RED ALDER (*Alnus rubra* Bong.) AS A BIOACCUMULATOR OF INDIUM AND DYSPROSIUM WITH AND WITHOUT DTPA TREATMENT AS MEASURED BY NEUTRON ACTIVATION ANALYSIS

A Thesis

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DEDICATED TO

MY

Brother Mohamed

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ABSTRACT

Bioaccumulation of indium (In) and dysprosium (Dy) by the roots of red alder (Alnus rubra Bong.) was tested in a small free-flowing stream in Oregon. Mixed solutions of the two elements were continuously introduced into the stream water as non-radioactive tracers for 1.8 hr and 2.1 hr in two separate experiments. In the first experiment, the two elements were added in a non-chelated form; in the second experiment, upstream from the first and at a later time, In and Dy were chelated with (carboxymethylimino)bis(ethylenedinitrilo)tetraacetic acid pentasodium salt (DTPA). Instrumental neutron activation analysis (INAA) was used to determine the quantity of each stable tracer in stream water and the quantity sorbed to the alder roots. Alder roots were found to sorb more Dy than In whether the elements were chelated or not. Non-chelated In was sorbed by roots up to a concentration of 1.7 μg/g of root (dry wt.), whereas the In-DTPA sorption rate was higher by a factor of approximately 2. Up to 29 μg/g of root (dry wt.) of non-chelated Dy were sorbed, while the sorption of Dy-DTPA was less by a factor of 8. The reported results point to the possibility that the elements with relatively large ionic radii, such as Dy, are most useful in elucidating the potential buildup of like elements in the environment, while elements with smaller ionic radii, such as In, may be more useful for tracing water over greater distances. It appears that metals chelated with DTPA are sorbed at low levels, regardless of ionic radii of the metals used.

The reported results also demonstrate the possibility of using the root structures of the red alder (Alnus rubra Bong.) in environmental pollution studies as a biological accumulator of heavy metals, chelated or non-chelated.

CHAPTER ONE

INTRODUCTION

As man discovers more uses of heretofore unimportant or curious elements, a need exists for a methodology to monitor those elements in the environment. Therefore, two experimental studies were designed to explore the accumulation of indium (In) and dysprosium (Dy) by red alder (Alnus rubra Bong.) root systems in a free flowing stream. These elements were chosen, in part, because of their increasing importance in modern industry and technology: their disposal into the environment is becoming a source of pollution, particularly to fresh water eco-systems. In addition, environmental constraints currently in force in the State of Oregon are so strong that all uses of radiotracers in the environment are prohibited. If this restrictive phenomenom grows, ecologists will be faced with the need to follow or trace pollutants in a field study, will be denied the sensitive tool of radiotracer methodology and will be forced to seek other tracer methods. One such alternative method is the use of stable elements such as rare earths and other elements like In in conjunction with neutron activation analysis.

Indium was chosen because it has a high thermal neutron cross section (203 barns with an ideal detection limit of 0.0034 parts per million (ppm) for no post irradiation radiochemistry) which makes it suitable for experimentation (Sunderman and Townely 1960). In addition In may mimic other elements in the trivelent metallic group III A, which might be introduced into fresh water ecosystems. Indium has an isotopic abundance of 95.8% of stable precursor indium-115 (^{115}In), which, with thermal neutron activation, will produce radioactive indium-116 (^{116}In) by the following nuclear reaction: $^{115}In(n,\gamma)^{116}In$. Indium-116 has a half life of 54.2 minutes and it has four easily resolvable photopeaks in its characteristics gamma ray spectrum, 0.417 MeV 36%, 0.819 MeV 17%, 1.09 MeV 53%, and 1.29 MeV 80%.

Dysprosium was chosen because it also has a high thermal neutron cross section (2700 barns with an ideal detection limit of 0.027 ppm) and to represent the rare earth group of elements, some of which are found in the heavy fission products derived from uranium (^{235}U) and plutonium (^{239}Pu). Dysprosium has an isotopic abundance of 28.2% of stable precursor dysprosium-164 (^{164}Dy) which when activated with thermal neutrons produces radioactive dysprosium-164 (^{164}Dy) by the following nuclear reaction: $^{164}Dy(n,\gamma)^{165}Dy$. Dysprosium-165 has a half life of 2.35 hr and two resolvable photopeaks in its characteristic gamma ray spectrum, 0.095 MeV 4% and 0.361 MeV 1.1%.

