of the island's land mass, and yields the most accurate positioning of sample sites for mapping. An accurate survey is important when using the grid method. The survey team first clears a line of sight through dense jungle areas to aid in measuring and staking each grid site for flagging and identification. Each grid is identified using a number, or preferably grid coordinates. Grid lines are measured to the beach high tide mark, and to other prominent land marks to accurately position the grid for subsequent mapping.

Using a tripod mounted Brunton compass, the survey leader directs the rest of the team members along the grid line compass coordinates.

A bulldozer, when available, is used to clear survey tracks along grid lines. A military pickup truck and a tractor-backhoe follow. An illustration of equipment deployment is shown in Figure 1. The pickup truck is used as a mobile platform for the plant and soil sampling crews. The tractor/backhoe is used to dig narrow soil profile trenches. The survey crew selects edible, fruit bearing plant species near the survey grid stake. The coconut, *Cocos nucifera*, is the dominant edible plant species in the Marshall Islands. Other edible plant species growing in close proximity to our sites are collected also.

Field Collection of Coconut Samples

In describing the condition of the coconut's growth development for data comparison purposes, a scheme to categorize them has been established. The artificial age development categories as shown in Figure 2 are drinking coconuts, copra, and sprouting coconuts. The category of drinking nut refers to a coconut normally utilized by the native Marshall Island population for drinking and eating. Proper age development characterization can only occur after the husk is removed and the inner nut is examined.

The senescent coconut leaf fronds and inflorescences bearing the older copra nuts are at the bottom of the tree canopy. The nuts preferred for sampling are referred to as "drinking coconuts."

The inflorescences are studied from the ground before climbing the tree. After identifying the area of the canopy containing "drinking coconuts," the climber selects the nuts for the sample. Where trees are collected annually, climbers collect the coconuts without the aid of pole climbing spikes to avoid damaging the coconut tree trunk. The nuts are dropped to the ground and husked. If the husker determines that the nuts are not at the optimum developmental stage, the climber may be directed to sample a different inflorescence. When drinking nuts are not available, younger drinking nuts with little meat or copra nuts may be taken instead.

In the field, the fibrous coconut husk is removed. A sample usually consists of eight coconuts. Occasionally, it is necessary to sample a tree which has only produced one or two nuts.

In the collection of coconuts, the juice is removed from each nut in the field. A small hole is made and the coconut juice is drained into a graduated cylinder. The juice volume is individually measured, recorded, and homogenized with others of the same sample. A 1 liter sample aliquot of the homogenized juice sample is collected in a labeled bottle, and frozen for laboratory processing. For quality control purposes, 10 percent of the coconut juice samples are collected in 2 liter aliquots for duplicate samples.

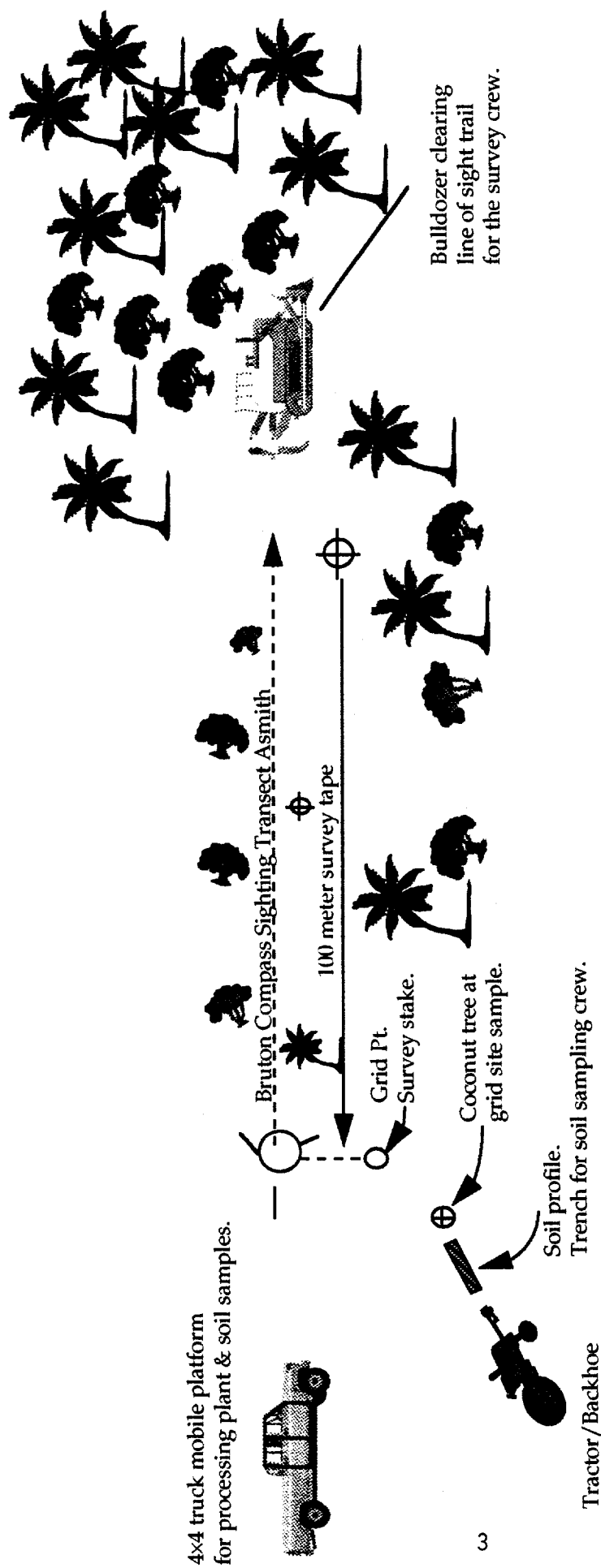


Figure 1. Grid Equipment Field Deployment.

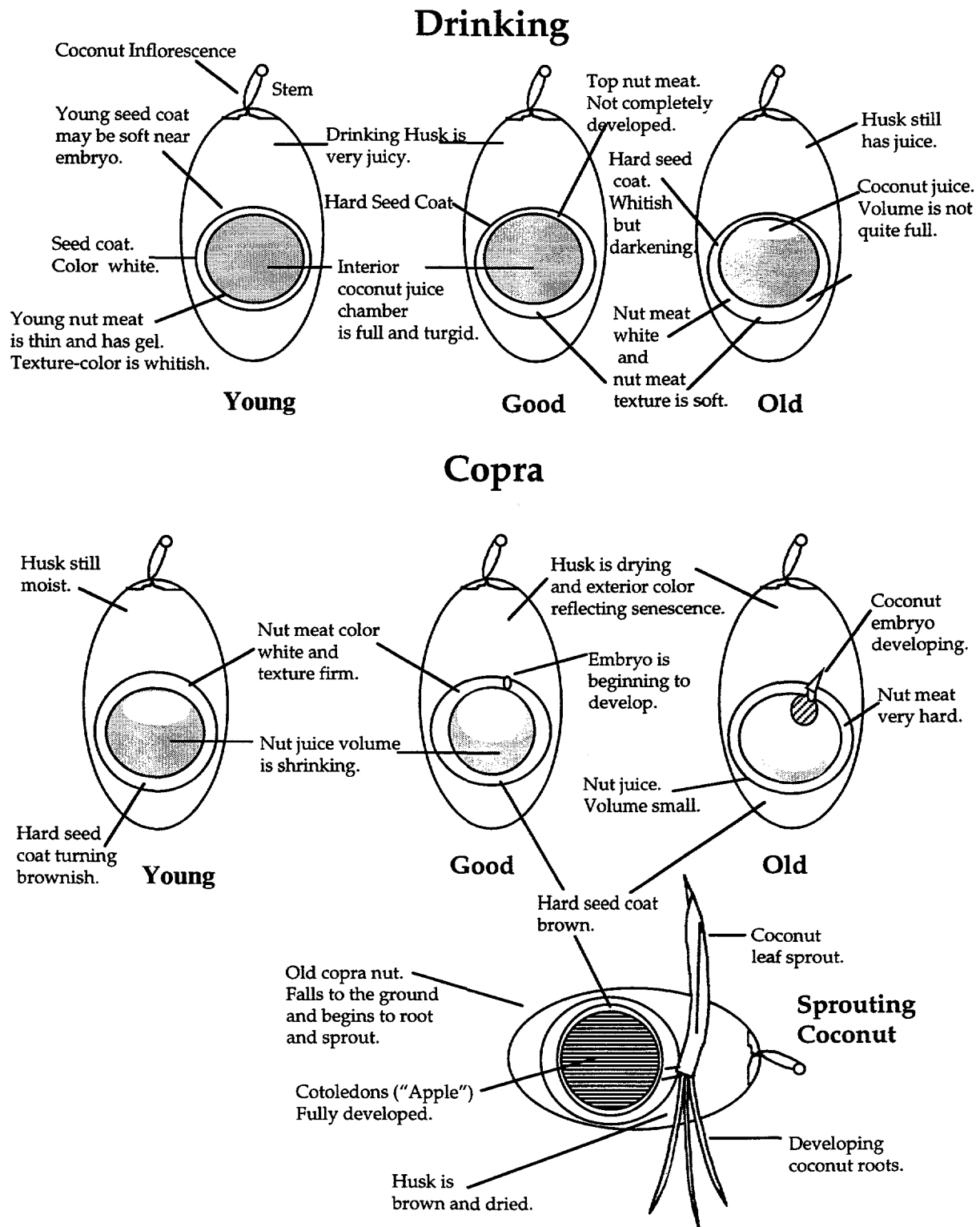


Figure 2. Cross-sections of LLNL coconut development categories.

