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Title: SOME FACTORS AFFECTING THE DISTRIBUTION OF ORGANIC ARSENICAL SILVICIDES IN THE FOREST ECOSYSTEM

Abstract approved: Michael Newton

The initial distribution of organic arsenical silvicides was measured in the trees, litter and soil of four forest types in the Pacific Northwest following a standard, precommercial chemical thinnning. Factors which influenced the persistence, movement or fate of these compounds were noted and used to evaluate differences in the generalized patterns of distribution after four different treatments. The treatments included two different organic arsenicals, monosodium methanearsonate (MSMA) and dimethylarsine oxide (cacodylic acid), applied during fall or spring seasons. The project was part of a cooperative study on the safety of organic arsenicals as thinning agents initiated by the U. S. Forest Service in 1970.

The choice of chemical and season of treatment both influenced the pattern of distribution and the absolute concentration of

that control arsenic distribution in conifers. Distribution of arsenic within trees is apparently related to an interaction between the chemicals and the season-dependent transport mechanisms in the tree. Mobility of arsenic in conifers seems to be regulated by the direction and degree of phloem transport at the time of application if the transport system is not immobilized by treatment. Chemicals that do not depend on temperature for phytotoxicity (e. g. cacodylic acid) probably concentrate at the ends of growing tips because they immobilize the phloem transport system but not the xylem. Strongly temperature-dependent chemicals (e. g. MSMA) apparently do not immobilize phloem transport during cool weather and result in equilibration of the chemical throughout various sink areas in the tree. In contrast, during warm seasons these chemicals would become more phytotoxic and immobilize the phloem, limiting basipetal movement. Thus, basipetal transport of temperature-sensitive toxicants in conifers is likely to be restricted to both cool weather and season of basipetal movement. In this context, fall treatments of MSMA can be employed to maximize concentration in roots, and minimize exposure to above-ground organisms.

Most plant samples in this study were analyzed for total arsenic content using a graphite tube adaptation on an atomic absorption spectrophotometer. The procedure developed was faster and more economical, but less precise, than standard arsenic determination

procedures. This analytical technique permitted the sampling intensity necessary for variable biological material in ecosystem studies.

Some Factors Affecting the Distribution of Organic Arsenical Silvicides in the Forest Ecosystem

by

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INTRODUCTION

In our finite world, ecologically sound management practices are needed to maximize the utilization and conservation of our resources. Resource managers are increasingly concerned with minimizing effects of organisms which appear to conflict with this objective. Synthetic pesticidal compounds are used in a wide variety of pest management programs. Among these are organic arsenical herbicides.

It is important to know the behavior of any synthetic compound introduced into an ecosystem; such information is needed to avoid inadvertent damage to fragile interactions among organisms, and to limit the exposure to non-target organisms. Unfortunately, research on herbicides used in forests has traditionally focussed on direct toxicological effects. There is very little information available about behavior and fates of some families of herbicides in forests, and about the importance of residual concentrations in various parts of forest ecosystems.

Inputs or alterations in an ecosystem produce more than single, dependent responses. All components are interdependent; a response in one component can eventually affect all the others. Thus, since

more than just the target organism may be affected, the behavior of the compound as well as its toxicity should be understood. When dealing with organo-metallic compounds such as organic arsenicals, it is important to know how the compound behaves regarding metabolites, because exposure to certain very toxic forms of arsenicals could lead to death of non-target organisms. In addition, metallic compounds are generally persistent. Therefore, the toxicity of a compound alone at some specific time and place is insufficient evidence to evaluate potential hazards to other organisms.

Organic arsenical herbicides have found increasing utility in forestry as the need for intensive management practices has increased. Relatively little work has been done on the behavior and toxicity of these compounds as used in forests. As the result of suspected adverse effects on application crews, cattle, and wildlife, a cooperative study on the safety of organic arsenical herbicides as precommercial thinning agents was initiated by the United States Forest Service in 1970. The overall objectives were to:

1. Measure the initial distribution of organic arsenical herbicides among several components of the forest ecosystem.
2. Determine their persistence, movement and fate in the forest.
3. Characterize the acute and chronic toxicity of these chemicals to large and small animals.

Results of these studies are to be used to determine and minimize the

risks of using organic arsenicals for chemical thinning. The overall project was divided into studies of specific toxicity to animals, and behavior in forest ecosystems. This thesis is a report on the results of one of the behavioral studies. Its purpose is to investigate the distribution of arsenic within and among forest trees, litter and soil after representative forest stands have been precommercially thinned with organic arsenical silvicides.

The general objectives of this study coincide with the first two of the aforementioned objectives of the overall project. The research approach entails the answering of several specific questions related to the fate of commonly-used arsenicals in a variety of forest types. To this end, four forest types were precommercially thinned with monosodium methanearsonate (MSMA) and hydroxydimethylarsine oxide (cacodylic acid). Both are systemic organic arsenicals. The following questions were asked regarding each:

1. What is the distribution of arsenic in trees injected with organic arsenicals?
2. How much of the applied arsenic reaches the forest floor in litter in treated stands?
3. To what degree does the arsenic content in the soils of treated stands change?
4. What factors influence the distribution of arsenic in the forest ecosystem?

The form of organic arsenicals in the soil is an important behavioral characteristic originally proposed for this study. This information is beyond the scope of this thesis, but is dealt with elsewhere (Newton, 1971). The results of this study should be useful in interpreting the behavior of all organic arsenical herbicides when used for thinning and for other silvicultural practices.

LITERATURE REVIEW

The Uses of Organic Arsenicals

Herbicides containing organic arsenicals have been used effectively in several silvicultural practices since they were first tested as chemical thinning agents. The most common organic arsenicals, cacodylic acid and MSMA, have been used successfully for thinning and for hardwood control on a wide range of coniferous and hardwood species. This method of thinning has generated interest because it creates less fire and insect problems than saw-thinning. In addition, chemically treated stands are more accessible, esthetically more pleasing and may have less crown damage after severe snow or wind storms (Newton and Holt, 1971a).

Newton (1970) and Newton and Smith (1974) have summarized treatment effectiveness on many of the tested species. In almost every instance, the crowns of treated trees were quickly defoliated and/or killed. In some cases, chemical, dosage and season of treatment were found to influence treatment effectiveness. Newton (1971) has also noted the successful use of these compounds as adjuvants for phenoxy herbicides in brush control work.

These compounds have also been tested for chemical debarking and preharvest drying of commercial timber. Holt (1967, 1971) achieved from ten to 20 percent weight reductions in preharvest

killing of small, commercial Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) trees treated with cacodylic acid. Holt cited other researchers who achieved similar results on other coniferous species using different arsenicals.

In a different capacity, organic arsenicals have been carefully examined as systemic insecticides and fungicides. Work on the suppression and prevention of bark beetles has centered largely around post-flight treatment of infected trees (Chansler and Pierce, 1966; Chansler et al., 1970; Buffam and Flake, 1971) and pre-attack (trap tree) treatments (McGhehey and Nagel, 1967; Newton and Holt, 1971b; Frye and Wygant, 1971; Buffam, 1971). Results from both types of treatments were mixed, but generally showed promise for suppression of beetle populations in several forest types. Once again, dosage and season of treatment were influencing factors. These compounds can be effective fungicides as well. Laird (1971) found a reduction in root and butt rot (Fomes annosus (Fr.) Karst) infection of stumps of trees treated prior to harvest with MSMA. In addition, several researchers have noted that these compounds are fairly specific because associated insects, parasites and predators are often unaffected by treatment (McGhehey and Nagel, 1967; Hinds and Buffam, 1971).

The Fate of Organic Arsenicals

Distribution

Despite the utility of organic arsenicals in silvicultural projects, little work has been done to follow the distribution of these compounds through the forest ecosystem after application. The studies that have been done will be presented here in a logical pathway of distribution from the injection point to the ultimate sink for arsenic.

Wort, as quoted by Holt (1971), examined the distribution of arsenic in the trunks of Western Hemlock (Tsuga heterophylla (Rafn.) Sarg.) and Douglas-fir trees treated with sodium arsenite. Although Wort did not use an organic arsenical, his work gives an indication of the arsenic gradients in the trunks of trees treated with arsenicals. The greatest concentrations of arsenic were in the outer one-eighth-inch of wood and in the first one-sixteenth-inch of bark adjacent to the sapwood. The concentration of arsenic decreased both horizontally with distance from the cambium and vertically with distance above the injection point. The gradient approached zero parts per million (ppm) arsenic at the center of the tree and decreased from 17.3 ppm arsenic six inches above the injection point to 1.75 ppm 100 feet up the trunk.

Data showing the distribution of arsenic in the crowns of treated trees is sketchy. Newton, as quoted by Norris (1971), sampled foliage in pines treated with organic arsenicals in November and detected

110 ppm, 139 ppm and 58 ppm arsenic the following April, June and August, respectively. Allard, also quoted by Norris (1971), found 116 ppm arsenic in dead pine needles from the upper branches of a treated tree and 2.5 ppm arsenic in green needles from lower limbs. Needles intermediate in damage and in crown position had arsenic levels within this range. Thus, the arsenic concentration in the crown of treated trees appears to be correlated with time after application, crown position and needle damage. Both studies indicate that substantial amounts of arsenic could enter the forest floor through fresh litter fall.

Macklin and Witkamp (1973) monitored changes in arsenic levels in the litter and soil beneath Tulip Poplar (Liriodendron Tulipifera L.) trees treated with cacodylic acid. Leaf litter beneath trees treated with the recommended label dosage ranged from 190 ppm arsenic initially after treatment to 42.8 ppm arsenic 16 weeks later. In comparison, control leaf litter ranged from two to four ppm arsenic during the experiment. The untreated leaf litter and the soil from the A_1 horizon beneath treated trees had 9.7 ppm arsenic and 15.1 ppm, respectively, when collected 16 weeks after treatment. On the other hand, corresponding control values were 6.5 ppm arsenic and 15.4 ppm, respectively. Apparently arsenic was leached from the treated fresh litter fall to the untreated litter layer. No significant increase in the arsenic level was detected in the soil beneath

normally treated trees. This suggests that the soil is a sufficiently large sink of native arsenic so that a pulse from a single treatment causes little change. The authors also noted that the greatest loss of arsenic occurred in leaves which received the highest treatment dose (nine times the recommended label dosage). Some of these same trends have been observed in coniferous stands. Holt (1967) collected fresh litter fall beneath Douglas-fir trees injected with cacodylic acid. Values in this study ranged from 171.0 ppm arsenic to 12.4 ppm beneath treated trees. Litter beneath control trees was between 1.53 ppm and 2.15 ppm arsenic. In both of these studies the arsenic level in the forest floor increased significantly at first, but decreased with time. Their results indicate that time and dosage are important factors which influence the arsenic content in the forest floor.

In tracing the arsenic from the litter to the soil, Norris (U. S. Forest Service, 1974a, b) has been quoted as finding that MSMA and cacodylic acid are readily leached from three inch columns of chopped ponderosa pine (Pinus ponderosa Dougl.), Douglas-fir, or mixed true fir and larch (Larix occidentalis Nutt.) needles. Once in the soil, Norris found MSMA was quite resistant to further leaching, while cacodylic acid was somewhat more mobile. Correspondingly Canutt and Norris, as cited by Norris (1971), did not find detectable quantities of arsenic in streams flowing through MSMA-treated stands.

They concluded that little, if any, of the arsenic was leached from the soil. These results and conclusions by others (Sandberg, 1973b, c) indicate that the soil is the ultimate sink for arsenic.

Behavior

Behavioral characteristics such as the form, persistence and degradation mechanisms of organic arsenicals determine the pathway and biological effect of their transfer toward the ultimate sink. Most of the behavioral studies on organic arsenicals deal with these characteristics in the soil, where they remain. Relatively little work has been done on these same factors in other components of the forest ecosystem, through which they must travel.

Newton and Holt (1971b) and Laird (1971) reported evidence suggesting that the distribution of arsenic within treated trees was influenced by season of treatment. These investigators determined that organic arsenicals are apparently dependent upon temperature above a minimum threshold for phytotoxicity. Their distribution within the tree appears to follow carbohydrate transport when temperatures are low enough so that phloem tissue is not immobilized by toxic action. During rapid sap flow in warm weather, the chemicals were quickly transported to the crown of the tree. Only narrow strips of cells were killed, in line with injection wounds, while the phloem tissue between these strips appeared normal. During periods

of low physiological activity, October through February, MSMA and cacodylic acid did not show phytotoxic symptoms until the following spring. The cambium in these trees was more uniformly killed and damage to cells was more generalized. Crown damage appeared less streaky in fall treated trees than in those injected in the spring or summer. Presumably more lateral distribution occurred in fall treated trees because low temperatures prevented destruction of conduction tissues, and there was more basipetal translocation. Such a pattern would suggest that cold-season applications of arsenic in these trees would probably be distributed evenly throughout the tree; in comparison, the authors suggested that one might expect to find high concentrations of arsenic in localized dead tissue in spring treated trees. The initial amount of arsenic in fresh litter fall would be influenced if these factors are operative. In the short term, more arsenic would probably reach the litter faster after spring treatments because less arsenic would be tied up in the wood of spring-treated trees. In the long run, arsenic from twigs and branches would be released when the tree decomposed. So the same amount of arsenic is released either way, but the time may vary. This may be an important factor in controlling arsenic levels in the forest floor.