Indium and Dy were also chosen because they form a very stable chelate with the chelating agent DTPA [(carboxymethylimino) bis(ethylenedinitrilo)tetraacetic acid pentasodium salt].

The experimental organism, red alder has the unusual characteristic of growing its roots directly into the flowing stream water. This root growth habit is not a result of stream erosion as is often the case with black willow (Salix nigra Marsh.). Alder roots were chosen for studying the accumulation of the stable tracers In and Dy, because they possess a large surface area in the water of the experimental stream, within the experimental site. Red alder roots are, therefore, ideal for the testing of possible bioaccumulation or sorption of the two biologically conservative, technologically enhanced elements in a stream environment. The site also offered the advantage of close its proximity to the Oregon State University Research Reactor.

The purposes of these studies were to establish a method of measuring the degree of In and Dy sorption under the natural stream conditions by unwashed alder roots, and to determine if there is a significant difference in the root sorption of these tracers in two different chemical forms, chelated and non-chelated.

REVIEW OF LITERATURE

The accumulation of the rare earth Dy in a laboratory indoor stream microcosm (800 ℓ capacity) containing living compartments of algae and snails (Physa spp.) was examined by Knaus (1976) in Texas. He found that stable Dy was quickly removed from water that was allowed to constantly recirculate through the microcosm. The coefficient of accumulation for Dy in algae and Physa spp. increased from 3,000 to 60,000 during a period from 4 to 55 days after the introduction of a solution of Dy into the microcosm as a single pulse. The coefficient of accumulation is defined as the ratio of the concentration of pollutants or materials sorbed by the bioaccumulator to the concentration of the same pollutants or materials in the surrounding medium or system. The bioaccumulator might be any organism which is able to concentrate a material of interest from the surrounding environment. Rediske et al. (1955), working with plants growing in soil, reported that a plant root system could be used as a bioaccumulator for pollutants due to its coefficient of accumulation which is greater than other plant organs. Curry (1976) reported that the roots of the black willow (Salix nigra Marsh.) growing in a recycling outdoor stream microcosm (1200 ℓ capacity) sorb the rare earth, lanthanum (La), up to a concentration of 24 μg/g of root (dry wt.) 1.8 hr after the pulse introduction of a solution containing 1 g of La. He also reported that willow roots sorb manganese (Mn) at a concentration up to 4,000 μg/g of root (dry wt.) 10 hr after tracer introduction into the stream microcosm. Using the same methodology as is employed in the present work, Knaus and Curry (1979) reported that S. nigra roots sorb Mn to a level greater than 1,000 µg/g of root (dry wt.) after 2 hr in a stream microcosm, but with the stream operating in a free flowing mode, that is, the stream water was allowed to pass by the roots only once. Knaus (1978) found La to be sorbed by S. nigra roots to a concentration of up to 101 µg/g of root (dry wt.) in a free flowing stream microcosm.

Loveland (1978) discussed the advantages that the stable, activable tracers possess over radioactive tracers in natural water studies. Jester and Uhler (1974) reported that bromine and iodine along with some studies of vanadium and cobalt ions could be used to mimic the movement of soluble species in natural water. Chennel and Kruger (1968) reported the use of rare earth tracers to follow pollutant dispersion in San Francisco Bay. The rare earths are easily detected by instrumental neutron activation analysis (INAA). This approach allows long-range pollutant tracing and the use of environmentally insignificant tracer levels. The use of short-lived activation products of collected samples allows rapid sample analysis (Loveland 1978). In a preliminary study (Schmitt and Wang 1968; Wang et al. 1969), the dispersal of lanthanum (La) and samarium were followed in a large river, but no attempts were made to systematically study the use of these elements as tracers. Indium and scandium were used as stable activable tracers for monitoring in-plant movements of water in wastewater treatment plants (Craft and Eicholz 1975). Dahl and co-workers (1970) have shown that indium nitrate [$In(NO_3)_3$] could be used to trace stream patterns and pollutant dispersal in and around the harbor of a Norwegian town. Smith et al. (1978) discussed In chemistry and the industrial influence in terms of pollution of In in the environment. They reported that as of 1970's, industrial releases of In to the environment annually comprise about 28 tons to the air, 100 tons of solid wastes to the land and about 230 tons to land and/or water. Wastes disposed of in water or on land result in the mechanical loss of insoluble In compounds from such wastes either by wind or water erosion. These actions probably account for more In entering natural water bodies by erosion than by leaching. They also reported that drinking water is unlikely to be a major source of In exposure to humans, but that the consumption of fish and shellfish that have bioconcentrated In from In contaminated waters is a potential source of human exposure.