The coconuts are double bagged in 12 × 30 in, 4 mil. plastic bags, and sealed with vinyl tape. The sample bag is labeled with field log number, sample description, experiment, tree identification, island name and date. Both the bottle of coconut juice and the packaged, drained coconuts are placed in frozen storage at the earliest opportunity.

Field Collections of Other Edible Plant and Animal Species

Other edible plant samples are collected as close as possible to a ripened state. Notes are recorded in the Field Log Book describing the precise condition of the vegetation samples. By collecting fruit in a similar growth stage as that utilized by the native population, the data accurately represents the input to the dose assessment models. Care is taken to eliminate soil contamination. In general, the dissection of the vegetation samples in the field is not done, because it is a very laborious, time consuming job and greatly limits the field work that can be accomplished. It also is undesirable because, it increases the chance of soil contamination. Dissection is done in the laboratory where the samples can be kept clean, thus preventing any contamination and ensuring sample integrity.

Pandanus, sometimes called "screwpine" (ref. Murai), is the second most readily available edible plant species grown in the Marshall Islands. *Pandanus* has both male (staminate) and female (pistillate) trees, both quite distinct. (ref. Murai). The female trees are inspected for ripe fruit by the climber. The ripe fruit is split open and the *Pandanus* "keys" are broken free of the central fruit stalk. They are placed into a doubled, labeled sample bag. A three quarter bag of *Pandanus* sample keys is taken as the sample aliquot. If the fruit is small, more than one fruit is required for a sample. If ripe *Pandanus* is not available, the green fruit is collected. The condition of the fruit and other relevant information is noted in the Field Log Book.

Breadfruit trees of the Batak dak (seedless) and the Mijiwan (seeds) varieties grown in close proximity to villages. The trees are often quite large. The climbers cut or break the breadfruit stem, dropping the breadfruit to the ground. Care must be taken to clean the fruit before placing it into the sample bag. Ripe fruit often become very soft after being frozen and are difficult to clean before laboratory processing. Six large ripe breadfruit make an adequate sample aliquot, but more are collected if the fruit is green and small.

Papayas, bananas, and limes are fruits occasionally encountered around village areas on the islands. They are also grown in experimental plots. These fruit are collected whole, and as ripe as possible. Between 10 and 20 papaya make an adequate sample. The sample aliquot for bananas range between 20 and 30 fruits. A lime sample is usually about 30 fruits. These fruits are bagged whole, and sent back frozen for laboratory dissection of tissues such as skin, edible flesh and seeds.

Another edible plant common to the Marshall Islands is *Tacca leontopetaloides*, commonly known as mokmok or arrowroot. The edible portion is the tuber which

develops on the plants root system. Their shape is irregular varying in diameter from 1 to 8 centimeters, with a thin brown skin and a white starchy interior. (Murai et al.,1958). The plants are dug up and the tubers are collected. The soil is washed in the field prior to packaging and freezing.

Squash of several types are occasionally encountered around villages and are collected whole, sealed into labeled sample bags, frozen and dissected back at the LLNL plant laboratory.

Other types of plant samples are routinely collected. Coarse vegetation, such as leaves, woody plant parts, and grasses are collected as part of our island characterizations. Leaf samples from edible plants are collected for comparison to the fruit.

Animals, coconut crabs, chickens, several species of birds and eggs are collected on the islands when available. Large animals, such as pigs, are dissected in the field, and aliquots of important tissues are taken. Crabs, fowl and birds are collected whole, bagged, labeled and frozen.

As soon as possible, all samples are placed into a freezer container aboard the research vessel. They are frozen to prevent fermentation, and molding of plant parts, and to kill insects living on the plants. Care is taken to ensure that the samples are not allowed to thaw. Freezing causes the cell wall to rupture.

Field Collection of Water Samples

Samples of ocean, lagoon and well water are collected as part of the island characterizations. These samples are collected in 30 liter plastic bottles. The bottles are steam cleaned and rinsed with 3 Normal nitric acid and water before being shipped back to the field collection sites for reuse.

Well Pumping Procedure

The following are the procedural steps used in well sampling:

1. The well pumping equipment array is laid out at the work area and the connections are checked for tightness (Figure 3). Sample identification and location are recorded in the pumping log book.
2. Lower the intake end of the tubing to the desired sampling depth, and record the depth in the log book.

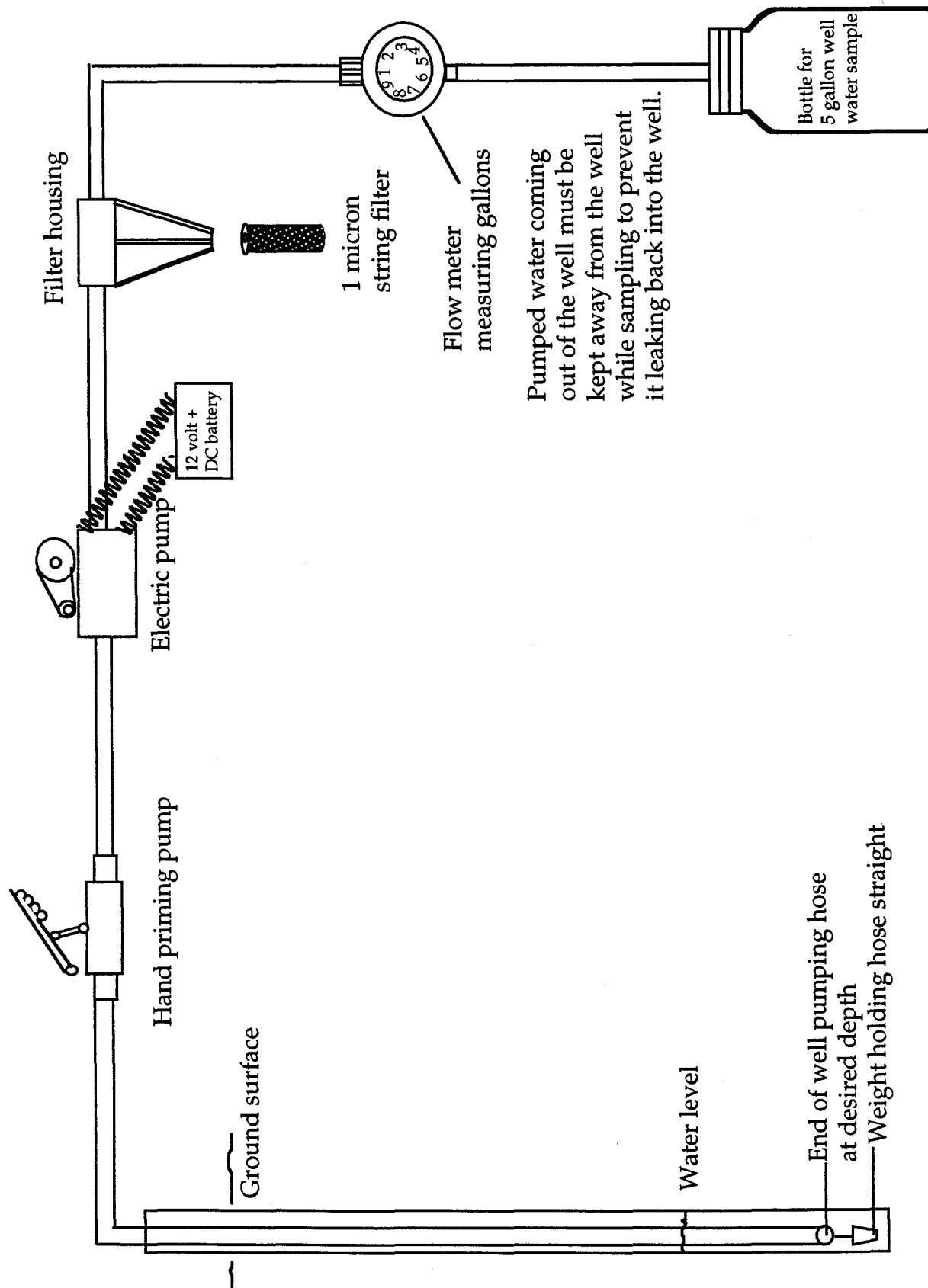


Figure 3. Well pumping array.

