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The Preparation of Plant Material and Determination of Weight Percent Ash

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ABSTRACT

A standardized procedure for the physical preparation and dry ashing of plant material has been developed. At the submitter's option, the sample is washed and rinsed in deionized water. After drying in a colander at 40°C for 24-48 hours, the sample is ground to minus 2 mm using a Thomas/Wiley or Christy/Norris Mill and mixed on a rotary machine for 30 minutes. A weighed aliquot of the sample is slowly ashed in a closed muffle furnace at 450°C for 8 hours. No ashing aids are added before or during this cycle. After cooling, the resulting ash is weighed and ash yield is determined. This calculation, expressed as weight percent, requires four decimal place measurement of the empty ashing vessel, vessel and ground sample combined, and cooled vessel containing ash. Net sample and ash weights are obtained by difference. Weight percent ash is found by dividing net weight ash by net sample weight and multiplying the results by 100. Four significant figures are recorded; three are reported.

INTRODUCTION

The method of plant preparation, dry ashing and calculation of weight percent ash presented here has become the standard procedure for the Branch of Geochemistry. It was developed largely from hands on experience and discussions with many users of the last 20 years as well as a recent survey of the scientific literature. It is capable of processing 5,000 samples per year (each weighing approximately 200 g) with a staff of 1.5 full-time workers.

The physical preparation of plant material consists of washing, drying, milling, and in most cases, dry ashing an aliquot of sample. Whenever ashing is done, a calculation of weight percent ash is recorded to four significant figures. The ashing temperature selected for the majority of samples is just above 450°C. Furnace controls are set so as to "ramp," or step up, to this temperture at the rate of 35°C per hour followed by 8 hours of ashing. A timer turns off the furnace after completion of the cycle.

The method of preconcentration of elements in plants by means of dry ashing is not without drawbacks. Some proportion of the total concentration of volatile elements, such as selenium, arsenic, mercury, phosphorus, tellurium, as well as some forms of lead and gold, will be lost at ashing temperature. In the case of gold, Hou (1987, p. 325) reports that silica is also concentrated by dry ashing and that in the form of an "amorphous...powder...turns again into siliceous colloids which adsorb and occlude Au in aqua regia solution, forming a protective coat around auric ions" thus lowering the analytically determinable content of gold by acid digestion techniques. It is generally agreed that nondestructive techniques such as Hydride Generation Atomic Absorption Spectroscopy and Instrumental Neutron Activation Analysis are preferable and more accurate for several of these elements. Plant ash is, however, required for Inductively Coupled Argon Plasma Atomic Emission Spectroscopy and Direct Current Arc Emission Spectroscopy, the most common reconnaissance techniques used in Branch work for plant analyses.

EQUIPMENT

Laboratory equipment consists of the following:

Quantity	
(1)	Thomas/Wiley Mill Standard Model 4, with 2 mm screen
(2)	Mellen Model B-222 muffle furnaces fitted with Cramer 24-hour timers
(1)	Christy/Norris pulverizer, 8 in
(3)	Laboratory drying ovens, 0-200 C, 8-10 cu ft capacity
(1)	Spex 8 000 Mixer/Mill
(2)	Box fans (4.5 in) mounted to ring stands
(36)	Vitreosil evaporating dishes (fused silica, 3.75 in I.D.)
(50)	Coors evaporating dishes (porcelain, 3.0 in I.D.)
(3)	Glass or plastic beakers, 4-liter capacity
(1)	Rotary mixer holding at least 36-pint sample containers
(1)	Mettler AC100 electronic balance
(1)	Scientech 3300 electronic balance

A supply of 0.5 ounce polycons (pillboxes), 5 mm solid borosilicate beads and waxed weighing paper is also needed. A small supply of acetone is useful as a cleaning aid.