Indium was discovered in 1863 by Reich and Richter. It occurs in commercially practical concentrations with the ores of zinc, iron, lead, and copper. Indium is a lustrous silver metal almost as soft as lead and is both ductile and malleable (Hilderbrand and Powell 1953). In 1924, Murray investigated In effects on silver base alloys, receiving a patent in 1926 for his indium electroplating process. He also founded the Indium Corporation of America in 1934. Indium, technologically enhanced as a result of the growth of modern industry and technology, has become an important commodity (Indium Corporation of America 1979). Indium combines with group V elements to form inter-metallic semiconductors which are widely used in infra-red detectors. It is also used for magneto resistors, solar energy conversion devices, and coating for bearings to improve corrosion resistance. Indium has helped make transistor production possible and speed the coming of computer age. In addition to these applications, In has been used as a stable environmental tracer of air dispersion (Shum et al. 1975) and in water flow studies (Hanson 1970; Chick 1979; Schmitt and Wang et al. 1969; Loveland 1978; and Smith 1978).

Dysprosium was discovered in 1886 by Lecoq de Boisbaudran. It is fairly well distributed in nature but at extremely low concentrations (Gschneidner 1964) and it was the last rare earth discovered. Dysprosium, which is also technologically enhanced as a result of the growth of modern industry and technology, has potential for becoming an important element. Dy belongs to group III B elements and is often found in uranium ores as a sesequioxide (R_2O_3). Dy is soluble in strong acids, but not in water (Yost et al. 1947). Reports of uses of rare earths (other than Dy) either singularly or as a class are common in the literature. However, technological uses of Dy specifically are not found in the literature, except for highly specialized laser devices and stable tracer experiments. Bibliographies of the uses of rare earth elements can be found in promotional articles of the Moly Corporation, Incorporated (Moly Corp. Inc., 1964, 1970, and 1973).

Metal chelates of amino carboxylic acids such as EDTA and DTPA represent some of the most stable chelates known. However

DTPA as a chelating agent possesses a greater thermodynamic stability constant than other chelating agents with metals (Reilly et al. 1959; Schwarzenbach and Anderegg 1954). Loveland (1978) reported that In and Dy form a very stable chelate with DTPA and that Dy-DTPA was the more stable chelate when compared to In-DTPA.

Hanson (1970) defined chelation as an equilibrium reaction between metal ions and a complexing (chelating) agent. The reaction ends with the formation of multiple coordinate bonds between the metal ion and a single molecule of the ligand forming an enclosing chelate ring which depends upon the acceptance of donated electrons by the metal from the ligand and the stability constant of the metal. Personal communication with Loveland and his co-workers at Oregon State University suggested the possibility of using In and Dy chelated with DTPA in water flow studies.