3. Attach the battery to the pump, and start the pump. Rinse the pump array and clean the filter chamber with the water that is to be sampled. If necessary, use the in line, hand pump to assist the small electric pump to initially prime the intake line.
4. Stop the pump, after priming and rinsing the pumping array, open the filter canister chamber and inspect it. It must be is clean, and the have it's o-ring gasket in place. The clean 1 micron string filter may then be positioned in the filter holder and the filter assembly re-assembled.
5. The initial water meter reading and start time is recorded in the pumping log book, and the pump is restarted. The filter canister, and the rest of the pumping array connections, must then be inspected and any leaks corrected. Well sample aliquots are monitored frequently throughout the collection period. Salinity, conductivity, temperature, and flow measurements are recorded in the pumping log.
6. The first 10 gallons are used to triple rinse the 30 liter Nalgene bottle. Pump 30 gallons of water through the 1 micron filter and collect the 5 gallon water sample in the bottle from the last 10 gallon through the filter.
7. The 5 gallon water sample is taken when the gallon meter reads 20 gallons of water through the filter. The tube is removed from the bottle, and the time recorded when the meter reaches the 30 gallon mark.
8. The filter canister is opened carefully and the 1 micron string filter is extracted, placed into a clean plastic bag and sealed with wide vinyl tape. The water filter, and water sample are labeled with the sequential field log number and sealed. Similarly the water sample is labeled with it's field log number, date, site identification and location information.
9. To ensure than any plutonium in the sample stays in solution, 50 milliliters of 4 molar hydrochloric acid is added to each 5 gallon water sample only if the sample is to be processed for plutonium.

The amount of water filtered and collected in the sample bottle is varied depending on the predicted activity. Usually 20 or 30 gallons are passed through the 1 micron string filter for well and lagoon water samples. These filters, collected from well and lagoon samples, are sent to our chemistry group for analysis. Water samples taken in open ocean are usually not filtered.

Field Collection of Soil Samples

Soil samples are collected as profiles in several depth increments. The usual increments are 0-5, 5-10, 10-15, 15-25, 25-40, and 40-60 centimeters. Sometimes soils are collected at 60-80 cm and 80-100 cm depth. Each increment is assigned a

separate field log number, and descriptions of each increment are recorded in the field log book.

Soil profiles are usually taken in conjunction with a plant sample. Ideally, the profile reflects the soil in the root zone of the plant. On occasion, multiple profiles may be taken around a plant sampling site to more accurately define the activity levels in those particular soils. Multiple profiles around the same tree are disruptive to the tree's root system and to existing soil horizons, and is avoided unless necessary. When resampling with greater density around a given tree or within a given experimental plot boundary, the use of less disturbing soil coring techniques is preferred.

After the narrow soil profile trench is made, it is examined for anomalies. An area of the trench exhibiting somewhat clear, undisturbed soil horizons is selected and cleaned so that discreet sample increments, free of contaminants, may be obtained. Undecomposed surface litter is removed and discarded, along with small herbs and grasses growing in the area to be sampled. Any layers of peat, covering the surface soil horizon are collected if found. The surface soil usually consists of some partially decomposed organic and dark organic soil, depending on the location. Areas closer to the lagoon or ocean sides usually have very little or no organic layer. An area about 25 sq cm is needed for the surface soil sample (0-5 cm increment), although more may be taken if the soil is very rocky.

A step method is used in sampling the rest of the increments. After the surface 0-5 cm increment is removed, the area of the sampling depression is enlarged to about 40 cm x 35 cm to the sample depth. The surface is skimmed to clean any particles of the layer above from contaminating the next sample increment. The 5-10 cm. increment is then taken from the front center area, avoiding the edges of the step which has been widened to contain any contaminating particles falling on to the step from the upper layers. Each step is cleaned and expanded similarly to its lower limit after the increment is taken. Sample increments routinely collected are 0-5cm., 5-10cm., 10-15cm., 15-25cm., 25-40cm. and 40-60cm. Samples are bagged, labeled, and sealed with tape. Approximately 2 kilograms of soil for each sample increment is collected.

Soil coring techniques are used to sample the soil in a given area and minimize the mixing of the existing soil column horizons that results using the trench profile method. Soil coring works best in sandy, damp soils between 0 and 30 cm. It is used to integrate all the soil taken with the one core, or for combining several cores taken over a broad area. The most accurate soil core is obtained in a given area by a single coring. When taking multiple cores at progressively deeper depths, the risk of soil contamination from upper layers is increased. It is advantageous to do multiple depth soil coring after a rainy period. The water helps keep the sand and soil particles from falling from the upper layers onto the deeper core surfaces, thereby decreasing the possibility of cross-contamination between core depths. It is important that the coring tube be decontaminated between coring increments.

Field Log Book

As samples are taken, information about each sample is recorded in the Field Log Book. Under each entry are notes regarding atoll, island, experiment name, sample description, location of sample collected, identifying tree or experiment plot numbers, date, remarks regarding the condition of the sample, and special instructions for processing the sample. Maps showing the location of the sample in relation to its surroundings are often included so that it is possible to return later the same location. The use of navigational charts, island maps and aerial photos, when available, help position the sampling locations as accurately as possible.

At each site, the trees are marked with a permanent stainless steel tag labeled with the tree number. To make the trees more visible, 12 inch wide, surveyors flagging is taped and stapled in a band around the tree's trunk. The tree number is spray painted on the trunk. Permanent sampling sites are marked near by with metal fence posts. The methods of marking the trees or sampling sites are subject to weathering. They must be checked and renewed every other year to ensure relocation of the site.

Labeling

Every sample is assigned one or more field log numbers in the field. If the sample is dissected into two or more parts, more than one number is required. For example, each coconut sample has two sample numbers—one for the coconut meat and one for the fluid. Each sample aliquot is labeled with a field log number. For plants not dissected in the field, the sample number of the fleshy edible portion of the fruit is used to identify it for initial storage and shipment. Reserved field log numbers for the expected remaining portions are recorded in the log book. When the sample is thawed in the laboratory, the numbers for the other sample tissue aliquots are determined by reviewing the field log book. The field log numbers are composed of two digits identifying the year, a single letter code identifying the atoll and another signifying the island.

Shipment of Samples to LLNL

Soil, plant, and animal samples are shipped frozen on barges from Kwajalein to Honolulu, and on to the Oakland Army Base. This method of shipment is the most cost effective. Surface shipments must clear U.S. Customs at the Oakland Army Base. Occasionally, high priority samples are shipped Military Air Command (MAC). This method of shipment significantly increases the shipping cost.

When U.S. Customs releases the frozen sample shipping container, the samples are transported by truck to LLNL. Upon arrival, the container is unloaded, inventoried, checked for shipping damage and organized by atoll, island and experiment. Vegetation and animal samples are stored in freezers.

Agricultural Permits

The importation of plant, animal, and soil samples requires several permits. The U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA), and the State of California, Department of Food and Agriculture Division of Plant Industry, Pest Exclusion Division (CSDA) are the issuers of the permits used in the program. The LLNL sample processing facilities have been inspected by representatives of the USDA, the CSDA and the Alameda County Dept. of Agriculture to approve the sample processing protocol. The field collection and laboratory processing procedures are in compliance with the regulating standards outlined by these agencies.

The plant importation permit requires that all fruit and vegetable parts be frozen below 20° F. The soil importation permit requires that, 1) all soil be shipped in sturdy leak proof containers, 2) containers must be clearly marked, 3) logbook records of date of arrival, amount, origin, method of treatment, and date of disposal are maintained, 4) shipments of samples to other laboratories must be approved by regulating agencies, 5) laboratory personnel take all precautions to prevent the escape of any potential pest, 6) standard operating procedures are followed, 7) and soil is handled as quarantined material until sterilized. Copies of the plant and soil permits and agreements are shown in Appendix A.

Laboratory Preparation and Processing of Edible Plant Samples

Preparation

Samples are removed from the freezer to thaw. They are placed in plastic trays to catch any liquid as they thaw. Pint containers are pre-labeled with sample information, and any special processing instructions are noted. Tissue samples are dissected, and each type cut up into small pieces and put in their pre-labeled containers. Small pieces maximize the surface area, and increase the rate at which samples can freeze-dried. Each container is weighed. The wet weight is recorded in the sample log book and entered in the computer. Table 1 shows the usual sample portions produced by dissection during sample processing.

Processing

Laboratory Processing of Coconut Samples

The dissection of the samples varies for the different plant species. Coconut is partially dissected in the field, and the juice fraction is processed by a separate procedure. The husk of the coconut is only saved for processing when it is edible

Table 1. Laboratory dissected portions of fruit, nut and vegetable samples for analysis.