PREPARATION PROCEDURE

Washing

A plant sample received for preparation usually undergoes a washing process to eliminate contamination from adhering particles such as dust. There are three methods for this: (1) "in the bag" washing by machine, (2) "beaker soak" hand washing in tap or deionized water, (3) "colander rinse" with tap or deionized water. The washing machine in use is a Maytag KA806S. Samples are run through at least one complete wash/rinse/spin cycle in gentle mode. No more than fifteen, are processed at a time. Sample bags must be tied, knotted, and strings cut short to prevent entanglement with the agitator. No detergent or hot water is used.

When the "beaker soak" method is used, water must be constantly changed since the sample is actually moved from one beaker to another over the course of a few minutes. The sample is rinsed in a colander, and the beakers are rinsed and refilled for the next sample. All samples rinsed manually are transferred to a colander for drying. Drying temperatures are held under 40°C unless specified otherwise. Material having a resinous coating on stems or leaves is dried without heat to minimize the possibility of its loss through liquifaction. Samples are dried to brittleness; usually 24-48 hours.

Milling

Dry samples can be put directly into the grinder. It has been found that the Wiley Mill (fig. 1) is best for young, woody growth up to a thickness of 5 mm. The Christy/Norris Mill is used for all thicker materials such as twigs, roots, and branches up to about 13 mm in diameter. Larger diameter material must be cut to prevent jamming of the mill. This is most easily accomplished with pruning shears or a band saw.