CHAPTER TWO

MATERIALS AND METHODS

Study Area

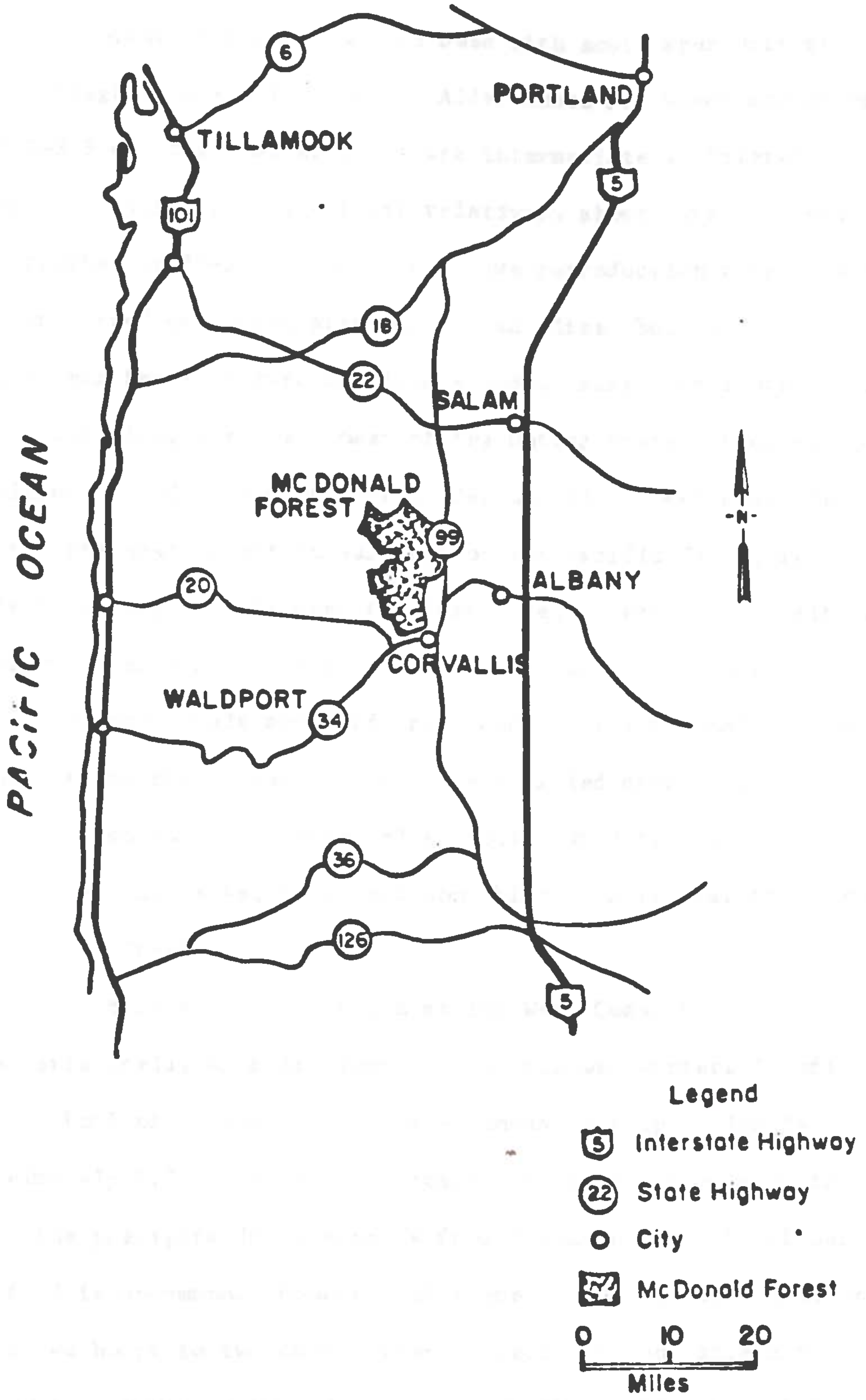
The experimental site is a small perennial stream flowing through the McDonald Forest, managed by the Department of Forestry at Oregon State University. The forest consists of 4.48×10^7 m^2 (11,000 acres) and is located 9.68 km (6 miles) west of Corvallis, Oregon (Fig. 2-1).

The vegetation in the McDonald Forest is mostly young Douglas fir (*Pseudotsuga menziesii* (Mirbel.) Franco.) with some 250 year old veterans, white fir (*Abies* spp.) and some very old oaks (*Quercus* spp.). Some other soft wood species present in the forest include grand-fir (*Abies grandis* (Dougl.) Forbes), western yew (*Taxus brevifolia* Nutt.), western red cedar (*Thuja plicata* Donn.) and hemlock (*Tsuga heterophylla* (Raf.) Sarg.), along with such hard woods as oregon ash (*Fraxinus latifolia* Benth.), big leaf maple (*Acer macrophyllum* Pursh.), vine maple (*Acer* sp.), wild cherry (*Prunus* sp.), willow (*Salix* sp.), madrone (*Arbutus menziesii* Pursh.), and red alder (*Alnus rubra* Bong.).

Red alder is a medium sized tree 24-30 m high and 0.3-0.9 m in diameter (maximum to 39 m high by 1.50 m in diameter), with a narrow rounded crown and pendulous branches (Preston 1975). Alder trees have smooth white or white mottled bark, with the inner bark red brown. Leaves are 5-15 cm long and 3-8 cm wide, ovate to

FIGURE 2-1

Location of McDonald Forest in the State of Oregon.