Plant Description	Flesh	Skin	Seed	Juice	Plant
Coconut (Drinking & Copra)	X			X	
Coconut (Sprouting)	X				
	(coco. apple)				
Coconut Palm (Young)					X (heart)
<i>Pandanus</i>	X				
Breadfruit	X	X	X		
Papaya	X	X	X		
Banana	X	X			
Lime	X	X	X		
Mokmok (Tacca)	X	X			
Taro	X	X			
Morinda	X	X	X		
Corn (Several Varieties)	X	X	X		X
	(cob)	(husk)	(kernel)		(husk)
Squash (Several Varieties)	X	X	X		
Bean (Several Varieties)		X	X		
		(pod)	(bean)		
Cucumbers	X	X	X		
Millet			X		X
					(stover)
Okra	X		X		
Pepper	X		X		

and is processed with the coarse vegetation procedures. The hard seed coat (shell) that lies between the husk and the whitish meat of the nut is left intact to support the meat during shipment. It is easier to remove the shell after the coconut has been frozen and thawed. A flow diagram of the coconut processing procedure is shown in Figure 4. The meat is diced into small chunks, and the hard coconut shell is discarded.

Other Plant and Animal Samples

Pandanus fruit are commonly used as food in the Marshall Islands. The fruits consist of numerous segments attached to a central stalk and only a bag of segments is taken as a *Pandanus* sample in the field. In the laboratory, the segments are thawed in a tray to catch the juice released during thawing. The end of the segment that was attached to the central stalk contains the sweet juice that is utilized for food.

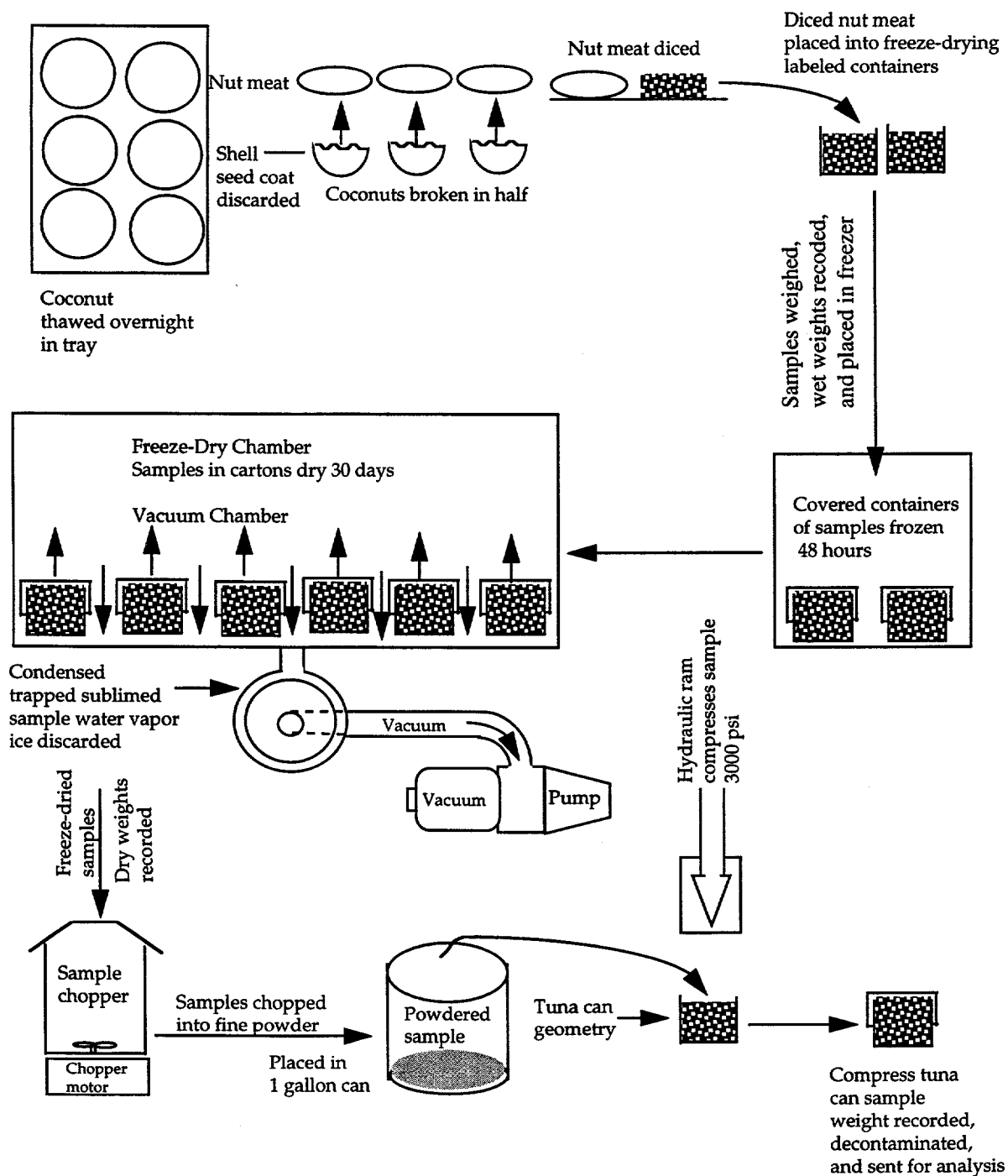


Figure 4. Laboratory flow diagram of edible vegetation sample processing procedure.

This section of the segment is dissected into smaller pieces and put with any juice into one pint sample containers. The rest of the segment discarded.

Breadfruit sample are allowed to thaw and the skin is removed. Both fractions are chopped into small pieces and put in separately labeled containers. The skin is not generally considered part of the edible fruit. If seeds are present, they are also removed and processed as a separate sample.

Papayas are divided into three separate sample fractions; skin, flesh, and seeds. Bananas are separated into skin and edible flesh. Limes are divided into three sample fractions; skin, flesh (and juice) and seeds. Tacca is peeled and divided into skin and edible starchy flesh. All of the fractions of each sample are processed, freeze-dried, milled and analyzed separately.

Animal species are collected when they are available. Free roaming pigs and chickens are the domestic stock commonly encountered. They are dissected, and their major organs processed as separate samples for analysis. Birds and coconut crabs are occasionally collected. Bird eggs are collected when available. and fish are taken periodically. Only enough is taken for a sample.

After weighing each container of diced sample, they are covered with filter paper and put in a freezer for at least 48 hours before freeze drying. The filter prevents cross-contamination of individual samples in case of accidental rapid changes in vacuum pressure. These sample containing significant amount of water and are routinely processed in our freeze dryers.

Freeze-Drying of Plant and Animal Samples

Three different freeze drying systems are in use in our processing laboratory. Each system is used at a different stage in the freeze drying process.

Northstar Freeze-Dryers

The Northstar freeze dryers remove the majority of the water. A modified model 3666 is being used. It has a very large capacity drying chamber. Modifications included a larger condenser compressor that uses AZ-50 refrigerant, and the addition of shelves to better utilize the space. The chamber size on the units is 1730 cm in length and 900 cm in diameter and accommodates approximately 550 containers full of dissected frozen sample tissue. Refrigeration coils in the wall ensure that samples will remain frozen even when the chamber is not under vacuum. Between the vacuum pump and the sample chamber is a freezer trap that catches most of the water vapor sublimated off the samples. Service and maintenance of the Northstar unit requires about two man hours per day, when it is operating correctly. The basic elements of the Northstar freeze-dryer is shown in Figure 5.