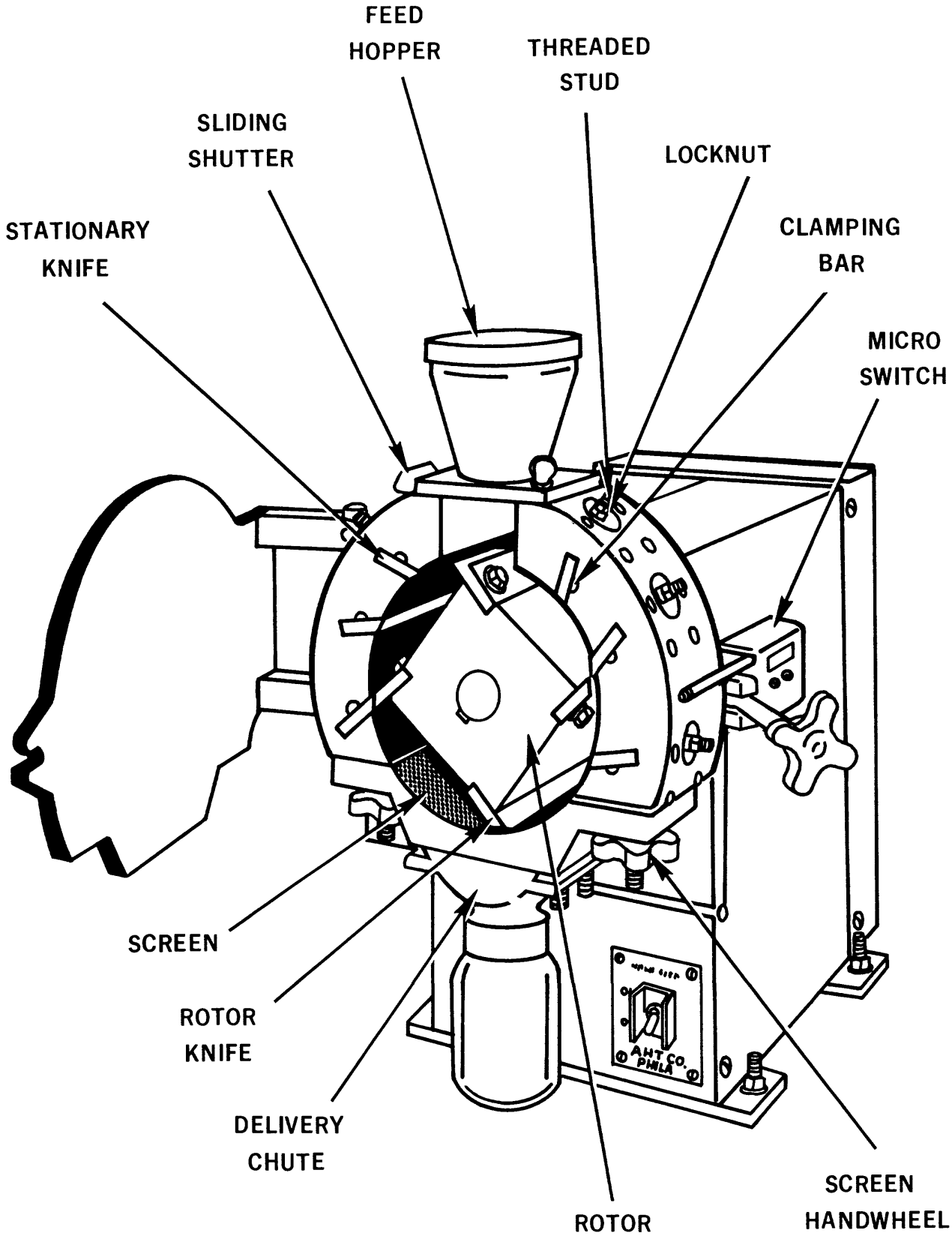

Figure 1. Pictorial diagram of the Thomas/Wiley Mill, Standard Model 4, Series 3375-E10. This mill is used for grinding nearly all types of vegetation up to 5 mm in thickness. The screen size most often used is 2 mm. Reprinted with permission of Thomas Scientific, Swedesboro, New Jersey.

Samples are mixed using the rotary type of tumbling device shown on figure 2. The holders are set to accommodate four 8.9-cm diameter sample containers in line. These sample holders form the circumference of the mixer. Samples are held in place by flat plates secured with wing nuts to long threaded bolts mounted in the holder frames. The mixer is driven by a gear reduction motor having an output of 45 rpm (39:1 ratio). A toothed timing belt is linked to a 1/3 HP., single phase, A.C. 1,725 rpm drive motor. The average weight of a plant sample after grinding is 200 grams. The top 2.5 cm of the pint container must remain unfilled to insure proper mixing.

Dry Ashing

Ashing requires only a portion of the sample, but enough to satisfy the analytical need and be representative of the entire sample. The amount of this "aliquot" is also determined by its density, estimated ash yield, and amount of sample available. Sample material is taken from its container with a teaspoon, off the top. An aliquot of 10 g is optimum for a 3.75 in. Vitreosil dish, although satisfactory results have been obtained from splits of 1-24 g. Using a spatula, the sample is spread evenly along the sides and bottom of each dish to enhance even heat distribution throughout the aliquot. The ashing vessels are arranged in each furnace three across, left to right, upper shelf first (nine per shelf). Any consistent arrangements is appropriate provided it is known precisely which dish holds what sample. There should be some space between each dish and the furnace walls. Breakage can occur from tightly packed arrangements. Shelving material is stainless-steel mesh wrapped over a similar framework forming a flat tray.

Ashing proceeds with the door fully closed. The small amount of oxygen necessary for the process enters through the imperfect seal between door and wall bricks and any hole drilled for thermocouple mounting. The furnaces are programmed to "ramp" up to the ashing temperature of 450°C over a period of 10 hours. Complete ashing is insured by maintaining this temperature for 8 hours. No ashing aids, such as nitric acid, nitrates of light metals (Mg, Ca, Al) or 10 percent sulfuric acid solution (Gorsuch, 1970) are being used. The furnaces are allowed to cool for 8 hours before sample dishes are removed. While cooling, the door should be slightly open but not swung away until the inside temperature dips below 200°C. Sample dishes should remain undisturbed until cooled to 100°C. Ashing vessels are removed using tongs and placed on a metal or insulated surface for further cooling. At least 20 minutes should be allowed for this. After sample removal, cooling of the furnaces is enhanced by box fans positioned in front of the interior (fig. 3). Due to limitations in the controller and programmer circuitry, the brick temperature must be reduced to 24°C or less before the next ashing cycle can begin. In all, furnace cooling requires about 10 hours to complete.