Numerous studies have examined the behavior of organic arsenicals in the soil. Woolson et al. (1969) reported that organic

and inorganic arsenic behaved similarly in the soil. They found that soils high in aluminum and iron bound the arsenic so tightly as to greatly reduce its availability. In fact, the water soluble (available) arsenic in a clay loam decreased by more than 90 percent within four weeks after application. Earlier, Dickens and Hiltbold (1967) had reported extensive adsorption of disodium methanearsonate (DSMA) by several soils. They achieved up to 100 percent adsorption in a clay soil column. Similarly, Ehman (1965) found only six percent of applied cacodylic acid leached through a sandy loam and nine percent through a sand column; his study used the equivalents of 15 pounds per acre cacodylic acid and 60 inches of water for leaching. He achieved similar results using 28 pounds per acre of DSMA. These arsenic concentrations are much greater than the amounts which would be added to the forest floor through fresh litter fall in treated stands. The time dependence of adsorption would suggest that a delay before leaching would minimize the free arsenical available for movement. Thus, very small concentrations of arsenic, if any, would be expected to leach from forest soil under actual use conditions.

Sandberg (1973a) concluded that arsenic did not accumulate in the soil after five annual applications of six to 7.5 pounds per acre of MSMA and cacodylic acid, respectively. He did caution that it was difficult to distinguish small accumulations of arsenic in some

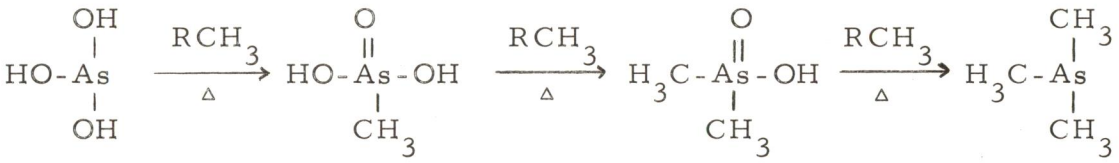
soils because of the inherent, wide variability of arsenic levels within these soils. On the other hand, arsenic levels in such soil, he reasoned, would show any significant increases in accumulation. In another study, Sandberg (1973b) reviewed the literature to determine if a buildup of arsenic normally occurs in the soils of forest stands treated with organic arsenicals. He concluded that normal applications of MSMA and cacodylic acid were not causing an accumulation of arsenic in soils.

Other researchers have examined the form of arsenic in the soil after application of organic arsenicals. Dickens and Hiltbold (1967) determined that a methyl carbon was removed from DSMA as CO_2 in the soil. The rate was related to the amount of organic matter in the soil. They presumed the arsenic was left as an inorganic oxide. Von Endt et al. (1968) also felt that MSMA was degraded to an inorganic form.

The fact that organic arsenicals are readily and tightly adsorbed in the soil, but are noncumulative over time may seem to be conflicting evidence. Even in an inorganic form, at least a temporary cumulative effect would be expected to occur because no pathways of arsenic loss have been accounted for yet. The apparent loss of arsenic from the soil must be occurring through some mechanism other than leaching from the soil horizon. A proposed mechanism is microbial degradation of organic arsenicals by soil microorganisms

and subsequent loss by volatilization.

According to Cox and Alexander (1973), the formation of an arsenic gas by biological action was first noted by Gosio in 1893. Gosio isolated a strain of Scopulariopsis brevicaulis and several fungi which were capable of producing what he presumed was diethylarsine gas from potato mash containing arsenic. Several decades later Challenger et al. (1933) correctly identified the gas as trimethylarsine. The postulated reaction sequence suggested by Challanger (1951) and simplified by Alexander (1961) is:



arsenious acid — methylarsonic acid — dimethylarsinic acid (cacodylic acid) — trimethyl arsine

Organic arsenical herbicides would be intermediate forms in this reaction sequence. The more oxidized the form of the organic arsenical, the more reduction that will have to occur. Thus, energy requirements for this reaction sequence vary by the state of oxidation of the arsenical.

Several studies including Zussman et al. (1961), Newton and Holt (1971b), Kearney and Woolson (1971) and Sandberg (1973a, b, c) have elaborated on the possible evolution of arsine analogs by reduction and methylation of arsenic compounds. The proposed analogs

are quite volatile, however, and are difficult to detect in recovery products. In one of the few successful studies, Cox and Alexander (1973) have positively identified trimethylarsine gas evolution from three sewage fungi growing in an organic arsenic-containing raw sewage media. In another study, Newton (1971) demonstrated that high concentrations of organic arsenicals are subject to attack by molds and possibly other microorganisms. After two weeks, he detected a garlic-like odor characteristic of methylarsines evolving from agar plates inoculated with methanarsonic acid (MAA). He deduced that the observed arsenic losses occurred through volatilization by microorganisms. Trimethylarsine gas was presumed to be the principal metabolite.

Kearney and Woolson (1971) have identified two metabolic pathways of cacodylic acid degradation. First, they detected an oxidative pathway which resulted in cleavage of the carbon-arsenic bond. Arsenate was the primary end product of the pathway. The second method they observed was reductive metabolism by molds such as Scopulariopsis. Their end product for this mechanism was an unidentified gaseous alkyl arsine. The arsine had a carbon-arsenic ratio identical to dimethylarsine. Therefore, there are probably two mechanisms of organic arsenical degradation. Newton (1971) suggested that both the oxidation and reduction may occur simultaneously. Researchers such as Von Endt et al. (1968) probably have not found

the evidence supporting this suggestion in their studies because they did not adapt their technique to determine the presence of volatile arsines. Nevertheless, the literature indicates significant amounts of organic arsenic may be volatilized by microbial degradation. Sandberg (1973b) and Woolson and Kearney (1973) among others agree that the reduction reactions occur in both aerobic and anaerobic conditions. They conclude that the arsines are so unstable, however, that they are quickly oxidized to a pentavalent form of arsenic. Thus, arsenates are probably the ultimate fate of organic arsenicals in the environment. Sandberg (1973c) arrived at a similar conclusion concerning the fate of organic arsenicals in an agronomic ecosystem. Sandberg concluded that due to microbial reduction of arsenicals, the input of arsenic into the soil phase is redistributed between treated and nontreated soils. This redistribution precludes a buildup of arsenic in the soil.

The total of these studies suggests that these compounds should be classed and treated separately from inorganic arsenicals which have been widely reported as persistent and cumulative compounds. Nevertheless, a complete knowledge of the behavior of organic arsenicals is still needed for accurate analysis of hazard.

Safety

The pentavalent forms of arsenic, including the organic

arsenicals, are much less toxic than trivalent inorganic arsenicals according to Clarke and Clarke (1967). They concluded that these forms of arsenic present no real threat to animals upon limited exposure. Also, Sandberg (1973c) concluded that arsenicals do not biomagnify in food chains. On the other hand, bioaccumulation of arsenic in animals is related to exposure. Sandberg pointed out that elevated arsenic levels in animals rapidly decreases when exposure to the compound is reduced, because most animals rapidly excrete arsenic.

Methylated arsines are highly toxic compounds. They are also gases and would be expected to leave treatment areas in low concentrations by mass air movement (Sandberg, 1973a, b, c). In addition, these volatile compounds are probably quickly oxidized to arsenates which are then tightly bound to soil molecules. This greatly reduces the availability of these compounds to various organisms (Von Endt et al., 1968).

Tarrant and Allard (1972) found no evidence of health problems or accumulation of arsenic in workers using MSMA and cacodylic acid for precommercial thinning. Arsenic was absorbed through the skin, but most of it was excreted from the body within one to two days. Arsenic levels in the body were correlated with amount of exposure and they recommended all necessary precautions to minimize exposure.

Other than studies of hazard to applicators, the exposure of

forest dwellers to arsenic residues has been ill-defined, especially in relation to tree-dwelling insects, birds and mammals. Preliminary results of a feeding study by Dickinson, as quoted by Norris (1971), indicates MSMA is more toxic to cattle than previously believed, while cattle fed similar quantities of cacodylic acid were not appreciably affected. Two of three cows died when fed ten milligrams per kilogram of body weight of MSMA. Norris (1971) also cites preliminary findings of Schroedel who detected no arsenic-induced lesions or arsenic residues in the tissues of miscellaneous birds, mountain beaver, porcupines, ruffed grouse and a deer shortly after chemical thinning. About 50 percent of the voles, shrews, mice and chipmunks collected had arsenic residues between 0.5 and 9.8 ppm between 2 and 30 days following treatment. Few animals collected after 30 days contained detectable residues. A total of 11 snowshoe hares were found dead on one treatment area.

The amount of arsenic within treated trees, litter and soil and the factors affecting its distribution among these components are questions which are not fully understood but are important for evaluating hazard to various forest dwellers. This study is designed to answer some of these questions.

METHODS AND PROCEDURES

Investigational Approach

This study examines the generalized patterns of distribution of organic arsenicals in several components of forest ecosystems when used for precommercial thinning. Comparisons are made in the patterns of distribution of elemental arsenic among stands and within trees which were treated with one of two different arsenicals, in two different treatment seasons. Some of the factors which influenced the distribution or concentration of arsenic within and among these components are examined to help evaluate the behavior of organic arsenical silvicides on a causative basis. Finally, the amounts of arsenic added to the litter and soil are derived to determine the amount of elemental arsenic which cycles through the forest ecosystem after a standard chemical thinning.

Plot Locations

The study sites were selected in four forest types. The locations include:

1. Frater Lake, Washington, Colville National Forest; lodgepole pine (Pinus contorta Dougl.) type, 65 years old, 3,200 foot elevation.
2. Twelve Mile Creek, Washington, Colville National Forest;

mixed conifer type, 50 years old, 2,800 foot elevation.

3. Pringle Falls, Oregon, Deschutes National Forest; ponderosa pine type, 65 years old, 4, 500 foot elevation.
4. Mt. June, Oregon, Eugene District, Bureau of Land Management (BLM); Douglas-fir type, 30 years old, 3, 500 foot elevation.

All plots were in second-growth stands with gently rolling topography. The sites were selected with the aid of United States Forest Service or Bureau of Land Management personnel in stands designated for standard chemical precommercial thinnings.

The treated trees on all plots were primarily codominant or intermediate except for a few, scattered, older, merchantable size, dominant trees. Most trees were less than full-crowned (i.e. crowns were one-half or less of the total tree height). The estimated number of trees per acre in the Douglas-fir type ranged from 500 to about 2, 000 in dense patches. The mixed conifer type had similar density, but both pine types were somewhat less dense. The trees on the Douglas-fir site appeared to be somewhat taller than the trees on the other areas.

Treatments

The experimental design for the study was set up in randomized blocks (Cochran and Cox, 1957). Each forest type is considered a

block. Within each block, ten one-acre plots were designated. Two one-acre replications each of five treatments were:

1. Cacodylic acid, fall treatment.
2. Cacodylic acid, spring treatment.
3. MSMA, fall treatment.
4. MSMA, spring treatment.
5. Control, no treatment.

Thinning was done according to typical contract specifications normally followed for such operations in each National Forest or BLM district. A Hypo-Hatchet tree injector, as described by Newton (1968) was used to make injections at Pringle Falls and Mt. June by a research crew. The hack-squirt technique (Finnis, 1967) was used at Frater Lake and Twelve Mile Creek by a Forest Service crew. Fall treatments were made in early December, 1971. Spring treatments were made in late April, 1972, for Frater Lake and Twelve Mile Creek and early June, 1972, for Pringle Falls and Mt. June. Cacodylic acid treatments were applied as Silvisar 510, an Ansul Company commercial solution, which contains the equivalent of 50-percent by weight cacodylic acid or 5.7 pounds per gallon. MSMA treatments used the Ansul Company product Silvisar 550 which contains the equivalent of 48-percent by weight MSMA or 6.0 pounds per gallon. Treatment dosages at Mt. June and Pringle Falls were one milliliter of chemical per two inches of diameter. Frater Lake and

Twelve Mile Creek plots received approximately one milliliter per inch of diameter.