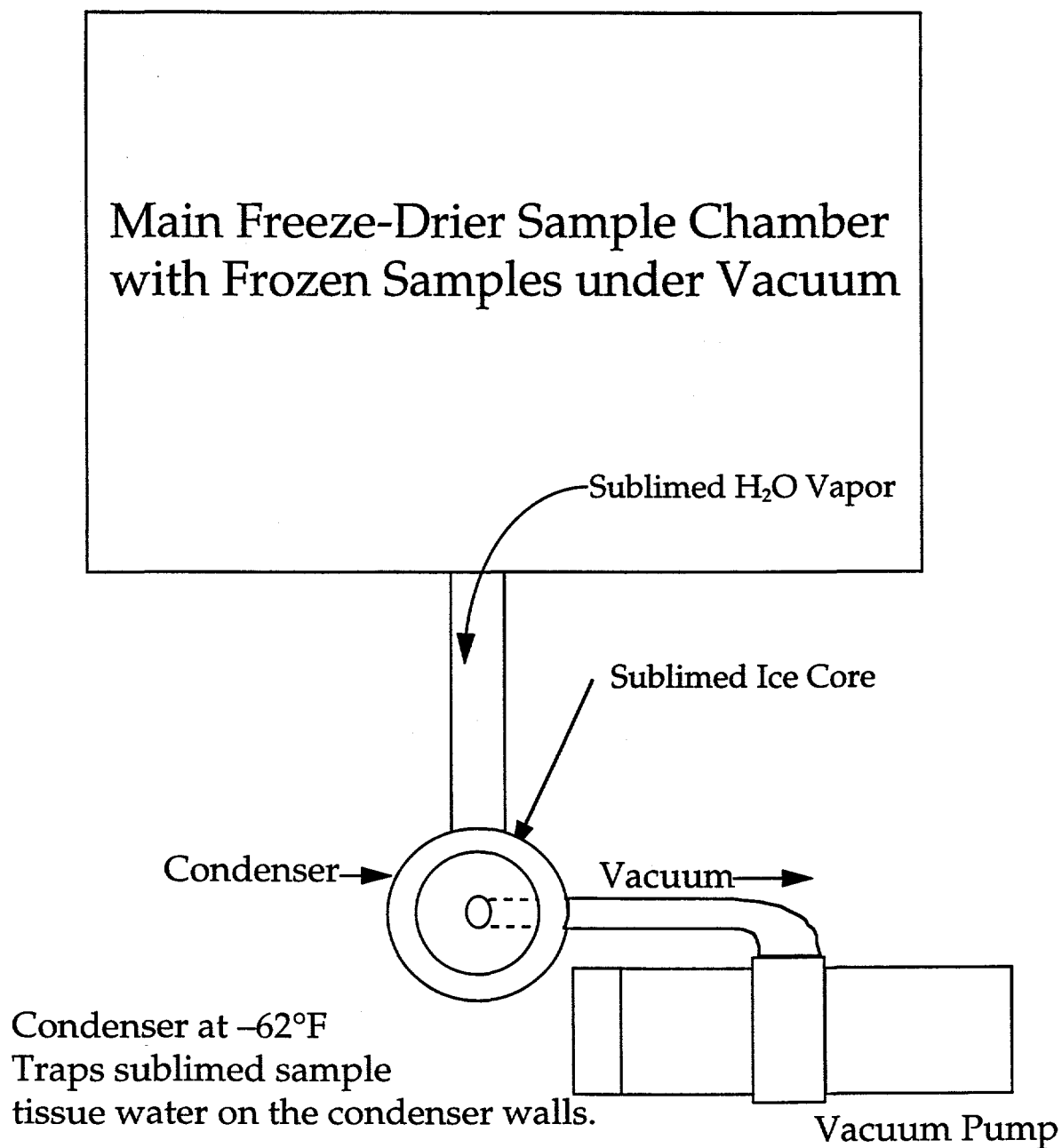


Figure 5. Northstar freeze-dryer.

It is important that the frozen sample containers be hard frozen before they are put onto the shelves of the freeze drying chamber. The drying chamber is loaded quickly, and the condenser is prechilled down to about -65° F temperature. The condenser compressor is working constantly to maintain the -65° temperature as the sublimated ice vapor is trapped on the walls. The trapped ice on the condenser walls should not be allowed to accumulate to more than approximately 3/8 of an inch in thickness. The freeze drying unit is shut down, and defrosted and the condenser cleaned at least twice in a 24 hour period. When the ice no longer exceeds the 3/8 of an inch limit for a 24 hour period, servicing the condenser once a day is adequate.

As the ice on the condenser walls becomes less than 1/4 inch thick, the samples are allowed to thaw slightly each day. The frozen tissue water is sublimed from the surface of the tissue cube inward. Tissue bound water, initially held in the cellular structure when hard frozen, ruptures the cell walls and is released through partial thawing to be readily absorbed into the previously freeze dried, porous surface layer. This careful thawing allows a pulse of tissue water to migrate from the center of the tissues frozen core to the porous outer surface, greatly increasing its re-frozen surface area. This small amount of tissue water is quickly refrozen as the vacuum is reapplied. Sublimation of this small pulse occurs slowly during the next 24 hour period. Using this technique a chamber full of samples can be freeze dried in a little more than a month.

After about a week, the sample containers are transferred to the main freeze-drying manifold, which holds about 510 one-pint containers.

Manifold Freeze-Dry System

The freeze-drying manifold is used for the final drying of the samples. It has eleven chambers, each able to hold 49 one-pint tissue containers. Each chamber is connected to the multiport manifold with its own CO₂ trap plumbed in between. The four vacuum pumps require oil changes every week. The system is designed so that the pumps operate in pairs; each pair has its own liquid nitrogen trap, in line, between the vacuum pumps and the manifold. This system requires a great deal of servicing. The two LN traps are cleaned once every 48 hours. Each trap uses 160 liters of LN twice during this period.

The CO₂ traps are cleaned of trapped ice every 24 hours. The ethylene glycol slurry is changed every two weeks, as it becomes diluted with water vapor. It takes about 14 days to completely dry tissue in the manifold.

Mobile II Freeze-Drying System

The Mobile II Freeze-Drying System is a small, low capacity freeze-drying system that can handle about 54 one-pint tissue containers. It is similar in operation to our

Northstar unit, and requires very little daily servicing. We use this unit for samples that may still require a little more freeze-drying after most samples on the manifold system are done. The Mobile II unit can finish drying these samples in two to four days, and, consequently, frees the drying chamber of the manifold to be refilled with samples from the Northstar unit.

Processing of Freeze-Dried Samples

The freeze-dried samples are re-weighed after removal from the freeze-dry units. The dry weights are recorded in the sample log book and entered into the computer. The containers are organized by field log number. When all the tissue containers from a sample are dried and accounted for, they are moved to the plant grinding laboratory.

In the plant grinding laboratory, the freeze-dried plant samples are organized by experiment priority. The contents of each container is ground individually in a blender, then combined with the contents of the others belonging to that sample. The resulting finely ground powder is homogenized and placed in a clean plastic-lined, tared can, and labeled with a barcode.

One or more aliquots are canned (salmon can geometry) from this homogenized powdered sample. Each sample is compressed with a hydraulic press to 3000 psi for greater sample density. Sample aliquots consisting of less than 300 grams dry weight are put into a smaller counting geometry (Prindle vial).

After each sample is ground, all equipment contacting the sample must be decontaminated. The final sample container aliquots are barcoded and weighed. The sample weights of the tuna cans and those of the residual sample are recorded in the sample gamma-log book and entered into the computer. Each sample is placed in a thin plastic bag and stored with others of the same experiment that are ready for gamma-spectroscopy analysis. Residual sample containers are organized by field log number and stored in our sample library at Livermore and the Nevada test site.

Laboratory Preparation of Coarse Vegetation, Leaves and Grass

Samples of coarse vegetation are wood, palm fronds, leaves and grasses. Those that don't contain much water are removed from their collection bags and weighed and placed into paper sacks for oven drying. Their weights are recorded in the sample gamma book and entered in the computer.

Leafy vegetation samples full of plant juice are left frozen and placed into labeled large grocery bags. These are weighed and their weights recorded. They are then placed into one of the Northstar freeze-drying units to pull off most of the water.

After this initial freeze drying, which may take 7 to 10 days, samples are placed into forced air ovens to complete drying.

Woody plant vegetation, and fronds with little juice, are placed directly into forced-air oven for about 2 to 3 weeks. When the sample feels dry, it is weighed and then returned to the oven. The following day the sample is re-weighed. When two similar weights are recorded, the sample is considered dry. This dry weight is recorded in the sample gamma-log book.

The dried bagged samples are taken to the coarse vegetation processing lab and organized by priority. Grinding is done at the outdoor milling facility in a Wiley mill. The Wiley mills are difficult to decontaminate and require thorough steam cleaning between samples.

The finely chopped sample is placed in a 1 gallon can, weighed, labeled, and recorded in the sample log book. The cans are covered with a tissue paper to prevent cross contamination, and returned to the forced-air ovens for a final drying. When dry, the final dry weight is recorded in the sample log book and the computer. Sample can aliquots are made along with 10 percent duplicates for our Quality Control program. The samples are compressed with a hydraulic press at 3000 psi into the sample can.

Samples are boxed for gamma spectroscopy as each experiment is completed. Each box contains 63 samples with 6 duplicates and 3 standards added. This box of 72 samples is then sent to the data management group for review. Presses, canners and other equipment are decontaminated between samples. Labeled, barcoded, residual samples are then weighed, organized and stored in inventoried boxes. Residual sample inventories of these serial numbered storage boxes are kept in a residual sample log book.

Laboratory Preparation of Liquid Samples

The radiochemical procedure for the determination of cesium 137 in liquid samples of coconut juice, ocean, lagoon, and well water samples, is based on the batch extraction of cesium onto a microcrystalline cation exchanger, ammonium molybdophosphate (AMP) developed at LLNL(Wong et al., 1994). A diagram of the coconut juice processing procedure is shown in Figure 6.