The ash is transferred to 0.5-ounce pillboxes using waxed weighing paper as funnels. The ash must then be mixed and reduced in volume as it tends to be highly charged with static. This is done through the use of a 5 mm solid-borosilicate bead (placed into the polycon prior to addition of the ash), and 10 to 60 seconds of shaking in a Spex 8000 mixer/mill. The ash is then ready for laboratory analysis.

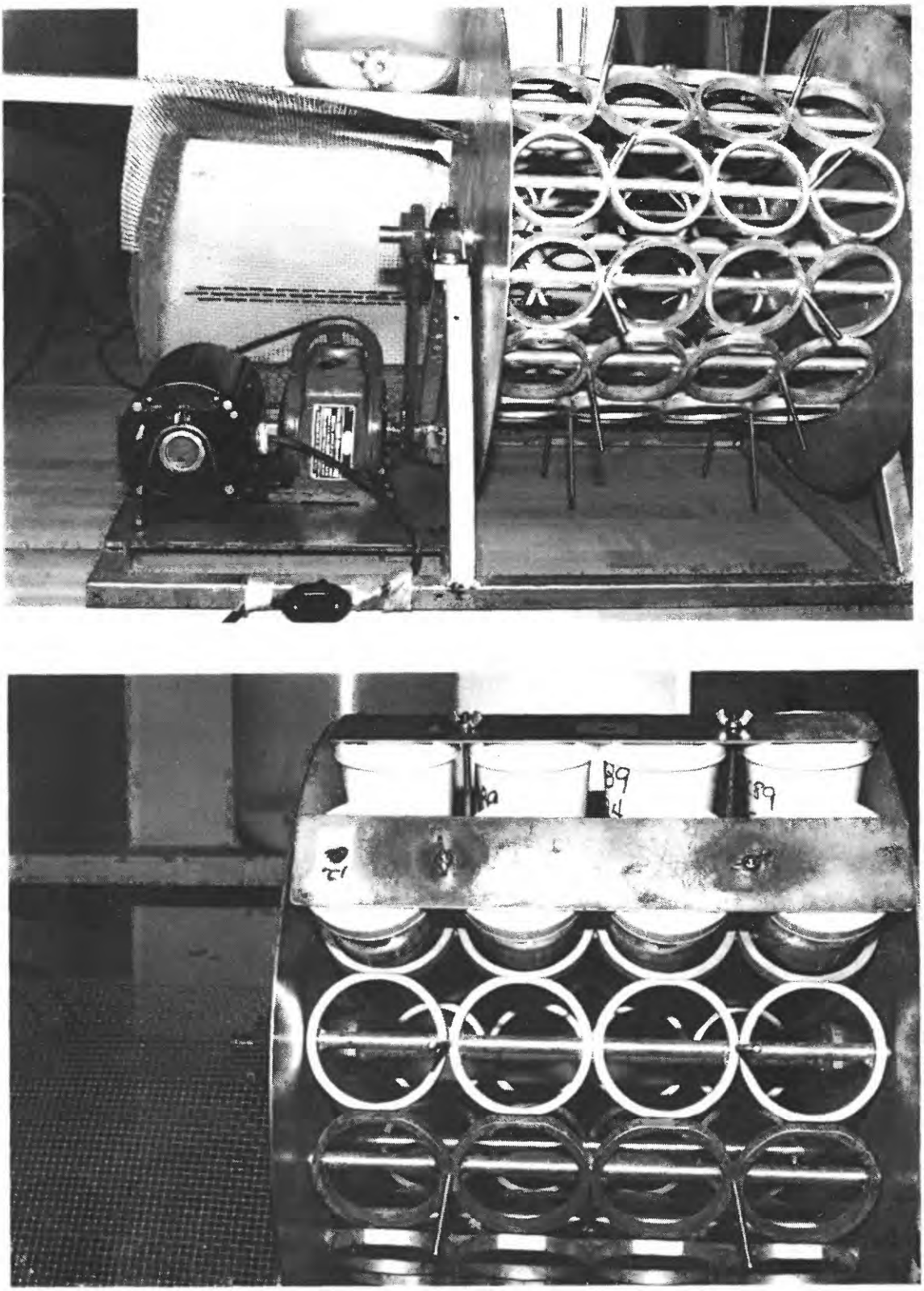

Figure 2. Photographs of rotary sample mixer showing principal parts. The holders are sized for pint sample