Field Sampling

Three major categories of samples were collected in the field for analysis of arsenic. They were taken from trees, litter and soil, with most of the observations taken from the tree layer. Sample collection and preparation will be discussed separately for each category.

Trees were sampled for both vertical and horizontal distribution of arsenic and for the partitioning of arsenic between wood and foliage. One tree from each plot was randomly selected during each collection. The tree was cut and subsampled according to the following specifications. Trunk samples from treated trees were taken in nine-inch sections directly above the injection point. Trunk samples of control trees were sampled at breast height (i. e. 4. 5 feet above the ground). Crown foliage and twigs from treated and control trees were sampled on the average from whorls 1, 4, 6, 9, and 12. Samples from whorls six and nine were composited from different ages of foliage and twigs along the branch. Samples from whorls one and 12 were similarly composited, but were separated into all wood and all foliage samples to examine the ratio in arsenic distribution between needles and twigs. The entire fourth whorl was collected

to examine horizontal distribution of arsenic in the crown. The sample was subdivided into each of the four growing years. Foliage and wood were not separated, but notes were taken regarding relative abundance of foliage. All crown samples were dried for 24 to 36 hours between 38 and 50 degrees Celsius and then ground to pass through a 20 mesh screen size in a small Wiley mill. Trunk samples were dried at room temperature for three to four months and then chipped and ground to pass through a 10 mesh screen size in a large Wiley mill.

Litter was collected from each plot in two 2.25 square foot litter traps which were randomly placed before treatment. The dry weight of four litter samples was taken to estimate the amount of fresh litter fall, although precise estimates of litter fall were beyond the scope of this study.

A composite sample of 50 soil plugs, 0.75 inch in diameter, between one inch and eight inches in depth, depending on the distribution of large stones and roots, was taken at random from each plot. Treatment replicates of soil samples for each forest type were combined for arsenic analysis because of economic limitations. The composited replicates were mechanically mixed and ground to pass through a 20 mesh screen size in a large Wiley mill.

Tree and litter samples were collected from late June to early July in 1972, and again from mid-September to mid-October in 1972.

In view of the stability of arsenicals in the soil (Dickens and Hiltbold, 1967), soil samples were analyzed from the fall 1972 collection only.

Observations

Besides sample collection, general observations were also noted in the field. The pattern of crown kill and treatment effectiveness were qualitatively observed, but not measured or described by any specific variable. The quantity of silvicide used was also noted to check differences between forest types.

In the laboratory, several additional observations were recorded. The diameter of the trunk samples was measured to the nearest quarter-inch. The percentage of foliage in each crown sample was rated on a four point scale in an attempt to compensate for any wood-foliage arsenic ratio that would bias samples of different foliage composition. Samples were rated according to rough percentages as follows:

1. 5% or less foliage.
2. 5% to 50% foliage.
3. 50% to 95% foliage.
4. 95% or more foliage.

In addition, a five point crown condition scale was reconstructed from notes to help determine the pattern of crown kill and treatment effectiveness. Trees were rated as follows:

Class 1 - Healthy tree.

Class 2 - Tree appearing abnormal; slight foliage discoloration.

Class 3 - Tree with top whorls dead, or crown appearing seriously discolored and deteriorated.

Class 4 - Tree with only a few branches of the crown with needles on them; needles all brown; death imminent.

Class 5 - Tree completely devoid of live crown; tree considered dead.

Arsenic Determination

A special procedure was worked out to determine the amount of elemental arsenic in plant tissue. Samples were atomized in a graphite tube furnace for atomic absorption. Manning and Fernandez (1970) describe the technique and applications of this new method. The specific procedural details developed in this study for working directly with arsenic in plant tissue are given in Appendix A. In general, however, one gram samples were digested in a nitric acid wet-ash digestion specifically adapted for this procedure. The digested samples were filtered, diluted to a constant volume and analyzed by atomic absorption using a graphite tube furnace.

The graphite tube furnace is an adaptation of the atomic absorption spectrophotometer developed by the Perkin-Elmer Company. The tube assembly replaces the usual burner unit in a standard

atomic absorption instrument. An electric current heats the tube to dry, ash and vaporize samples placed within the tube. The first step evaporates the solvent; the second step chars and removes the organic matter present; the final step vaporizes the solid residue. Light absorbance of the atomized samples is then calculated in the usual manner within the atomic absorption spectrophotometer.

Most of the samples in this study were run in triplicate. The mean absorbance of each sample was then used to determine elemental arsenic in parts per million (micrograms of arsenic per gram of digested material) from a standard curve. Details of the standard curve preparation are in Appendix B. The equation of the standard curve was fitted by a least squares regression analysis and appears with the curve itself in Figure 1.

Soil samples gave erratic results when run by this method. I would speculate that the digestion matrix of soil samples contained salts that did not readily char and interfered with the absorbance signal upon atomization. Therefore, the soil samples were sent to the Washington State Division of Health for arsenic analysis.[1]

The results of both arsenic determination methods gives the total amount of elemental arsenic present in the sample. The amount

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[1]The assistance from Dr. Logan A. Norris, U. S. Forest Service Forestry Science Laboratory, in processing and financing these analyses is gratefully acknowledged.

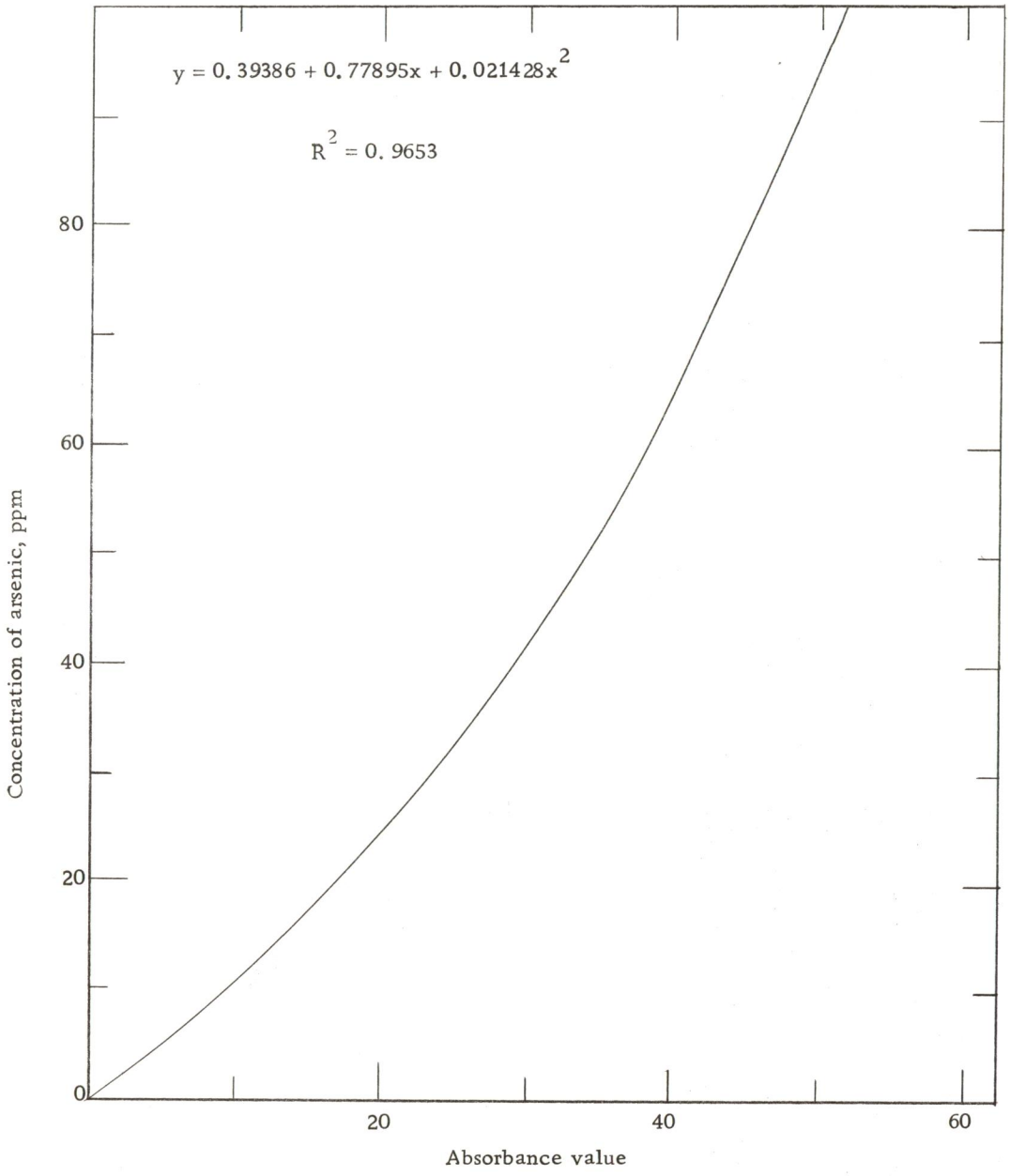

Figure 1. Arsenic standard curve and equation for digested samples analyzed in the graphite tube furnace. The equation was derived from a least squares regression analysis using the linear and quadratic functions of absorbance as the independent variables.

of arsenic greater than the average level of arsenic in control samples can be assumed to be the amount of arsenic added as the result of treatment.

Long-term Observations on Distribution and Degradation in Wood

Two trees were collected to determine if elevated arsenic levels were still present in the wood more than four years after treatment. A larch and a lodgepole pine, both about three inches in diameter, and with two injections of cacodylic acid apiece, were collected in early spring, 1973. The trees were near the Frater Lake, Washington, lodgepole pine block. The trees were sampled at breast height, the upper lateral whorls and the leaders. The bole sample of each tree was divided into phloem, outer one-eighth-inch of xylem, remainder of xylem and a wafer of bole including bark. Another bole sample near the base of the crown was taken on the lodgepole pine and subdivided similarly. All samples were ground in a Wiley mill to pass through a 20 mesh screen. The samples were analyzed at the Oregon State University Radiation Center by neutron activation.

Statistical Analyses

The data derived from observations in this study take several forms, each requiring different statistical procedures for evaluation.

The analysis of each set of data was based on the best fitting statistical techniques consistent with the form of the material. Arsenic concentration gradients within individual trees were fitted to distribution curves by multiple regression, using the partial contributions of the linear and quadratic effects of crown position and the proportion of foliage included in each crown sample to explain variance. An analysis of variance was used on complete sets of data to evaluate differences in arsenic concentrations among trees. Covariance was employed where an adjustment for uncontrollable variation due to diameter differences among sampled trees was necessary. Comparisons among unweighted means were made on some data with unequal sample size which did not fit these techniques.

The range of arsenic concentrations appeared large and variable, even among individual trees and means of replicate plots. This high random variability in sampling material precluded precise estimates of differences among treatments or close fitting prediction models. Nevertheless, the estimates of differences are precise enough to be useful for evaluating general trends and distinguishing general conceptual differences between sites and treatments.

Standardized Observations

A preliminary examination of the data indicated that each crown observation contained an effect of site, treatment, collection date,

position in the tree crown, proportion of foliage in the sample and tree diameter, in addition to random error. In order to compare the effects of various treatments on an equal basis, some of these factors had to be partitioned out, quantified, and used to adjust observations within trees to a standardized basis for comparison.

To begin with, it was necessary to sort out the within-tree variation so some observation could be derived that would be representative of the entire tree. This observation also had to be consistent for all treatments and sites despite variations in distribution. The derivation of such a standard observation also had to be adjusted on the basis of an average-sized tree in order to be more effective in comparing treatments involving a range of sizes. Regression and covariance methods were used to this end.

A stepwise least squares regression analysis (*STEP) was employed to fit a regression line to the observed arsenic concentrations within individual trees as a function of the linear and quadratic effects of whorl position and the amount of foliage per sample. The overall average foliage content of all samples was substituted for foliage proportion observations where this data was missing. Equation 1 shows the general form of the equation of each tree.

$$Y = B_0 + B_1 x_1 + B_2 x_1^2 + B_3 x_2 \qquad (1)$$

where Y = concentration of arsenic in ppm; x_1 = whorl position; x_2 = proportion of foliage in the sample.

To adjust for variation due to differences in size of the trees collected, the regression equation for each tree was adjusted by

covariance on the basis of an average-sized tree. The average diameter of the collected trees from the corresponding site was substituted for trees with missing diameter observations. The mean coefficients of determination and residual mean squares indicate the relative fit of the covariance-adjusted regression models by site and treatment and are listed in Tables 1 and 2.

The predicted value at the sixth whorl was then calculated from the adjusted-regression curve for each tree. This value was chosen as the basis of comparison between trees because the sixth whorl approximates the center of both the sampled crowns and the fitted regression lines and had the lowest relative error. The sixth whorl is also representative of a typical crown response since it avoids potential environmental or treatment response extremes at the tip and base of the crown.