Coconut Juice Sample Processing Procedure

The coconut juice samples are collected in one and two liter plastic bottles in the field. They are frozen within 7 hours of their collection for shipment back to LLNL. At the LLNL plant processing laboratory, the coconut juices along with other samples are inventoried, organized by experiment, and stored in order of processing priority in freezer containers. The samples are kept frozen until they are to be

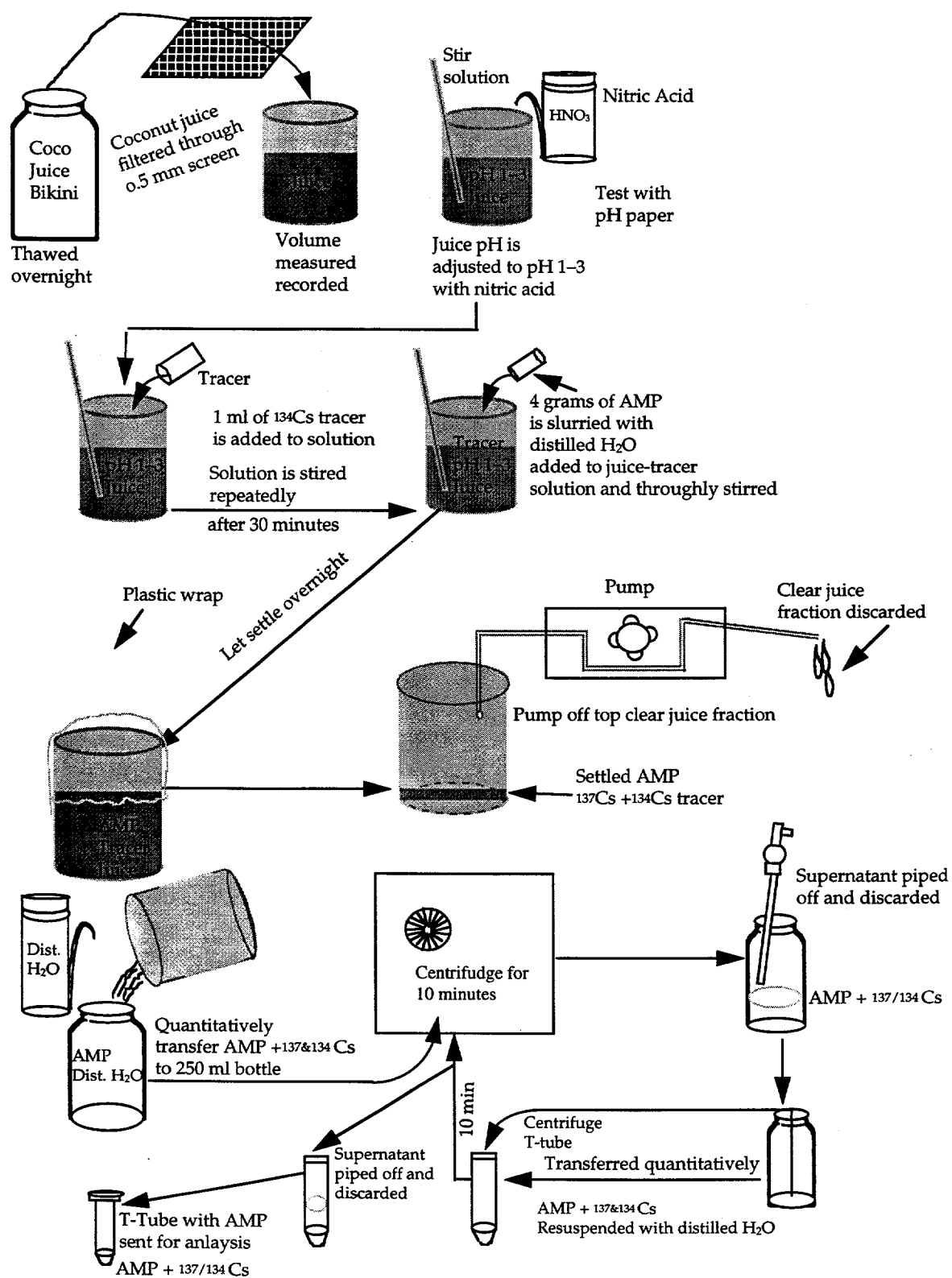


Figure 6. Coconut juice processing procedures flow diagram.

processed. The selected juice samples are removed from the freezer and allowed to thaw over night.

The thawed coconut juice sample size is measured by volume, and recorded in the sample log book and entered in the computer data base. The juice sample is filtered through a 0.5 mm nylon screen into a labeled 2 liter beaker. The juice in the beaker is adjusted to pH 1 - 3 by adding 4 N nitric acid. One milliliter of ^{134}Cs standard tracer is added to each juice sample in the beaker. An I.D. code for the ^{134}Cs tracer is recorded in the sample log book. The sample solution in the beaker is stirred and allowed to stand for at least 30 minutes. A separate beaker containing 4 grams of AMP is mixed into a slurry with distilled water. After the 30 minutes, the slurry of AMP and distilled water is poured and rinsed into the coconut juice sample beaker. The sample is stirred, covered, and left to settle overnight. The clear juice fraction is pumped off the top of the settled AMP. Care must be taken to remove as much of the clear juice fraction as possible without disturbing the AMP. The settled AMP and the clear juice fraction remaining are transferred into bottles. The samples are centrifuged for ten minutes. After the first centrifuging, the supernatant is discarded. The AMP in the bottle is resuspended and transferred into a labeled 50 ml tube (t-tube). Pairs of sample t-tubes are centrifuged for another ten minutes. After this final centrifuging, the supernatant is again discarded and the t-tube with the AMP is sent to the Gamma Spectroscopy Lab for analysis.

With each batch of 24 coconut juice samples processed, an extra beaker of 1500 ml. of distilled water is processed as a control blank sample using this same procedure. Duplicates are also prepared from large 2 liter samples. Control blank and duplicates represent approximately ten percent of the total samples processed. Standards are also prepared using 4 grams of AMP and spiked with pre weighed Cs-134 tracer. Notes documenting the details of processing each sample are recorded in the sample log book. This information includes the cesium standard tracer codes, AMP batch I.D. information, and the height of the AMP in the t-tube.

Ocean, Lagoon and Well Water Processing Procedure

The ocean, lagoon, and well water samples are collected and processed using similar processing procedures as for coconut juice samples. Larger samples of well water, usually 5 gallons are collected. An eight gallon container is used to hold the water sample for the addition of nitric acid, cesium tracer, and 4 grams of AMP. The rest of the procedure is essentially identical.

Laboratory Preparation of Soil Samples

Soil samples are taken to the soil processing facility, unpacked, and sorted according to field-log numbers. The samples are placed in labeled, weighed 1 gallon cans. Wet

weights are recorded in the sample log book and entered in the computer. A flow diagram of our soil processing procedure is shown in Figure 7.

The soil cans are covered with a filter paper to prevent contamination and placed in forced-air ovens for about one week. At the end of the week, dry weights are taken daily until two similar weights are achieved at which point the sample is considered dry. The soil cans are sealed and put back in the ovens for at least two hours at a temperature of 300 degrees F. These sterilized, dried samples are then removed from the ovens and put on the rolling mills for about 15 minutes to loosen clods and stuck particles. The rolled soil is then sieved for 20 minutes on a shaker table. The sieve containers are reusable and have been designed to accept common window screen which has a 1.5 mm opening. These clean disposable sieve screens prevent cross contamination. The sieving process divides the sample into two fractions: the coarse particles on the screen and the fine particles that pass through. These fractions are weighed and the weights recorded onto their containers, in the sample log book, and in the computer data base. The fine particle fraction is placed back in the gallon can, and the coarse material is stored in a labeled canister. Steel grinding balls are added to the fine fraction, then the cans are put back on the rolling mill until the particles have been ground into a fine powder.

Sample can aliquots of the resulting powdered soil are canned, decontaminated, weighed, and labeled. The residual sample weights are recorded into the sample log book and in the computer. For each experiment, 10% duplicates are made. Each box of 63 soil sample cans has 6 duplicates and 3 standards added to it before it is sent to the data management group to be reviewed for gamma counting.

Rolling mills are monitored frequently. Periodic maintenance checks and repairs are performed routinely by our staff. Decontamination of the building and equipment is also performed between individual island processing runs. Sample sieve containers and canning equipment are decontaminated between each sample, and sieve screens are replaced.

Purchasing and Shipping Field Equipment and Materials

At the completion of each field trip, inventories of existing equipment and materials are recorded in the field log book. Estimates of materials needed for the next field trip are made. Shortly after returning to LLNL, the supplies are ordered. In addition to shipping new equipment and materials, recycled bottles are also shipped. These used bottles are washed, labels removed, pressure tested, and packed for one of our shipments to the field. Electronic equipment still undergoing repairs or recalibration, and supplies not available for barge shipment must often be shipped using Military Air Command (MAC).