containers not more than 8 .9 cm in diameter at the bottom. Each row is secured with a metal slat and wing nuts as shown. The drive motor is a single phase, 1/3 H.P., 1725 RPM. A.C. unit. The reduction motor has an output of 45 RPM (nearly 1:39).

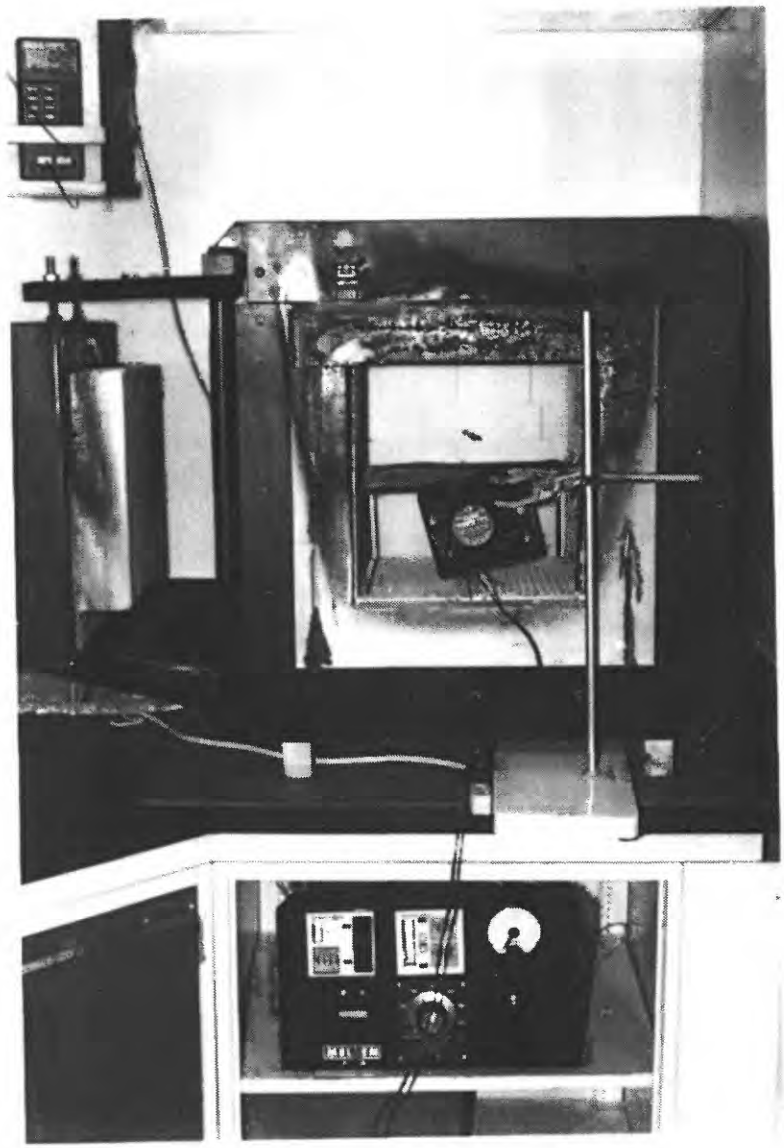

Figure 3. Mellen Model B-222 muffle furnace arrangement. The controller unit on the cabinet shelf also houses a programmer and Cramer 24 hour timer. All ashing cycles are monitored with the Fluke 52 digital thermometer shown in the upper left corner of the picture.