6
PORTLAND
TILLAMOOK
5
101
18
22
SALAM
PACIFIC OCEAN
MC DONALD FOREST
99
N
20
ALBANY
CORVALLIS
WALDPORT
34
36
126
5
Legend
5 Interstate Highway
22 State Highway
City
McDonald Forest
0 10 20
Miles

elliptic, obtuse or rounded at the base with acute apex with the margins slightly crenately lobed. Alder cones are woody and oblong ovoid 1-3.5 cm long. Alder trees are intermediate in tolerance, and exhibit rapid growth, but are relatively short-lived, maturity being reached in 60-90 years. Aggressive reproduction often takes place in burned or logged areas on varied sites, but is typical along stream banks in pure or mixed stands. Alder trees are widely distributed along the West Coast of the United States, from Alaska to California and east to Colorado (Japson 1957). Red alder is probably the most important hardwood on the Pacific Coast, its wood is light, strong, and is used for furniture, veneer, and novelties. Red alder is also known to be a nitrogen fixer (Brill 1979).

Common mammals and birds recorded for the McDonald Forest and populating the stream area are black tailed deer, bob cat, raccoon, brush rabbit, nutria, blue grouse, wild turkey, turkey vulture, various hawks, owls, and song birds. Cutthroat trout are found in Oak Creek.

The McDonald Forest has a marine West Coast climate with characteristically warm dry summers and cold wet winters (Draft report School of Forestry). The mean annual precipitation is approximately 1.2 m with a significant variability from year to year. The precipitation season is from November through February. Snow fall is uncommon. However, if there is any, it will last only from a few hours to two days. Yearly precipitation, stream flow, and water temperature records were not available for the Oak Creek area where the present work was done. However, seasonal records of such measurements are usually recorded for the period from October to May. The mean annual runoff of Oak Creek during the winter months is estimated to be about 0.1 m^3/s.

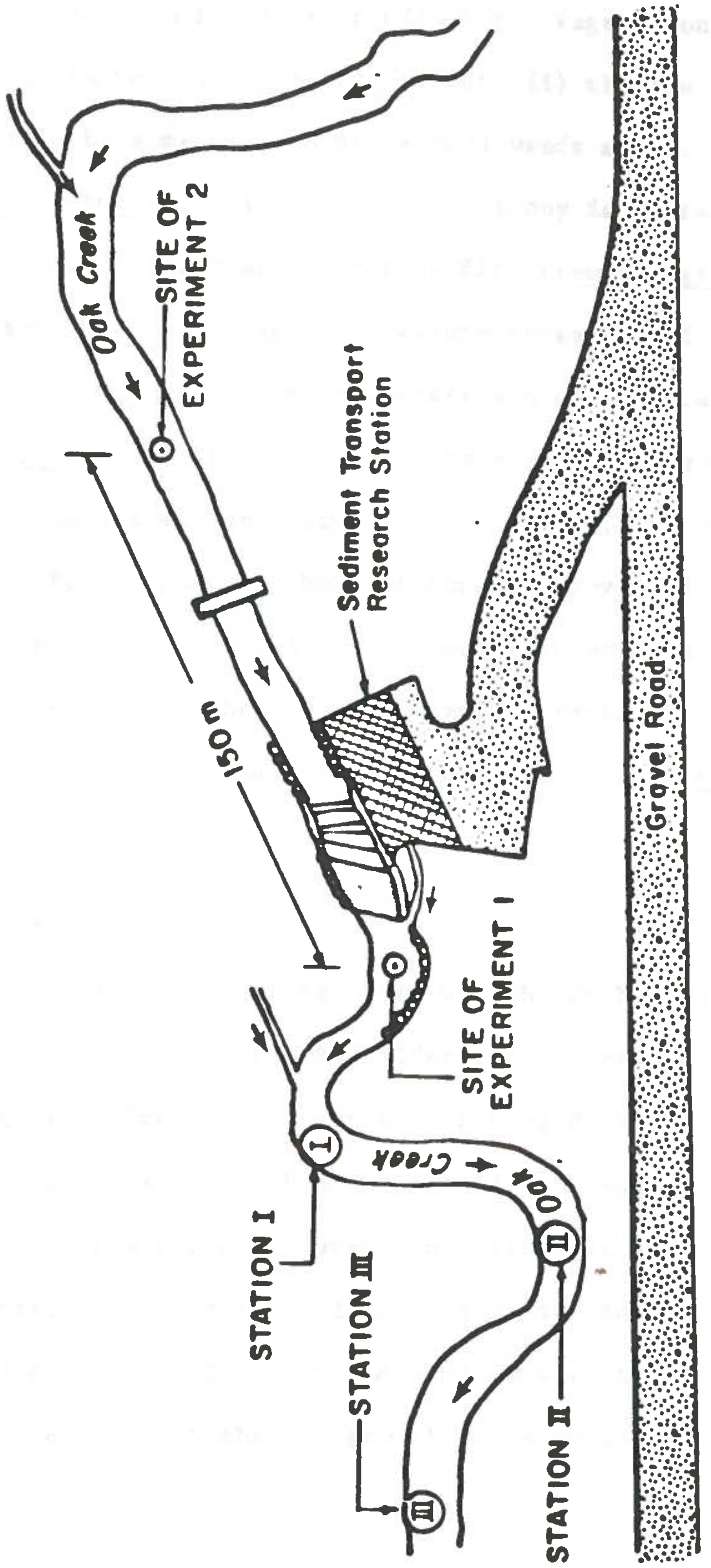
The Oak Creek area is dominated geologically by volcanic formations. The drainage pattern above the experimental site reflects the jointing of the underlying basalt bed. Weathering of such rocks has produced a thick to moderately thick mantle of silty-clay-loam soils. The major soil types present on the experimental sites are Dixonville, Price-Ritner, and Jory soil series. At the sediment research facility, the soil is of the Witham soil series with dense clay and very slow infilration rates. Stream channel changes typically occur when bank erosion undermines a tree and causes it to topple, partially blocking the original channel and letting the stream cut behind the tree into the bank.