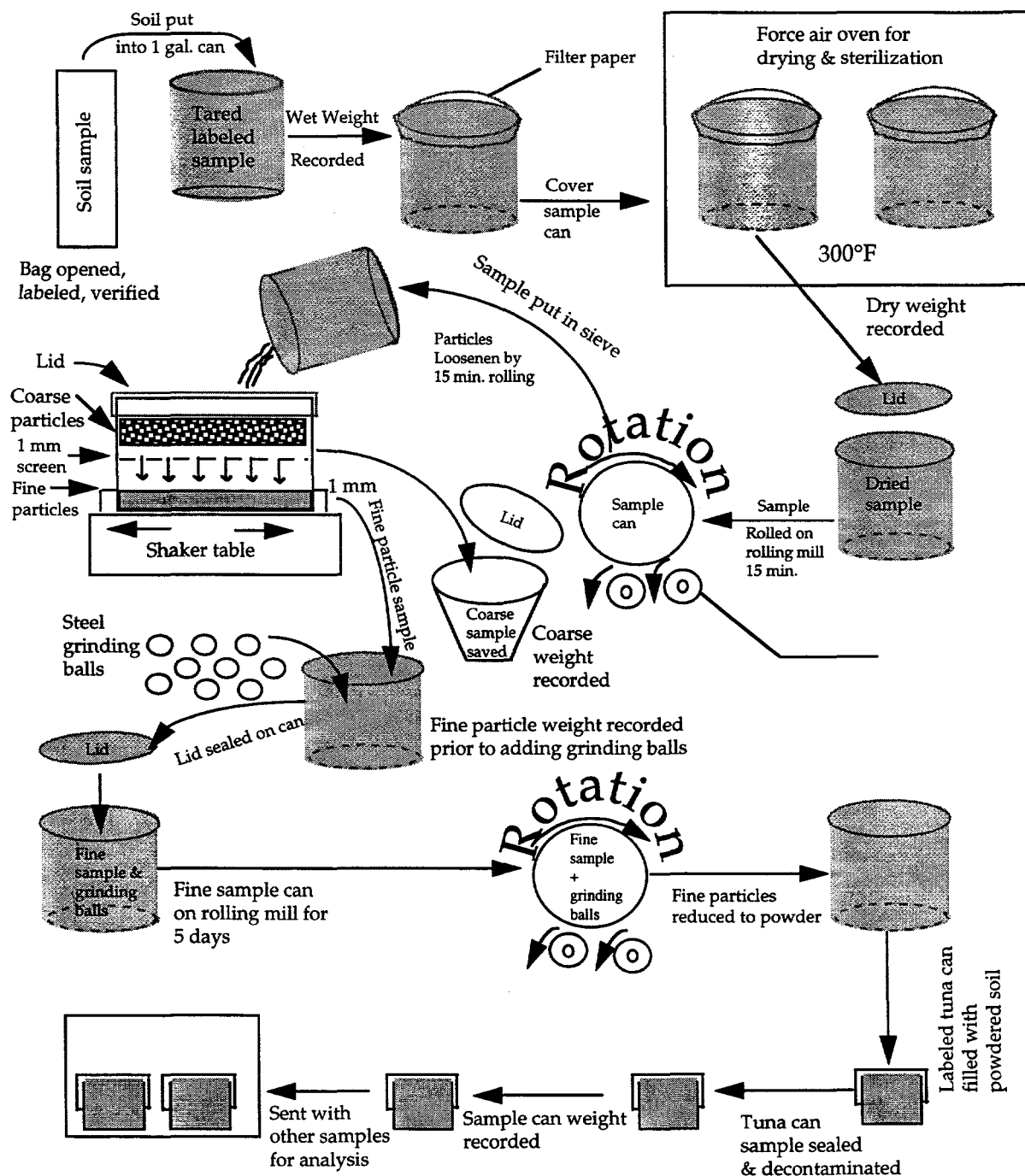


Figure 7. Soil sample processing procedures flow diagram.

Biannual Decontamination and Overhaul of Laboratories and Equipment

When the sample processing from a field trip is completed, the laboratories and equipment undergo thorough maintenance .

The manifold freeze-dryer is disassembled. The aluminum vacuum chambers are steam cleaned and repainted with epoxy paint. All O rings and chamber gaskets are inspected and replaced as necessary. Vacuum pipes and LN traps are thoroughly cleaned. Flexible vacuum hoses from the vacuum chamber, CO₂ trap and the vacuum manifold are steam cleaned and inspected. Dry ice extended reservoirs are cleaned and repaired. The system is reassembled and leak-checked. The automatic LN filling system is inspected and repaired, and solenoids and LN tank valves rebuilt as necessary.

The Northstar and the Mobile II freeze-drying units are thoroughly steam cleaned. Vacuum pump oil is changed, the oil sumps cleaned of debris and the internal parts are evaluated for wear. If necessary, the vacuum pump is exchanged with a rebuilt unit and sent out for repair. Rusts spots on the chamber and shelving are cleaned and repainted. Vacuum gaskets and hoses are inspected and replaced if necessary. The units are leak-checked and cycled through freezing and defrosting modes to make sure they are functioning perfectly. The units are then continuously run to prevent malfunctions caused by being left inoperative for more than 2 weeks.

The three vacuum pumps of the manifold system are inspected for oil leaks and the pump shaft seals replaced. The pump vee belts are replaced and the pulley inspected for wear. Pump shafts are inspected for bearing wear, and the pump sent for repair when necessary. Pump buildings and secondary oil containment trays are cleaned. Fifty-five gallon drums of used pump oil and ethylene glycol are removed and taken to hazardous waste disposal. New drums of pump oil and ethylene glycol are ordered as needed.

Both of the 20 ft walk-in freezer containers are defrosted and steam cleaned, and refrigeration units inspected. Smaller laboratory freezers are defrosted and washed. Automatic canners, hydraulic presses and canning dies in all labs are disassembled, moving parts oiled, adjusted and tested. All fume hoods in the labs are decontaminated and painted where necessary, then relined with clean 4 mil plastic and paper.

The vegetation chopping Wiley mills are inspected. The cutter heads may be removed, and the blades sent out to be sharpened as needed. All shafts and bearings are inspected for wear and bearings refilled with grease. Resharpened blades are reinstalled and adjusted to tolerance. The mills are retested and sprayed with light oil on non-painted surfaces to prevent rusting until they are needed.

A proper maintenance program ensures a minimum of delays when the samples are being processed. For optimum sample processing efficiency, the various procedures must be sequenced in proper order as shown in Figure 8.

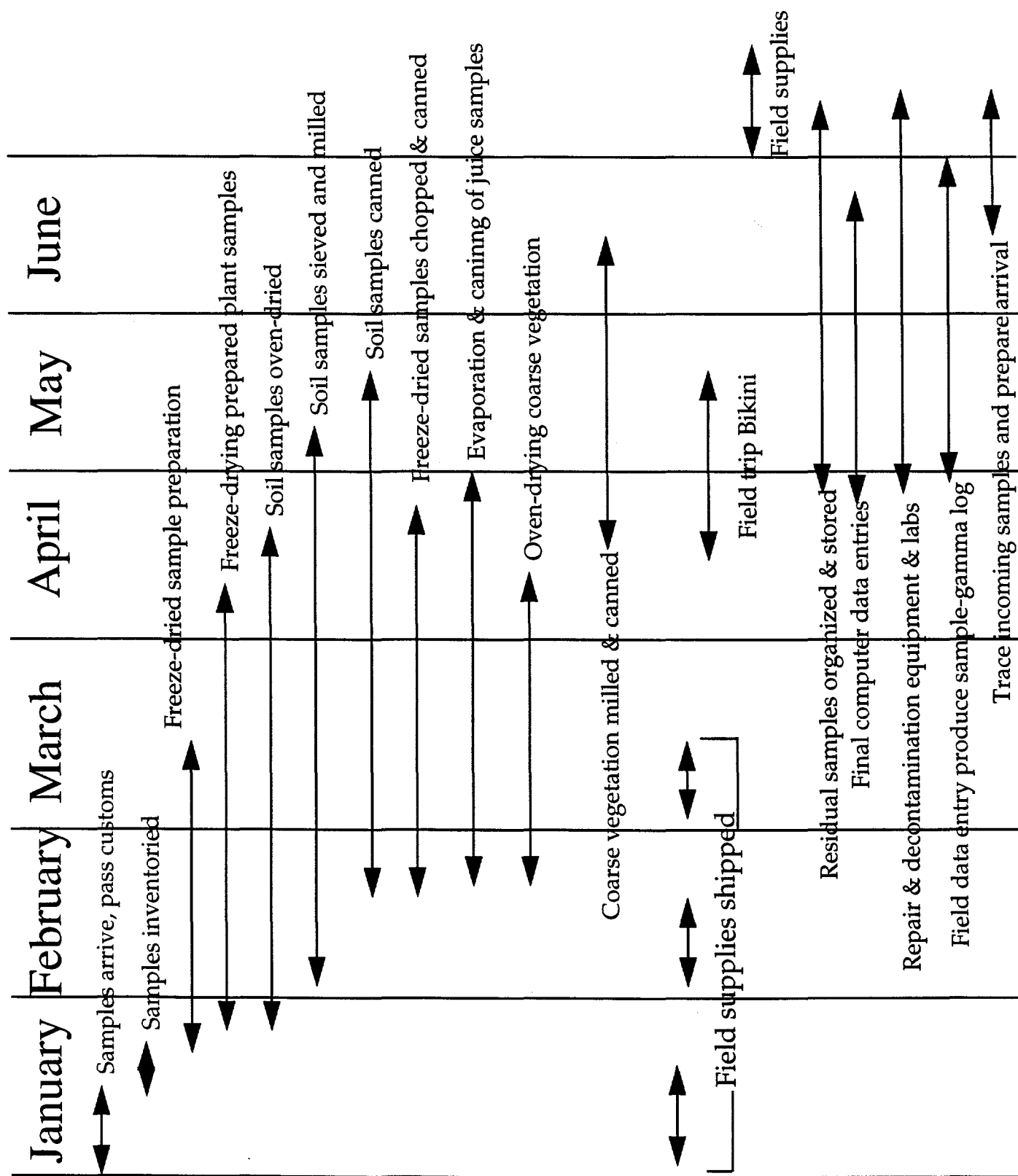


Figure 8. Time line.