The fitted values thus derived were used as the standardized observations on which comparisons among sites, treatments and collection dates were made. A three factor analysis of covariance program (ANCOV3) was used to partition the effects of these variables from among-tree observations. Tests for the homogeneity of adjusted means between sites and between treatments were made according to the procedure specified by Snedecor (1956). The vertical concentration gradients for each treatment were derived from the treatment means of the regression coefficients after adjustment for diameter by

Table 1. Mean coefficient of determination (R^2) values of regression of arsenic content on crown position and proportion of foliage by site and treatment.

Treatment	Site				
	Lodgepole pine	Mixed conifer	Ponderosa pine	Douglas-fir	Mean
Caco. -fall	.7950	.5125	.7025	.5875	.6494
Caco. -spring	.6275	.2150	.8150	.5525	.5525
MSMA-fall	.5275	.6075	.4100	.5925	.5344
MSMA-spring	.4775	.5925	.5525	.3400	.4906
Mean	.6069	.4819	.6200	.5181	

Table 2. Average residual mean square values of regressions of arsenic content on crown position and proportion of foliage by site and treatment.

Treatment	Site				
	Lodgepole pine	Mixed conifer	Ponderosa pine	Douglas-fir	Mean
Caco. -fall	59	131	486	175	213
Caco. -spring	223	456	166	200	261
MSMA-fall	122	132	165	17	109
MSMA-spring	371	334	296	285	322
Mean	194	263	278	169	

covariance. The horizontal concentration gradient data did not fit these techniques, but were derived from the means of actual observations from the fourth whorl.

FINDINGS

The following presentation of results requires prior consideration of some experimental discrepancies that were beyond control, but which influenced the findings. Treatment specifications were different for the Washington blocks (lodgepole pine and mixed conifer stands) and the Oregon blocks (ponderosa pine and Douglas-fir). Twice as much silvicide was applied by the Forest Service crew per tree on the Washington plots as was applied by the research crew on the Oregon plots. The average amount of silvicide applied per acre and the average amount of elemental arsenic added by treatment were generally greater in the lodgepole pine stand than in the other stands (Tables 3 and 4). The implications of this variation will be discussed in conjunction with the appropriate analyses of results. Despite the differences in dosage, treatments were equally successful in severely damaging or killing the crowns of nearly all treated trees. The crowns of several trees treated in the spring, primarily with cacodylic acid, were only partially killed. The pattern of crown kill in these trees was spotty, but the greatest damage was generally in the tip and upper-mid sections of the crown. Damage in these trees was judged sufficient to result in either the death of the tree or suppression of the tree by released dominant trees much sooner than if left untreated.

Table 3. Average amount of silvicide applied by site and treatment. (Quarts per acre)

Treatment	Site				
	Lodgepole pine	Mixed conifer	Ponderosa pine	Douglas-fir	Mean
Caco. -fall	3.33	2.17	2.25	3.13	2.72
Caco. -spring	2.38	2.17	2.25	3.42	2.56
MSMA-fall	4.08	1.75	2.25	2.50	2.65
MSMA-spring	3.83	2.75	2.25	1.63	2.62
Mean	3.41	2.21	2.25	2.67	

Table 4. Average amount of elemental arsenic added by site and treatment. (Pounds per acre)

Treatment	Site				
	Lodgepole pine	Mixed conifer	Ponderosa pine	Douglas-fir	Mean
Caco. -fall	1.27	0.84	0.87	1.21	1.05
Caco. -spring	0.92	0.84	0.87	1.32	0.99
MSMA-fall	1.35	0.58	0.75	0.83	0.88
MSMA-spring	1.28	0.92	0.75	0.54	0.87
Mean	1.21	0.80	0.81	0.98	

Results of treatment are expressed in terms of defoliation in Table 5 according to site, treatment and collection date. An analysis of variance on these values showed that the only significant visible difference in the crowns of treated trees was between the average degree of defoliation between fall and spring collected trees. As would be expected, the fall collected trees had had longer to defoliate and thus reflected more serious crown deterioration. Differences between treatments and sites did not result in significant differences in crown damage (Table 1, Appendix C).

No tree mortality was observed due to root grafting between treated and untreated trees (backflash).

Table 5. Mean defoliation index values by site, treatment and collection date. Defoliation index is based on a 5-point scale, where 1 = a healthy tree; 5 = completely defoliated tree; considered dead.

Site		Treatment		Collection	
Lodgepole pine	3.8 a[1]	Caco.-fall	4.1 a	Spring	3.4 a
Mixed conifer	4.1 a	Caco.-spring	3.8 a	Fall	4.1 b
Ponderosa pine	3.6 a	MSMA-fall	3.7 a		
Douglas-fir	3.6 a	MSMA-spring	3.6 a		

[1] Defoliation values followed by the same letters are not statistically different at the 95-percent level of probability. Those not followed by the same letter are statistically different within the same column.

Variation Among Trees

The variation among some sets of trees can be seen in the arsenic concentration means of the standardized observations from the sixth whorl which are listed by site and treatment in Tables 6 and 7, respectively. The means of standardized observations from the fall and spring collection dates were 46 ppm arsenic each. The variation among trees due to these factors was partitioned out by an analysis of variance (Table 2, Appendix C). The significant differences were between sites (99-percent probability), treatments (90-percent probability) and site by treatment interactions (95-percent probability).

Site

The significance of variation between sites is apparently due to the highly significant differences between the average treatment concentration from the Douglas-fir stand compared to the other three stands as measured by the standardized observations. The average arsenic concentration after treatment on the Douglas-fir stand was 29 ppm, or almost half the mean arsenic concentration encountered on any of the other stands. The mean standardized arsenic concentrations on the other sites were nearly identical. This implies that the variation among sites is due primarily to

Table 6. Mean arsenic concentrations in the sixth whorl, foliage and twigs, from weighted mean trees exhibiting average defoliation, by site, ppm.

Site	Concentration of Arsenic
Lodgepole pine	52 a[1]
Mixed conifer	50 a
Ponderosa pine	52 a
Douglas-fir	29 b

[1]Arsenic concentrations followed by the same letters are not statistically different at the 99-percent level of probability. Those not followed by the same letter are statistically different.

Table 7. Mean arsenic concentrations in the sixth whorl, foliage and twigs, from weighted mean trees exhibiting average defoliation, by treatment, ppm.

Chemical by Season		Season		Chemical	
Caco.-fall	38 a[1]	Fall	39 a	Caco.	46 a
Caco.-spring	54 b	Spring	53 b	MSMA	46 a
MSMA-fall	40 ab				
MSMA-spring	52 ab				

[1]Arsenic concentrations followed by the same letters are not statistically different at the 95-percent level of probability. Those not followed by the same letter are statistically different within the same column and hierarchial level. The difference between concentrations by season is significant at the 99-percent level of probability.

biomass differences between the Douglas-fir stand and the other stands. Part of this difference may also be attributable to dosage differences between the Oregon plots and the Washington plots. The ponderosa pine stand was not similarly lower in concentration compred to the Washington plots, despite receiving a lower dosage, because the ponderosa pine stand was the shortest in stature, and had the lowest biomass and dilution factors.

Treatment

The mean response of trees to different treatments also resulted in significant variation in arsenic concentrations as determined by the standardized observations. Trees treated with cacodylic acid in the spring resulted in a mean arsenic concentration 16 ppm higher than that of trees treated in the fall with cacodylic acid. Although this was the only significant difference among individual treatments, the arsenic concentration of MSMA spring treated trees was similarly greater than MSMA fall treated trees by 12 ppm arsenic. The cacodylic acid treatments resulted in the highest mean concentration, 54 ppm arsenic for spring treatment, and the lowest mean concentration, 38 ppm arsenic for fall treatment. These values, however, are only slightly different than the corresponding mean MSMA treatment values and are not significantly different than the MSMA values. Spring treatment values in general were 14 ppm arsenic higher than

those of fall treatment, a highly significant difference. The differences in mean concentration response to cacodylic acid and MSMA treatment, in general, was imperceptible. Thus, the significance of treatment variation seems to be attributable to the season of application, but the absolute magnitude of the mean concentration values varies insignificantly by chemical.

Collection Date

The mean concentration of all standardized observations from trees sampled in the spring was not perceptibly different from trees sampled the following fall. Both values were 46 ppm arsenic. Thus, the effect of collection date on the arsenic concentrations among trees was negligible.

Variation Within Trees

Arsenic concentrations within trees followed vertical and horizontal concentration gradients in the crown. Differences also occurred among arsenic concentrations in various tissues in the tree. The results are generally illustrated by graphical patterns representing treatment means in order to evaluate the behavior of each chemical by season of treatment.

Vertical Distribution

The mean vertical concentration gradients in the crowns are presented in Figure 2 by treatment, along with their corresponding equations. Each gradient was determined by the regression coefficients of the linear and quadratic effects of whorl position from the standardized observation calculations, corrected for diameter variation among trees. Individually sampled conifers will vary from these general functions because of differences between sites and random error, but the overall average of many sampled trees for a treatment would follow the general gradient depicted by the graph of that treatment. These functions are not presented to give precise estimates; rather, they can be used to clarify basic trends and differences among treatments.

Each treatment appears to have a different vertical concentration gradient. Trees treated with cacodylic acid in the fall or with MSMA in the spring have the most similar patterns of arsenic distribution in the crown. In both of these treatments, the concentration of arsenic decreases from the tip of the crown to the base of the crown in a slightly parabolic curve. The MSMA spring treatment gradient has much higher concentrations throughout the crown than the cacodylic acid fall treatment gradient but follows the same pattern. The distribution patterns of the other two treatments differ somewhat

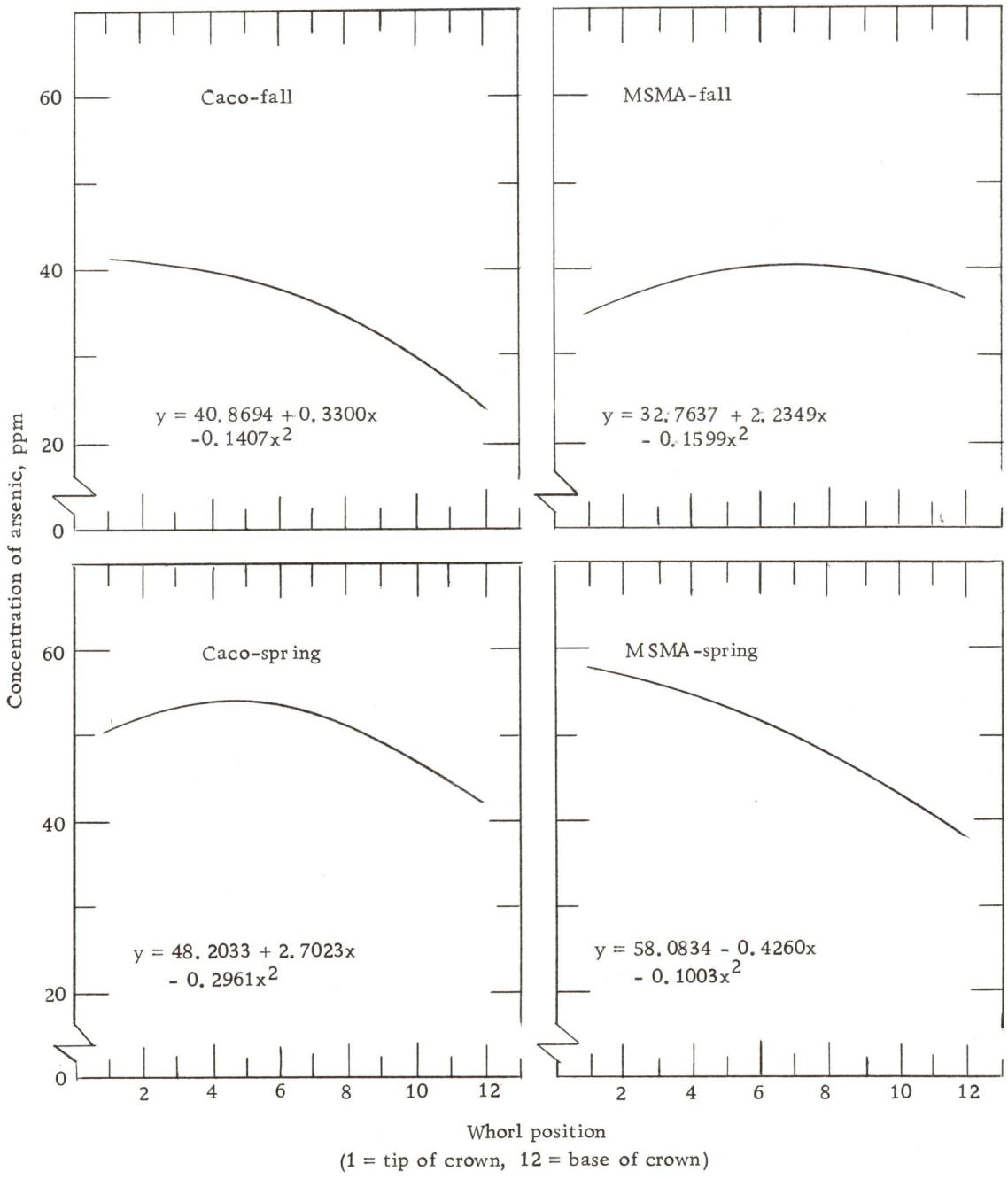

Figure 2. Mean functions of the vertical distribution of arsenic in the crown by treatment for the spring and fall collections combined. Values based on standardized observations corrected for proportion of foliage and diameter variation among trees.

from the first two. Both the cacodylic acid spring treatment gradient and the MSMA fall treatment gradient have stronger parabolic curves, with the highest arsenic concentrations in the upper-mid to mid-crown of the tree. The cacodylic acid spring treatment gradient is steep, while the MSMA fall treatment gradient is the flattest of the four treatments, ranging between 35 and 40 ppm arsenic. In general, the spring treatments appear to produce generally higher arsenic concentrations throughout the crown. For a given chemical, mean spring treatment values range up to 20 ppm arsenic more than mean fall treatment values at comparable points in the crown.