Percent ash calculation

Weight percent ash is determined for all ashed samples. The method used for this should be accurate to three significant figures. It requires the measurement of the empty vessel, the combined weight of vessel and sample aliquot before ashing, followed by the weight of the cooled vessel containing the ash. All are recorded to a maximum of four decimal places (.0001 g). The net weight of the aliquot and resulting ash must be determined by difference. Weight percent ash is calculated by dividing net ash by aliquot weight and multiplying the result by 100. This is recorded to four significant figures. Rounding gives the reported values in three significant figures.

SAFETY PRECAUTIONS

As with all machinery, care must be taken while grinding or ashing the sample material. The Wiley mill has stationary and rotating razor-edged knives that can easily remove finger tips. The Christy/Norris mill (fig. 4), becomes very hot after just a short period of operation. Both mills must be cleaned between samples. This is usually done with compressed air (40 psi) and a toothbrush. Before cleaning, the mill should be at rest. Occasionally, a sample will leave a resinous buildup on the spinning cutting head, door, or screen. It can often be removed with the careful use of a razor knife and acetone. Due to dust, noise in excess of 80 dba, and heat hazards, the worker should be fully protected with a lab coat or smock, leather work gloves, dust mask, safety glasses, and hearing protectors. A muffle furnace also creates a potential burn hazard. **Never** load or unload a hot furnace.

UNCERTAINTIES

Saiki and Maeda (1982) have reported success with a washing technique utilizing 0.2 M HCl solution and a distilled water rinse. They found the method useful for all species tested (Japanese cedar, Japanese white oak, Japanese red pine). However, evergreens with protective cuticular outer layers seem best suited for this technique because of their resistance to leaching by the acid solution. Whether a separate washing procedure for evergreens is desirable and justifiable is still open to question.

The use of the Maytag machine has opened a lively debate regarding its efficiency, reliability and possible point-source cross-contamination of up to 15 samples at a time. At present, it is used only at the specific request of the sample submitter(s). Obviously, additional experimentation and adaptation will be necessary to validate this washing method.

As Gorsuch reports (1970), deionized water contains some organic matter, but its nature, concentration, and effects on raw sample material have not been determined. Where ashing is done to determine weight percent of the ash only, porcelain dishes are used because the reported problems with glaze contaminants such as copper (Elvehjem and Lindow, 1929), lithium (Harms, 1988 oral commun.; Vaughn, 1988 oral commun.); lead and other heavy metals (Sandell, 1944) are of minimal consequence. All ash to be chemically analyzed should be prepared in fused silica (Vitreosil) vessels. Volatilization losses and retention reactions between sample material and the silica dishes have not been investigated.