Acknowledgment

I would like to recognize the excellent support of our field collection staff; Henry Jones, Cynthia Conrado, Steven Kehl, Robert Weidman, William Steele, Steven Hall, Miguel Granillo, Carol Stoker and John Rehder. As members of the Marshall Island field team, they have assisted in every facet of our field collections insuring the continued success of our sampling missions. In our plant and soils laboratories, Miguel Granillo, Steven Hall, William Steele and Robert Weidman continue to process the thousands of samples collected every year constantly striving to produce samples of the highest integrity. I would like to recognize Kai M. Wong for his excellent technical support in the development of our laboratory chemical sample processing procedures. All of these personnel have contributed to the success of our field and laboratory effort and I would like to thank them for their support. I would also like to thank Rayla Bradsher for her technical assistance in producing this paper.

References

Murai, M., F. Pen and C.D. Miller (1958), *Some Tropical South Pacific Island Foods*, University of Hawaii Press, Honolulu, pp. 12-13, 67.

Wong, K.M., T.A. Jokela and V.E. Noshkin (1994), *Radiochemical Procedures for Analysis of Pu, Am, Cs and Sr in Water, Soil, Sediments And Biota Samples*, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-ID- 116497.

Appendix A: Soil and Vegetable Permit and Agreement

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE
HYATTSVILLE, MARYLAND 20782

IMPORT PERMIT FOR PLANTS AND PLANT PRODUCTS

1. PERMIT NUMBER
56-23460 - REVISED

Transmit Permit Number to your Shipper but Retain this Permit

2. EXPIRATION DATE
JUNE 30, 2000

3. NAME AND ADDRESS OF PERMITTEE (Include Zip Code)
Permittee should notify Plant Protection and Quarantine, APHIS, USDA, promptly
of change of address.

Lawrence Livermore National Laboratory
(Marshall L. Stuart)
P.O. Box 808
Livermore, CA 94550

TEL: (415) 422-1100

4. UNDER AUTHORITY OF THE PLANT QUARANTINE ACT, AS AMENDED, PERMISSION IS HEREBY GRANTED TO PERMITTEE TO IMPORT IN
ACCORDANCE WITH

7 CFR 319.56

5. THE PLANTS OR PLANT PRODUCTS SPECIFIED BELOW, GROWN OR PRODUCED IN

Various Approved Countries

6. THROUGH THE PORT(S) OF

San Francisco

7. DESIGNATION OF PLANTS OR PLANT PRODUCTS

FROZEN FRUITS AND VEGETABLES. Frozen fruits and vegetables, including those
not otherwise admissible, are authorized entry when quick frozen as prescribed
in Section 319.56-2c and not above 20 F. at time of arrival.

Items not admissible under these regulations include:

Avocados with seeds from Mexico, Central American, and South America.

Mangoes with seeds from Barbados, Dominica, French Guiana, Guadeloupe,
Martinique, St. Lucia, or any country outside of the Americas.

Citrus with peel from countries listed in Section 319.27 or 28.

Black currant when consigned to prohibiting States. Refer inquiries to
Permit Section for current list of states prohibiting black currant
fruit.

Corn-on-the-cob from nations bordering the Mediterranean Sea.

Potatoes with the peel and/or buds (eyes).

This permit does not authorize the importation of any genetically engineered plants or products thereof. To import such plants (or to move them interstate),
write to the Biotech Unit, Biological Assessment Support Staff, PPQ, APHIS, USDA, Federal Building, Room 634, Hyattsville, Maryland 20782.

8. SIGNATURE OF AUTHORIZING OFFICIAL

(301) 734-8645
KAREN BRADY

9. DATE ISSUED

6-15-95

ENCLOSURE



**UNITED STATES
DEPARTMENT OF
AGRICULTURE**

**Animal and Plant
Health Inspection
Service**

**Plant Protection and
Quarantine**

Soil Permit

Permit
Number:

S-4621

Lawrence Livermore National Laboratory
(Marshall L. Stuart)
University of California, H.E.A. Division
Box 808, L-524
Livermore, California 94550
(510) 422-9723

Issued To:

Under the authority of the Federal Plant Pest Act of May 23, 1957, permission is hereby granted to the facility/individual named above subject to the following conditions:

1. Valid for shipments of soil not heat treated at the port of entry, only if a compliance agreement (PPQ Form 519) has been completed and signed.
2. To be shipped in sturdy, leakproof, containers.
3. To be released without treatment at the port of entry.
4. To be used only for laboratory analysis, and only in the facility of the permittee at Lawrence Livermore National Laboratory, located in Livermore, California.
5. No use of soil for growing purposes is authorized, including the isolation or culture of organisms imported in soil.
6. All unconsumed soil, containers, and effluent is to be autoclaved, incinerated, or heat treated by the permittee at the conclusion of the project as approved and prescribed by Plant Protection and Quarantine (PPQ).
7. Permittee shall notify the office of the Alameda County Agriculture Commissioner upon arrival of shipments at the facility at Area Code (510) 670-5232.

SEPTEMBER 30, 2000

Expiration Date

Victor Harabin
Approving Official
VICTOR HARABIN

COMPLIANCE AGREEMENT

1. NAME AND MAILING ADDRESS OF PERSON OR FIRM Marshall L. Stuart Lawrence Livermore National Laboratory HEA Division, Box 808, L-524 Livermore, CA 94550	2. LOCATION Phone 510-422-9723 or 510-423-3174
3. REGULATED ARTICLE(S) SOIL SAMPLES - from foreign sources or regulated areas within the United States.	
4. APPLICABLE FEDERAL QUARANTINE(S) OR REGULATIONS 7CFR330.300 and 7CFR330.302 are regulations which restrict the movement of soil into or through the United States as well as from State to State. The State of California also restricts movement of soil from other states into California.	
6. I/We agree to the following: <ol style="list-style-type: none"> 1. All soil will be shipped in sturdy leak proof containers and clearly marked "Contents - Soil Samples" on the outside of the shipping containers. If the sample is of foreign origin then the import sticker and/or permit number must also be displayed. 2. Notify the County Agricultural Commissioners Office upon arrival of all shipments or maintain logbook records of date of arrival, amount, origin, method of treatment, and date of disposal for inspection by County, State, or Federal Agricultural Inspector. 3. Do not ship samples to other labs unless they are on the approved list. Contact the County Agricultural Commissioner for information on approved laboratories for reshipment. 4. The laboratory personnel will take all precautions to prevent the escape of any potential pest which may be in the soil. A <u>standard operating procedure</u> (SOP) should be developed for lab personnel to follow. 5. The soil will be handled as quarantined material until sterilized. This will include keeping the soil enclosed in containers when not in use and labeling all containers and/or storage areas; "Quarantine Soil - Sterilize Before Disposal". 6. See the attachments for sterilization schedules and further instructions and information. 	

7. SIGNATURE <i>Marshall L. Stuart</i>	8. TITLE Environmental Scientist	9. DATE SIGNED 30 June 1995
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The affixing of the signatures below will validate this agreement which shall remain in effect until cancelled, but may be revised as necessary or revoked for noncompliance.

12. PPQ OFFICIAL (Name and Title) Dan Hamon, PPQ Officer	13. ADDRESS USDA, APHIS, PPQ P.O. BOX 1866 Stockton, CA 95201 Phone (209) 946-6252
14. SIGNATURE <i>Dan Hamon</i>	10. AGREEMENT NO. 11. DATE OF AGREEMENT June 30, 1995
15. STATE AGENCY OFFICIAL (Name and Title) Michael A. Greene, Ag Commissioner	16. ADDRESS Alameda County Dept. of Agriculture 224 W. Winton Avenue, Room 184 Hayward, CA 94544 Phone 510-670-5252
17. SIGNATURE <i>Michael A. Greene</i>	