A more refined estimate of the differences between total arsenic concentrations in the entire tree crown by treatment was obtained by integrating the area beneath each of the curves in Figure 2 between whorls one and 12. The results (Table 8) verify that the spring treatments result in much higher total arsenic concentrations throughout the crown than fall treatments (i. e. up to 75-percent higher). Individually, the MSMA fall treatment resulted in a higher total arsenic concentration than the cacodylic acid fall treatment, but there was little difference between the two chemicals during the spring treatment season.

Table 8. Integrated area beneath vertical concentration gradients between whorls one and 12 by treatment.

Treatment	Area Units
Caco. -fall	311.12
Caco. -spring	552.99
MSMA-fall	428.15
MSMA-spring	550.72

Horizontal Distribution

The mean horizontal concentration gradients across the fourth whorl of the crown are presented in Figure 3 by treatment. The gradients represent the average of the observed arsenic concentrations for the respective treatment by nodal position from all sites. Since only the fall collection of data from the fourth whorl contained a complete set of treatment means for each node and for all sites, the gradients for the fall collection are compared to the gradients for the overall collection in Figure 3 to determine if the fall data alone can be used as an unbiased estimator of variance for the horizontal gradient analysis. The gradients appear to be nearly identical for each treatment except the overall means are generally higher than those of the fall collection means. Nevertheless, the fall collection of data follows the same general trends as the overall set of data and appears to be a reasonably acceptable estimator. Although the

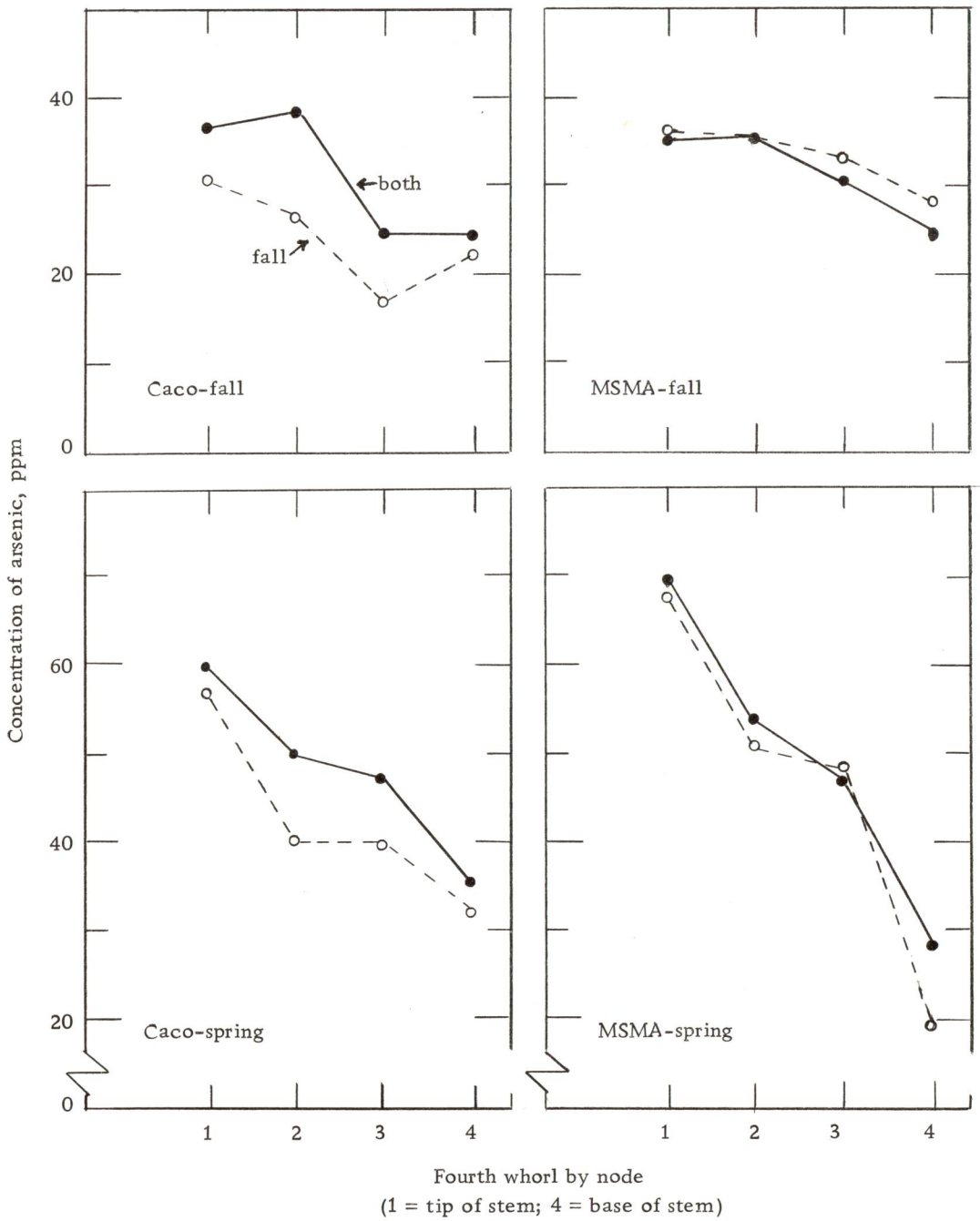

Figure 3. Means of the horizontal distribution of arsenic in the fourth whorl by treatment for the fall collection and for both spring (incomplete) and fall collections combined. Values are based on actual observations and are not standardized for proportion of foliage or diameter variation among trees.

spring collection means were not higher than the fall collection means in the standardized observations, the fourth whorl values show a relatively small difference by node for each treatment, which was not analyzed statistically, and is not thought to be important.

The mean arsenic concentrations for each node from the fall collection of data are presented by site and treatment in Tables 9 and 10, respectively. A three factor analysis of variance was used to evaluate differences between sites, treatments, and nodes and their interactions (Table 3, Appendix C). The three factor interaction term was used for the F-tests in lieu of an error term. The difference between sites, treatments, nodes and the interaction of treatment and node were all highly significant. In addition, the interaction of treatment and node was significant in the three factor analysis of variance, but not in a two factor analysis of variance using node and treatment.

The differences among the fourth whorl values by site were similar to these found for the standardized values. Mean arsenic concentrations on the Washington plots ranged between 7 and 19 ppm arsenic higher than means on the Oregon plots. Comparison among individual nodes by site were more variable, but still showed the same trend. Once again the Douglas-fir stand had the lowest mean aresenic concentration (28 ppm arsenic) of all sites and the ponderosa pine site was similarly lower (32 ppm arsenic) than the Washington plots (39 and 47 ppm arsenic).

Table 9. Mean arsenic concentrations of the fourth whorl by node (1 = tip of branch; 4 = base of branch) for each site and the overall means by site, ppm.

Site	Node				
	1	2	3	4	Mean
Lodgepole pine	42	42	38	32	39
Mixed conifer	74	49	40	27	48
Ponderosa pine	43	30	31	22	32
Douglas-fir	35	32	25	21	28

Table 10. Mean arsenic concentrations of the fourth whorl by node (1 = tip of branch; 4 = base of branch) for each treatment and the overall means by treatment, ppm.

Treatment	Node				
	1	2	3	4	Mean
Caco.-fall	32	27	17	21	24
Caco.-spring	57	40	40	32	42
MSMA-fall	36	35	33	28	33
MSMA-spring	68	51	44	19	46

Treatment differences were also similar to the findings from the sixth whorl standard observations, in that the spring treatments resulted in mean concentrations up to 21 ppm arsenic more than fall treatments. MSMA treatments seemed to be slightly higher in concentration (i. e. about ten ppm arsenic) than cacodylic acid treatments, but definitive differences cannot be described with confidence because very few trees were sampled per treatment and variation due to dosage and diameter differences precludes precise estimation in the lower range of differences between trees.

There was a general decreasing trend of arsenic concentrations from the tip of the branch to the base in all treatments. The rate of decrease varies by treatment. An examination of the curves in Figure 3 confirms this finding. The spring treatments appear to result in much steeper gradients than fall treatments. Trees treated with MSMA in the spring had the steepest gradients. The same chemical in the fall resulted in the flattest or most constant arsenic distribution gradients of all treatments, which agrees with the fairly uniform gradient found along the vertical axis in MSMA fall treated trees.

Wood-Foliage Relationship

The average concentration of arsenic in needles and branches for the tip and the base of the crown were calculated by treatment

in order to further evaluate treatment differences in organic arsenical behavior. The results in Figure 4 reveal that the concentration of arsenic is consistently higher in the needles than in the branches. Also, most of the arsenic concentrations at the tip of the crown are higher than at the base of the crown. In each treatment, the needle-to-wood concentration ratio is somewhat higher at the base of the crown indicating a stronger gradient exists between foliage and wood at the base of the crown. A comparison among treatments indicates the spring treatments have larger foliage-to-wood concentration ratios than fall treatments, while cacodylic acid treatments result in a greater foliage-to-wood concentration ratio than MSMA treatments. Thus, trees treated with MSMA in the fall had the most uniform distribution of arsenic throughout the crown. At the opposite extreme, trees treated with cacodylic acid in the spring had the largest amount of arsenic in the needles, and the largest differences between absolute concentrations and wood-to-foliage concentration ratios between the tip and base of the crown. These results agree with the findings from the vertical and horizontal distribution gradients. Although these ratios are not precise estimators, they do help elucidate the chemical and seasonal differences in the functioning of foliage as an arsenic sink.