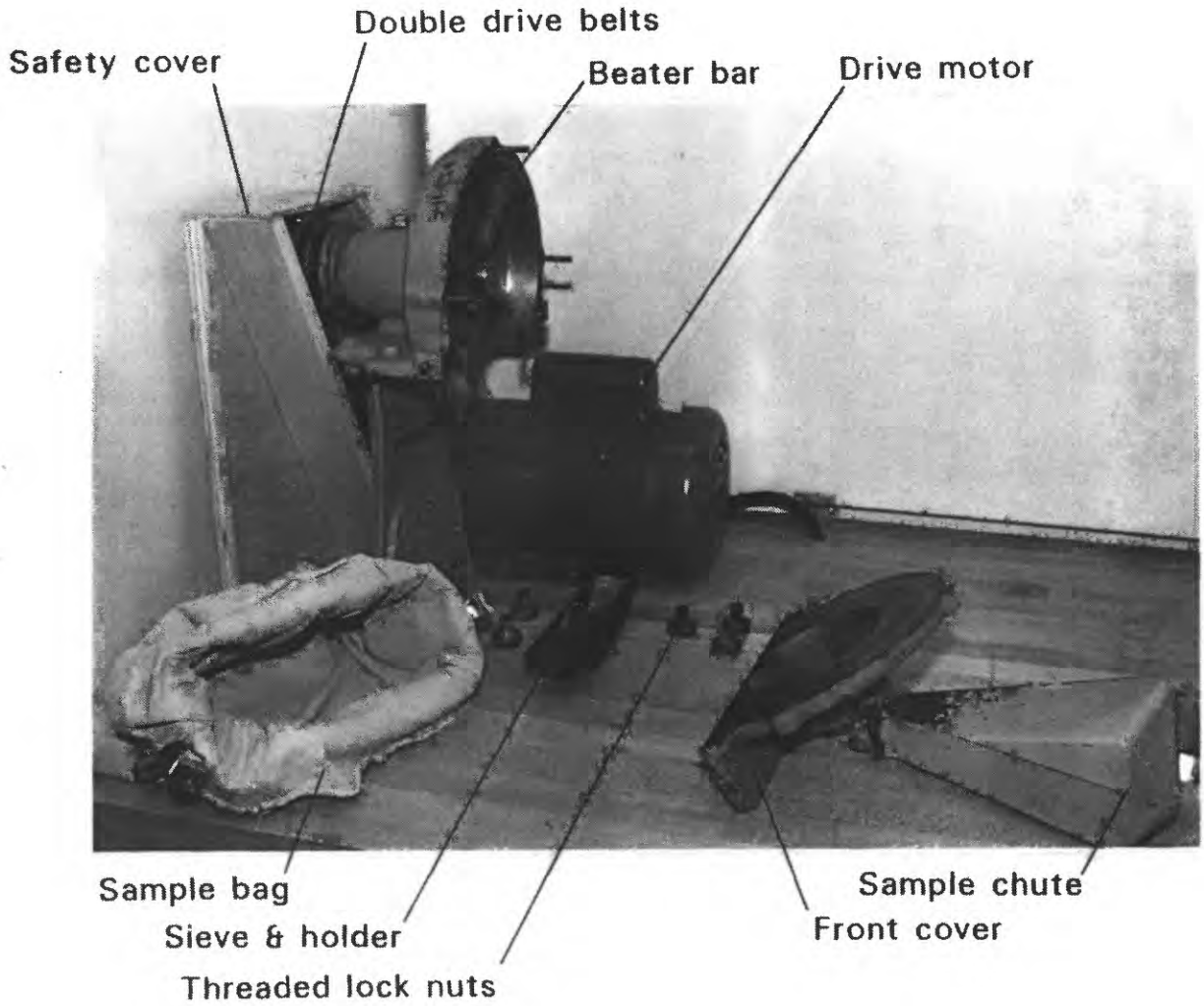

Figure 4. Christy/Norris 8-inch mill and principal parts. This mill is used for grinding all types of vegetation up to 13 mm in diameter. Manufactured by Christy & Norris Ltd., Chelmsford, England.

SUMMARY

The procedures described in this report are useful in the preparation of most plant material prior to laboratory analyses. As many as 5,000 samples can be prepared per year using these techniques. The selection of 450°C as a standard ashing temperature, while based on the literature available and practical experience, in no way implies immunity from future scrutiny, study, and modification. As long as plant ash is needed for chemical analysis, there will be a need for improvement of technique.

REFERENCES

Elvehjem, C.A., Lindow, C.W., 1929, The determination of copper in biological materials: Journal of Biological Chemistry, v. 81, p. 435-443.

Gorsuch, T.T., 1970, The destruction of organic matter—International Series of Monographs in Analytical Chemistry, Great Britain Pergamon Press LTD, v. 39, 152 p.

Hou, Zhihui, 1987, Influence of the siliceous component in determination of gold in plant materials: Journal of Geochemical Exploration 27, p. 323-328.

Saiki, H., Maeda, O., 1982, Cleaning procedures for removal of external deposits from plant samples: Environmental Science and Technology, v. 16, no. 8, p. 536-539.

Sandell, E.B., 1944, Colorimetric determination of trace metals, *in* Chemical Analysis—A series of monographs on Analytical Chemistry and its applications: Interscience Publishers, Inc., New York, 487 p.