Arsenic concentrations found in the trunks of sampled trees were insignificant. Over 80 percent of the values ranged between

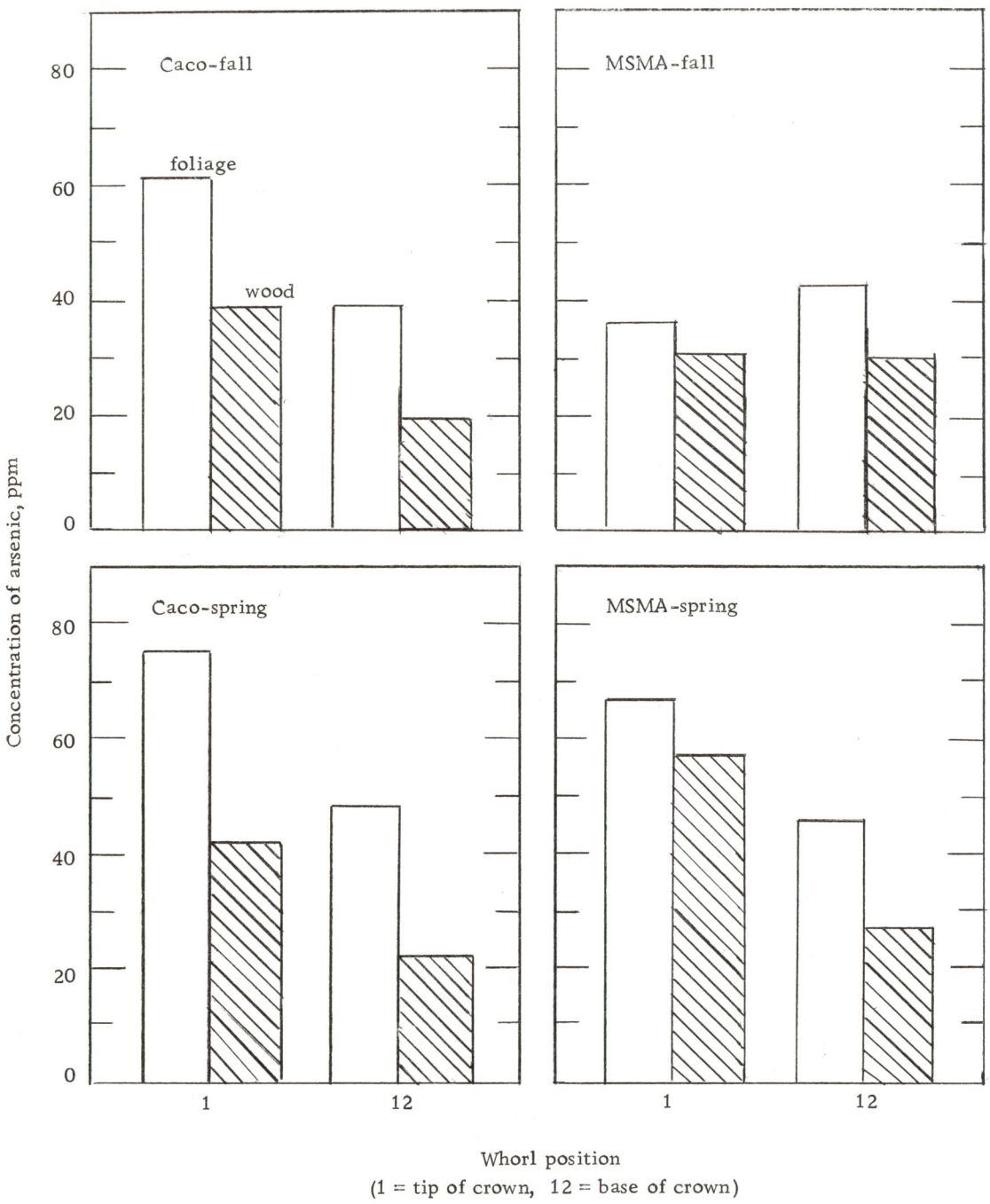

Figure 4. Mean concentration of arsenic in the foliage and in the wood at the top of the crown and at the base of the crown by treatment.

zero and three parts per million arsenic. Differences between sites or treatments were all within experimental error. The concentration of arsenic in the boles of treated trees is small and apparently not influenced by differences among sites and treatments.

Xylem Versus Phloem Distribution

Results from the neutron activation of the larch and lodgepole pine trees treated in August, 1968, are presented in Table 11. Each tree was completely defoliated. In both trees, the phloem had the highest concentration of arsenic and the concentration of the outer one-eighth inch of xylem was greater than the inner xylem. Thus, there appears to be a radial concentration gradient within the bole. The over-all arsenic concentration in the entire bole subsample was between the values observed in the phloem and xylem. The bole sample represents the approximate weighted average arsenic concentration of the entire bole at that point.

The upper-bole lodgepole pine samples had slightly higher arsenic concentrations than the corresponding lower bole samples. This further documents the vertical distribution trends already discussed, where the highest arsenic concentrations were in the upper-mid to top of the crown while the bole concentrations were generally lower. In addition, the leader or tip of the crown of the larch had concentrations about twice those of the upper lateral whorls. The

Table 11. Concentration of arsenic in trees over five years after treatment, unreplicated observations, ppm.

Tree Species	Sample Description	Concentration of Arsenic	Error
Larch	bole section	46.4	0.9
	phloem	670.0	10.4
	outer xylem	24.9	0.6
	inner xylem	18.8	0.5
	leader	77.8	1.3
	upper laterals	35.2	0.8
Lodgepole pine (lower bole)	bole section	16.5	0.5
	phloem	122.0	6.7
	outer xylem	18.3	0.5
	inner xylem	10.9	0.4
Lodgepole pine (upper bole)	bole section	27.6	0.6
	phloem	124.3	8.6
	outer xylem	25.0	0.8
	inner xylem	24.0	0.6
	leader	34.7	1.0
	upper laterals	35.8	1.0

lodgepole pine tip was partially missing, but the concentration of the remaining leader was not significantly different from the upper lateral whorls. There appears to be a general relationship among all these values. The concentration of arsenic is apparently correlated with the type and amount of tissue comprising the sample. Since the more active phloem tissue had higher concentrations of arsenic, samples with the highest proportion of phloem had the highest concentrations. In contrast, the inner xylem tissue is the least active and is exposed to the least translocation or diffusion of arsenic from the phloem. Consequently, the inner xylem has the lowest concentrations. Thus, as the proportion of inner xylem per sample increases, the arsenic concentration decreases. This hypothesis helps account for both the observed horizontal and vertical distribution gradients. The overall bole concentrations ranging from 16.5 ppm to 46.6 ppm partially reflect the very high treatment dosage compared to trees treated for this study by either treatment crew.

Litter

The overall means of arsenic concentrations in the litter by site, treatment and collection date are presented in Table 12. Prior to an analysis of the results, it should be noted that the sampling procedure had some inherent biases which affected the interpretation of this data. First, an undetermined amount of leaching occurred

Table 12. Means of arsenic concentrations in the litter by site and treatment (means of both collection dates) and by collection date for each treatment, ppm.

Treatment	Site					Collection	
	Lodgepole	Mixed	Ponderosa	Douglas-fir	Mean	Spring	Fall
Caco. -fall	27	20	23	5	19	15	22
Caco. -spring	42	29	8	7	22	12	33
MSMA-fall	46	23	23	5	24	18	26
MSMA-spring	39	44	9	9	25	14	38
Mean	39	29	16	7	--	15	30

between the fall and spring treatments and this leaching may have varied between sites, resulting in a variation in arsenic concentrations in the litter. Also, the fall collection measured two different time spans and leaching regimes in relation to fall and spring treatments. Differences so measured may be confounded with degradation and cycling mechanisms over time. Two unseasonably high litter values from the Douglas-fir site were not included in the data analysis because these values were probably the result of a saturated graphite tube which gave abnormally high absorbance values. Several litter samples were lost or destroyed by animal damage during the study. The resulting unequal sample sizes prevented an analysis of variance on the complete set of data. An analysis of variance was performed, however, on the complete set of fall collected litter values using the mean of each plot's replicate values. The statistical analysis was used to check the relative differences observed among litter value means from the overall collection of data.

Means of litter by site show that the concentration of arsenic on the Washington sites was more than twice as great as that of litter on the Oregon sites (39 ppm and 29 ppm versus 16 ppm and 7 ppm, respectively). The analysis of variance (Table 4, Appendix C) on the fall collection of data indicated the differences in litter concentrations by site are highly significant. Much of this variation may be attributable to the differences in treatment dosage between Oregon

and Washington. In addition, within dosage regimes, the pine stands in Oregon and Washington appeared to have litter concentrations about nine to ten ppm arsenic higher than the corresponding Douglas-fir and mixed conifer stands. This may be due to a lower biomass in the pine stand which resulted in more total silvicide per tree and less dilution of treated litter with untreated fresh litter fall. The lodgepole pine stands had the highest litter concentrations, but the stand also had the highest average treatment dosages per tree and per acre, with a low biomass dilution factor. The Douglas-fir stand had the lowest litter concentrations despite its having received the second highest amount of silvicide per acre. The Douglas-fir stand had a low dosage per tree, but a high dilution factor from its greater biomass. Thus, the amount of silvicide applied per acre and per tree, the biomass of the site, and the amount of leaching all appear to have some effect on the arsenic concentration in the litter.

The differences between treatments are not distinguishable in the litter on any site. The analysis of variance on the fall collection of data indicated no significant differences between treatments. The spring treatments appeared to result in nonsignificantly higher litter concentrations than the fall treatments. The litter from MSMA treatments in each season had somewhat higher arsenic concentrations than the corresponding cacodylic acid treatments, but again was not significant.

The difference between collection dates of 15 ppm arsenic does

appear significant, but since the analysis of variance was run on the fall collection of samples only, this difference cannot be statistically verified.

The dry weights of four litter traps were randomly selected from the spring collection to estimate the litter fall due to thinning and to reconstruct the approximate amount of elemental arsenic being added to the litter after treatment. The equivalent weights of fresh litter fall per acre on treated plots ranged from about 4, 600 pounds to 5, 800 pounds, or approximately two to three tons per acre. By contrast, a control plot from the lodgepole pine stand had about 1, 250 pounds of fresh litter fall per acre during the same period. By assuming the average litter fall in treated stands to be about 5, 000 pounds per acre for the first one half year after treatment and by using the highest and lowest concentrations of arsenic in the litter from the site and treatment means in Table 12 (i. e. 5 ppm and 46 ppm), a very rough estimate was made of the range of total elemental arsenic in the fresh litter fall immediately after treatment. Total arsenic in the fresh litter fall using these values ranged from 0. 025 to 0. 230 pounds per acre. These estimates appear reasonable, given the amount of elemental arsenic added per acre (Table 4). The spring litter collection was noticeably heavier than the fall litter collection, so it is likely that this range accounts for 50 percent or more of the fresh litter fall for the first year after treatment.

Soil

The soil arsenic values are presented in Table 13 as the mean concentration of arsenic added by treatment on each site. The values were calculated by taking the total arsenic concentration of the sample and subtracting the control arsenic concentration from each site. Each value represents the composited fall collection samples from replicate plots. No analysis of variance could be performed because there were no replicate observations from which to estimate random variance. Therefore, the validity of observed differences between sites or treatments cannot be confirmed. The presence of three questionable values suggests that either the precision of the arsenic determination method for soils was not accurate enough to detect small changes, or the variability of background arsenic levels in the soil was not sufficiently uniform within sites to justify the use of an overall control level by site for the pretreatment soil values on each acre. The positive values of 7.2 ppm and 5.6 ppm arsenic added by cacodylic acid fall treatments in the lodgepole pine stand and the Douglas-fir stand, respectively, are much larger increases than would be reasonably expected. For example, assuming a soil bulk density of about 0.8, there are approximately one million pounds of soil in the top six inches of each acre. Since the soil samples were collected from this layer, the two arsenic-added values above

represent additions of over seven and five pounds of arsenic per acre, respectively; that is more than the total elemental arsenic applied per acre. Part of this variance is attributable to the sampling procedure. Each composited soil sample contained an unmeasured small amount of fresh treated litter. Thus, all the values in Table 13 are slightly higher than the actual arsenic levels in the soil alone. The other questionable observation is a negative 3.2 ppm arsenic-added value for the cacodylic acid spring treatment in the Douglas-fir stand. The control value from this stand (6.9 ppm) compared to the cacodylic acid spring treatment value (3.7 ppm, total) suggests considerable inherent within-site variability because a negative discrepancy of this magnitude cannot be accounted for through sampling or arsenic determination method errors. This apparent variability was great enough to prevent precise interpretation of the soil arsenic because most treatment-related differences in soil values were smaller than this discrepancy. It can be concluded that within-site and between-site variation influence the total arsenic concentration in a soil sample sufficiently to mask differences due to treatment. In order to detect changes in the soil, pretreatment values from each acre would need to be taken.

Table 13. Net change in soil arsenic concentration in the top eight inches of soil, by site and treatment, ppm.

Treatment	Site				
	Lodgepole pine	Mixed conifer	Ponderosa pine	Douglas-fir	Mean
Caco.-fall	7.2	0.6	1.5	5.6	3.7
Caco.-spring	2.0	0.9	0.4	-3.2	0.0
MSMA-fall	1.3	2.2	2.2	2.0	1.9
MSMA-spring	1.2	2.4	1.7	0.0	1.3
Mean	2.9	1.5	1.5	1.1	

Controls

The arsenic concentrations to the nearest whole part per million for the untreated samples are presented in Table 14 as the means by site for the trees, litter and soil. The Douglas-fir and mixed conifer stands had slightly higher background arsenic levels than either pine stand, but the soil arsenic concentration for the mixed conifer stand was not higher. The variation in these values may be due to actual differences in arsenic background concentration between sites, or may reflect random fluctuations of the data. Random fluctuations could be attributable to either the method of arsenic determination, or inherent variation in arsenic levels within and between sites.

Table 14. Arsenic concentrations in untreated trees, litter and soil by site, ppm.

	Site			
	Lodgepole pine	Mixed conifer	Ponderosa pine	Douglas-fir
Trees	1	4	2	5
Litter	1	3	1	6
Soil	3	2	1	7

DISCUSSION OF RESULTS

Both external and internal factors affect the distribution and concentration of arsenic in trees treated with organic arsenical silvicides. The choice of chemical and the season of treatment both influence the pattern of distribution and the absolute concentration of arsenic in the tree. The intensity of a treatment on a given site also had its obvious effect on the over-all concentration of arsenic. The phenomena that control within-tree concentration gradients after different treatments also affect the rates of deposit in litter and soil. Therefore, they influence the behavior of organic arsenicals in the forest ecosystem. The behavioral relations observed indirectly were the interaction of the organic arsenicals with the season-dependent transport mechanisms in the tree, and the cycling and degradation mechanisms which influence the concentration of organic arsenicals in various components of the forest ecosystem through a period of time. Each of these relationships will be discussed individually.

Alteration of Transport Mechanisms

Results from this study indicate that the different patterns of arsenic distribution between treatments in conifers are influenced by a chemical x season-of-treatment interaction. These are probably

dependent upon temperature and the direction of transport in the system of phloem sinks.

The upward transportation of solutes in xylem tissue is passive and is not dependent upon the metabolic activity of the tree. Solutes such as organic arsenicals are therefore translocated upward in the transpirational stream regardless of the season of treatment. The most exposed foliage exerts the greatest transpirational pull and thus attracts the greatest quantities of xylem-carried solutes.

Phloem transport is largely an active process (i.e. a function of energy-consuming metabolic activity). Translocation of solutes in phloem is downward during most seasons other than spring. Thus, during most of the year, the transport mechanisms in the tree tend to equilibrate the concentration of solutes within the metabolically active parts of a tree when both systems are functional. If the phloem system is immobilized for any reason, the solutes tend to accumulate at the ends of growing tips and are not redistributed to various other growing areas of the tree.

The functioning of the phloem transport system can help account for the observed patterns of arsenic concentration. Relatively uniform distribution and over-all lower concentrations occur consistently in some trees; the steeper concentration gradients, increasing toward the extremities of branches are consistent in others. These differences appear to be related to the season of treatment, but also

vary in degree between chemicals.

Chemicals that do not depend on temperature for phytotoxicity (e.g. cacodylic acid) would be expected to concentrate at the ends of growing tips because they immobilize the basipetal phloem transport system in all seasons of treatment. This pattern would be consistent for all except the very low temperatures during which very little upward translocation would occur. This would explain the similarity between the steep gradients of cacodylic acid in both fall and spring treated trees. In contrast, chemicals that are strongly temperature-dependent (e.g. MSMA) would not immobilize phloem tissue during periods of moderately low temperatures and would result in an equilibration of the solutes throughout the various sink areas in the tree. Thus, these concentration gradients would be fairly uniform throughout the phloem system. During warm seasons, temperature dependent chemicals would behave more like cacodylic acid, immobilizing the phloem and leading to stronger concentration gradients, culminating toward stem tips. Logically then, basipetal transport of temperature-sensitive toxicants (MSMA) in conifers should therefore be restricted to both cool weather and season of basipetal movement. The integrals of the calculated functions of the vertical concentration gradients showed higher over-all crown contents of arsenic associated with steep gradients. In this case, higher levels of arsenic in the crowns of spring-treated trees are probably attributable to the relatively fast

not cite the importance of seasonal differences, it now seems likely that the differences in incidence of backflash may be attributable to the dependency of organic arsenicals on low temperatures for downward translocation; such phloem transport would depend on minimal injury to the conducting tissue, and seasonal basipetal direction. The higher number of backflash deaths in trees adjacent to the fall-treated trees is a logical consequence of grafts to roots functioning as arsenic sinks. In contrast, arsenicals applied in the spring are rapidly transported to the crown and less backflash was observed. Thus, there is indirect evidence which suggests the translocation of organic arsenical silvicides and the related effects such as backflash are influenced by the chemical-temperature-season interactions that influence the transport mechanisms within the tree. It is unclear at this point exactly how temperature affects the translocation of these compounds, but the apparent differences in concentrations and distributional gradients in treated tree crowns does appear to be related to the mobility of the chemicals in the tree as determined by their own toxicity.

This hypothesis also helps explain the inconsistent or mixed results of the use of organic arsenical silvicides for other silvicultural practices such as bark beetle control. If the phloem transport is immobilized, most of the arsenic is transported to the crown where it remains. Very little of the silvicide remains in or is

redistributed to the base of the treated trees where it may be needed most for suppression of beetle or fungi populations. These studies have been performed in different seasons and under different temperature regimes. In all cases, decreased activity of insects and fungi reflected more uniform concentrations of phloem arsenic when associated with fall and winter treatments, especially with MSMA (Newton and Holt, 1971; Laird, 1971). Thus, in some cases the phloem may have been immobilized, resulting in poor distribution and poor control.

Cycling and Degradation

Cycling, as used herein, refers to the flow pattern of applied arsenic through various components of treated stands. An arsenic cycle *per se* is not demonstrated, but has been discussed in the literature. It should also be noted that organic arsenical compounds are subject to degradation, but that elemental arsenic is not.

The dosage per acre, rather than dosage per tree (*i.e.* treatment intensity) affects the ultimate amount of elemental arsenic cycling through the ecosystem. To a certain degree, the amount of arsenic applied can be minimized as long as the treatments are effective in relation to management objectives. Newton and Smith (1974) reported data which showed that to achieve the same effective kill on increasingly larger trees, it takes more than a proportionately greater dosage of silvicide per inch of diameter. The relationship

they found appears to be about a three-halves power function of diameter. When the volume of a tree is roughly the five-halves power of diameter and dosage is the three-halves power, then the larger the trees, the lower the dosage per unit of biomass. In practice, stands with the largest trees generally get lower doses than smaller, denser stands. This may explain the slight significance of the effect of diameter of the tree on arsenic concentrations in crowns, despite a linearly proportional dosage scheme. It also helps account for the arsenic concentration variation between sites observed in this study.

Arsenic moves from the tree to the litter layer in fresh litter fall and then leaches into the soil. Components of treated trees break up and decompose into litter over an interval of time. Crude estimates from the litter analysis in this study indicate that most fine material in the crown ends up in the litter within one year after treatment. The medium and large material of the tree eventually breaks up and decomposes in the litter layer also. Thus, by controlling the relative amounts of arsenic in the different components of the tree, the distribution in time of arsenic cycling can be managed. Nevertheless, the absolute quantity is reasonable constant, barring degradation in place.

The trees collected for the xylem-phloem study had been standing for approximately four and one-half years after treatment. Many

of the smaller twigs and branches had broken off and presumably were decomposing in the litter layer at the time of collection. By the time the trees were analyzed, it was over five years after treatment, but the concentrations of arsenic in the wood were still comparable or even higher than those recently treated. It was impossible to reconstruct how much of the organic arsenic had degraded within these trees because initial concentrations within the tree were unknown. Comparable samples from the leader and upper laterals from trees treated specifically for this study from the Washington sites showed that arsenic concentrations were in the same range, so it is unlikely that substantial degradation occurred during the five-year interval. The arsenic determination procedure was quantitative only for elemental arsenic, and did not reveal the form of the arsenic. Regardless, it seems unlikely that much, if any, of the original wood-bound arsenic was volatilized and lost from the tree. Organic arsenic molecules bound in the wood are apparently not exposed to major leaching or degradation processes until the tissue decomposes. In this case, trees were temporary sinks for arsenic for at least five years after treatment.

There was no indication in this study as to what degradation or leaching processes may occur in the litter. In most cases the arsenic concentration in fall-collected litter was higher than in spring-collected litter, but the fall collection was lighter in volume.

The smaller amount of fresh litter fall from untreated trees during the summer months resulted in less dilution of treated litter from treated trees.

Since most of the fine crown material seems to defoliate within the first year after treatment and since the greatest accumulations of arsenic are in fine material, the greatest increment of added arsenic to the litter probably occurs within the first year. The remaining arsenic in the wood is released slowly as the wood decomposes. After the first year, only small quantities of elemental arsenic would be expected to transfer annually from the tree to the litter. In the meantime, it is likely that the main influx of arsenic via the litter layer would have leached into the soil (Norris, 1971), or would have been released and acted upon chemically by other mechanisms as the litter decomposed.

The speed with which leaching occurs is probably related to the amount, duration and type of precipitation. In this study, little precipitation occurred between the spring and fall collections, so loss of arsenic was minimal during the droughty months. Nevertheless, retention time of organic arsenicals in the litter is thought to be relatively short.

Numerous studies have indicated that organic arsenicals are quickly adsorbed by soil particles, but the noncumulative effect of organic arsenicals in the soil has not been completely resolved (see

Literature Review). Results from this study reveal only that it is difficult to distinguish small accumulations of arsenic in the soil because of the apparently large variability of background soil arsenic levels, even within sites. Sandberg (1973a) reached a similar conclusion. Since typical precommercial thinnings result in a total addition of elemental arsenic to each acre of only 0.54 to 1.35 pounds, an addition to the soil would be hard to detect without a comparison of pretreatment and post treatment values on each treated acre. Assuming there are approximately one million pounds of soil in the top six inches of each acre, the total increase in arsenic soil levels, even if all the added arsenic was immediately dumped in fresh litter fall, would be only 0.5 to 1.5 pounds of arsenic per acre. Since up to half the added arsenic could remain in the wood of treated trees, according to the litter calculations, it is likely that the arsenic added to the forest floor (from a given treatment) will probably be much less than one pound per acre (i.e. an addition of less than one ppm arsenic in the top six inches of soil). By the time all the wood-bound arsenic is released, the postulated degradation mechanisms may have reduced the original influx of added arsenic to near normal levels. In fact, a variation in degradation rates between sites and treatments could be a partial explanation for the observed inherent variability in soil arsenic levels. In addition, Ehman (1965) reported the inactivation of both DSMA and cacodylic acid in the soil within one month

after crop application at rates of five to ten pounds per acre. He also indicated that the rate of inactivation was a function of the amount of precipitation, the arsenical used and the amount applied. The lower levels of organic arsenicals used for silvicultural practices would probably be inactivated in less than one month after reaching the soil. Prior to this most of the applied arsenic is tied up in wood or foliage tissue which decreases its availability. Further research on organic arsenic degradation processes in the soil would help resolve how long and in what form soil arsenic remains. For the present, individual links in the degradation process have been studied, but the entire mechanism is not fully understood. It appears that most, if not all, of the arsenic added to the soil in the organic form is biodegraded. According to Sandberg (1973c), the compounds are volatilized and redistributed, so that arsenic levels return to near normal levels, perhaps as part of a global arsenic cycle.

The relationships discussed, in addition to treatment intensity, are the primary factors which influence the absolute arsenic concentrations at various points in ecosystems, and in time related gradients involved in the transfer of arsenic from site to site. There is an apparent discrepancy, however, between the distribution gradients actually derived and the gradients we might expect to find based on these relationships. The cacodylic acid spring treatment vertical concentration gradient would be expected to have steadily decreasing

concentration from the tip to the base of the crown. Arsenic distribution should tend to follow a transpirational gradient, and redistribution of arsenic to other sinks in the tree is minimal. The gradient derived in this study, however, shows the highest concentrations of arsenic are in the upper-mid crown for this treatment. This discrepancy in gradients can be explained in several ways. First, many of the needles with the highest concentrations of arsenic were defoliated and were not included in the sample, particularly from the top of the crown. This would tend to lower the gradient at the upper end of the crown. Secondly, only parts of each whorl were sampled from whorls six to 12. A maximum proportion of foliage was included in these samples, so the concentrations of arsenic would be higher starting at the mid-crown than if the entire whorl had been collected and subsampled. This would tend to elevate the concentration gradient, especially at the mid-crown, where arsenic concentrations were indeed fairly high. Transpiration also may have slowed as foliage was injured, equalizing concentrations among the very exposed whorls. This bias does not invalidate the trends of this study, however, since the discrepancies were relatively uniform between all sampled trees.

Evaluation of the Graphite Tube Procedure

The graphite tube adaptation on the atomic absorption spectrophotometer provided a fast, economical, arsenic determination procedure. After grinding and digesting in a manner similar to most determination procedures, each sample was run in two minutes or less, depending on the time it took for the drying and charring steps. An average of 30 to 35 samples per hour was accomplished in this study. Although cost varies according to many factors, the use fee on the equipment for this study was approximately $7.50 per hour. The low cost and time factors make this determination procedure attractive for large, first approximation studies where large numbers of samples of unknown concentration need to be determined.

The most accurate range developed for use in this study was from zero to about 100 parts per million of arsenic. This is a larger range than is estimated without adjustment by most other procedures, which may require dilution and/or recalibration within this range. Absolute variance increased with concentration in this study, resulting in a proportional error of approximately plus or minus ten percent plus some electronic noise. Actual error per sample was greatly reduced by running replicates of each sample. This way, if there were widely divergent values between replicates, more replicates could be run in order to approach the actual value

by a statistical average, or the questionable replicate(s) could be excluded as machine error. Multiple replicates are possible in this procedure because of the low cost and time factors.

The procedure developed for this study emphasized a wide range of concentrations, but by changing the voltage, the absorbance scale, and other machine variables, a smaller range with less variance could be used. The larger range was chosen for the sake of efficiency.

Precise estimates are less important in a behavioral study, than general relationships which elucidate the principles that are operative in the system. The ability to run replicates within the constraints of the study reduced the error to an acceptable level. For example, the results of other researchers using standard methods of arsenic determination (Macklin and Witkamp (1973); Holt (1967); and both Newton and Allard, as quoted by Norris (1971)) are in the same range. The principal benefit of this system over the others is that it permits extensive sampling in the field. Precise analyses would have been meaningless on a small number of samples because of the great variability in field samples. The large number of field samples taken in this study substantially reduces the chance of major sampling bias. Thus, this procedure adds an important capability for biological research on a variable matrix.

CONCLUSIONS AND MANAGEMENT IMPLICATIONS

The relationships discussed in this study influence the pattern of distribution and absolute concentration of arsenic in trees treated with organic arsenical silvicides. In addition, several implications of arsenic cycling and degradation mechanisms were elucidated. These relationships and their implications aid in interpreting the behavior of organic arsenical silvicides. The primary conclusions, whether found directly or inferred, are listed below:

1. Treatments ranging from 0.5 to 1.5 pounds per acre of elemental arsenic applied in the form of organic arsenical silvicides generally result in arsenic concentrations from 20 ppm to 60 ppm above background levels in the branches and foliage of trees, and in litter. Elevated arsenic levels in the soil are of smaller magnitude, one to three ppm, and are not readily detectable.
2. The temperature dependency of organic arsenicals for phytotoxicity influences the pattern of arsenic distribution in the crown when applied at different times of the year.
3. The mobility of arsenic in conifers appears to be regulated by the direction and degree of phloem transport at the time of application, independent of temperature, provided the phloem transport system is not immobilized by treatment.

4. Organic arsenicals tied up in woody tissues may not be exposed to substantial degradation or cycling mechanisms until the wood decomposes.
5. The relative amount of applied arsenic added to the litter and soil can be managed in the short term by controlling the distribution of organic arsenicals within components of the tree by choice of treatment.
6. There appears to be substantial inherent variability in background soil arsenic levels. Observations in treated areas were generally within the range of naturally-occurring concentrations.
7. The graphite tube furnace analytical process permits sampling intensities needed for variable biological material in ecosystem studies.

The findings of this study suggest that silvicultural practices using organic arsenical silvicides can be managed to achieve the desired results and to minimize the exposure and risks to other components in the ecosystem. Managing the distribution of arsenic by appropriate choice of chemical and season of treatment can affect the pattern of crown damage of the treatment and the behavior of the compound in the ecosystem after application. Treatment intensity can be minimized in keeping with treatment objectives to reduce the amount of elemental arsenic cycling through the ecosystem. In this way, the risk of chronic or toxic exposure to nontarget organisms

is lessened.

On the basis of this study, a fall treatment of MSMA would be the safest and most beneficial treatment for chemical thinning or other silvicultural operations. This treatment results in the most even distribution of arsenic throughout the tree. In thinning operations this minimizes partial crown kill and maximizes the effectiveness of the thinning operation per unit of arsenical used. Also, since more basipetal movement occurs with this treatment, more arsenic is tied up in the various sinks of the tree and this lowers the initial concentration of arsenic reaching the litter. In turn, there is more time for the initial input of organic arsenicals in the litter and soil to degrade before the arsenic in the wood is released. Thus, the exposure to terrestrial animals and soil microorganisms is minimized. Finally, fall treatment permits use of chemicals when more manpower is available for application. When temperatures are below freezing, the silvicide may become highly viscous, making application more difficult unless the chemical is diluted.

In conclusion, the objectives for any chemical silvicultural practice should be closely matched with the type of treatment and the treatment intensity. More than a minimally effective kill wastes the silvicide and increases the recognized risks of use. The relative amount of exposure to arsenic within components of the forest ecosystem can be partially managed to maximize treatment effectiveness

and to minimize the exposure to various groups of nontarget organisms.

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APPENDIX A

Arsenic Determination Equipment and Procedures

The following equipment and procedural specifications were used to determine the concentration of elemental arsenic in plant tissue. The equipment was rented from the Department of Soils, Oregon State University.

Equipment:

Perkin-Elmer model 303 atomic absorption spectrophotometer
Perkin-Elmer HGA-70 graphite tube furnace
Perkin-Elmer, Hitachi model 159 recording graph

Settings:

Programme VII (temperature regulation)
8 volts
Drying cycle - 30 seconds
Charring cycle - 45 seconds
Atomization cycle - 10 seconds (until peak)
Ultra-violet
194 Angstroms (wavelength)
Slit 4
Absorbance level scale - 3 times absorbance
Sample size -20 microliters

Procedure:

The nitric acid wet-ash digestion procedure used to digest plant tissue samples is listed below. Replicates of each sample after the digestion procedure were injected directly into the graphite tube for analysis. The graphite tube was burned off at maximum temperature for a few seconds after each sample to vaporize any sample residue left in the tube. If the residues are not burned off, the graphite tube becomes increasingly saturated and the resultant absorbance signals become erratic and imprecise. The spectrophotometer and recorder were warmed up for 20 minutes before

running samples. Trial samples were generally run for an additional 20 to 30 minutes until the background noise in the machine settled down to give reproducible results. Most of the erratic background noise was speculated to be the result of sputtering of the molten arsenic in the arsenic lamp during warm-up. A few check samples of known concentration were also run before the unknowns to ensure the machine was properly calibrated.

Modified[2] Wet Ash Digestion Procedure:

(1) Weigh out 1.000 gram of the sample. Place the sample in a 100 ml. Kjeldahl flask.

(2) Add 6 mls. of concentrated HNO_3 and 3 glass beads to the flask.

(3) Set the current interrupter on electric hot plates at 20% and heat the samples until foaming ceases and solutions bubble freely. Concentrate samples down to about 1 ml. apiece.

(4) Add 3 mls. of concentrated HNO_3 to each sample and concentrate the samples down to about 1 ml. apiece. Heat can be increased slowly by advancing current interrupter in integrals of 3% to 5% up to about 35%. Avoid excessive bumping by controlling the temperature or by adding extra glass beads to the flasks.

(5) Add 3 mls. of concentrated HNO_3 to each sample and concentrate each sample to near dryness (about 0.25 ml.). Current interrupter can be advanced to 40% maximum.

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[2]Adapted for arsenic determinations on an atomic absorption machine with a graphite tube furnace. Original Wet Ash procedure was adapted by Physiology Lab, Forest Research Laboratory, Oregon State University.

(6) Cool samples and add 30 mls. deionized H_2O to each. Cap flasks overnight or go on to number 7.

(7) Filter each sample using Whatman #41 filter paper into a 100 ml. volumetric flask. Rinse Kjehdahl flask twice with about 2 mls. deionized H_2O. Rinse filter paper twice with about 3 to 5 mls. deionized H_2O. Dilute to 100 mls. with deionized H_2O.

(8) Store samples in capped, polyethylene vials until analysis.

APPENDIX B

Standard Solution and Standard Curve Preparation

An arsenic standard solution was prepared as follows. Dissolve 0.1320 gram of reagent grade primary arsenious oxide, As_2O_3, in 25 ml. of 20% (w/v) KOH solution. Neutralize with 20% (w/v) HNO_3 solution (pH 7.0). Dilute to 1 liter with 1% (v/v) HNO_3. The standard stock solution is 100 μg/ml. or 100 ppm.

Individual standards of varying concentrations were made as follows. Take x mls. of 100 μg/ml. solution and add 100 - x ml. of deionized H_2O to get an x μg/ml. solution or x ppm.

Standards of 0, 5, 10, 20, 30, 40, 50, 70, 80, and 100 ppm of arsenic were prepared. Exactly one milliliter of each of these standards was added to 1.000 gram of untreated foliage. These samples were digested by a modified nitric acid wet-ash digestion procedure and analyzed for total arsenic concentration in a graphite tube furnace on an atomic absorption spectrophotometer (Appendix A). An average of 11 replicates were run at each concentration level.

The absorbance value and the absorbance squared from each concentration replicate were regressed against the concentration of arsenic using a least squares regression analysis. The resultant equation and its curve are shown in Figure 1. Mean absorbance values for each unknown sample were substituted into this equation to determine the concentration of arsenic in parts per million.

APPENDIX C

Tables 1-4

Table 1. Analysis of variance by site, treatment, collection date and interaction terms on the assigned terms on the assigned defoliation value for each tree.

Source	DF	SS	MS	F-Value
Total	63	34.9375		
Site	3	2.0625	0.6875	1.6923
Treatment	3	1.8125	0.6042	1.4872
Collection	1	7.5625	7.5625	18.6154**
Site x Treatment	9	8.0625	0.8958	2.2051*
Site x Collection	3	1.3125	0.4375	1.0769
Treatment x Collection	3	0.5625	0.1875	0.4615
Site x Treatment x Collection	9	0.5625	0.0625	0.1538
Error	32	13.0000	0.4063	

** - significant at 99% level

* - significant at 95% level

Table 2. Analysis of covariance and F-values by site, treatment, collection date and interaction terms on the predicted sixth whorl concentrations of each tree; x = diameter, y = predicted arsenic concentration at the sixth whorl.

Source	Sum of Products			Adjusted SS			
	DF	X*X	X*Y	Y*Y	DF	SS	MS	F-Value
Total	63	28.2869	-123.1678	33193.9375				
Site	3	7.7328	-57.4284	6697.3125				
Treatment	3	.1706	-22.5097	3489.3125				
Collection	1	.9001	-5.4553	33.0625				
Site x Treatment	9	3.3566	87.4878	7205.8125				
Site x Collection	3	.4155	-8.4953	721.8125				
Treatment x Collection	3	.7455	8.3509	513.8125				
Site x Treatment x Collection	9	2.5335	-14.3228	1636.8125				
Error	32	12.4324	-110.7950	12896.0000	31	11908.6137	384.1488	
Site + Error	35	20.1651	-168.2234	19593.3125	34	18189.9423	534.9983	
Treatment + Error	35	12.6030	-133.3047	16385.3125	34	14975.3188	440.2506	
Collection + Error	33	13.3325	-116.2503	12929.0625	32	11915.4372	372.3574	
Site x Treatment + Error	41	15.7890	-23.3072	20101.8125	40	20067.4072	501.6852	
Site x Collection + Error	35	12.8478	-119.2903	13617.8125	34	12510.2175	367.9476	
Treatment x Collection + Error	35	13.1778	-102.4441	13409.8125	34	12613.4146	370.9828	
Interaction + Error	41	14.9658	-125.1178	14532.8125	40	13486.7974	337.1699	
Regression on Error					1	987.3863	987.3863	2.570
Site Adjusted for Average Error Regression					3	6281.3285	2093.7762	5.450**
Treatment Adjusted for Average Error Regression					3	3066.7051	1022.2350	2.661
Collection Adjusted for Average Error Regression					1	6.8235	6.8235	.018
Site x Treatment Adjusted for Average Error Regression					9	8158.7935	906.5326	2.360*
Site x Collection Adjusted for Average Error Regression					3	601.6038	200.5346	.522
Treatment x Collection Adjusted for Average Error Regression					3	704.8009	234.9336	.612
Site x Treatment x Collection Adjusted for Average Error Regression					9	1578.1837	175.3537	.456

* - significant at 95% level

** - significant at 99% level

Table 3. Analysis of variance by site, treatment, node and interaction terms on the fall collection of fourth whorl arsenic concentration values.

Source	DF	SS	MS	F-Value
Total	63	26733.7344		
Site	3	3457.1719	1152.3906	11.096**
Treatment	3	4314.2656	1438.0885	13.847**
Node	3	4471.3906	1490.4635	14.351**
Site x Treatment	9	7367.7969	818.6441	7.882**
Site x Node	9	1898.1719	210.9080	2.031
Treatment x Node	9	2420.8281	268.9809	2.590*
Site x Treatment x Node	27	2804.1094	103.8559	1.000

Three Factor Interaction Term is Used for F-Tests.

* - significant at 95% level ** - significant at 99% level

Table 4. Analysis of variance by site and treatment on the arsenic concentration values from the fall collection of litter.

Source	DF	SS	MS	F-Value
Total	31	17164.4922		
Site	3	11171.4609	3723.8203	16.7710**
Treatment	3	760.6484	253.5495	1.1419
Site x Treatment	9	1679.7578	186.6398	0.8406
Error	16	3552.6250	222.0391	

I - significant at 95% level ** - significant at 99% level