The Stream

Oak Creek is a mountainous stream flowing from the eastern edge of the Coast range, owned and maintained by the Oregon State University School of Forestry. Two experiments were carried out near the location of the Sediment Transport Research Station (Fig. 2-2). The first tracer experiment was conducted below the station, while the second experiment was conducted above the station. The two experiments were separated by about 200 m to avoid tracer contamination from the first experiment. The section of a natural winding narrow channel approximately 1-2 m wide (Fig. 2-2). The stream bed is naturally lined with coarse gravel and small rocks, while its sides at the Sediment Transport Research Station are lined

FIGURE 2-2

Detailed map of the experimental area in the McDonald Forest. The sites of Experiment (1) and Stations I, II and III are sampling points for non-chelated In and Dy. The site of Experiment (2) is sampling point for chelated In and Dy.


Oak Creek
SITE OF EXPERIMENT 2
Sediment Transport Research Station
150 m
SITE OF EXPERIMENT I
Gravel Road
I
Oak Creek
II
III
STATION I
STATION III
STATION II

with rocks up to 15 cm in diameter. Vegetation around the stream consists mainly of the following: (i) the low canopy is represented mainly by some grasses and annual weeds as well as horsetail (Equisetum sp.); (ii) the upper canopy is represented mainly by vine maple (Acer sp.), douglas fir (Pseudotsuga menziesii (Mirbel.) Franco.) of mixed age, and mature trees of red alder (Alnus rubra Bong.). Along the water courses are widely scattered willow trees (Salix sp.). The stream flow rate at the time of the two experiments was approximately 0.1 m^3/min. Personal communication with P. Klingaman, School of Forestry revealed that during the summer, the stream flow has been measured at approximately 0.1 m^3/min at the section where the Sediment Transport Research Station is located. The stream water temperature was 18 ± 2°C and the pH was 7.1 ± 0.2.

Red Alder Roots

The experimental site was chosen because of the abundant growth of red alder. Red alder growing beside streams produces large masses of free floating roots growing directly in the stream water during all seasons of the year. In the summer, when this work was done, these roots produced a multitude of fast growing pink-colored roots, which are approximately 1 mm in width and vary in length from 2-3 mm buds to 12 cm strands and lack root hairs (Fig. 2-3). All samples were of the new growth roots over 1 cm in length.

FIGURE 2-3

Red alder (*Alnus rubra* Bong.) roots within the experimental area.

Indium and Dysprosium Experiment Without DTPA

Five g of In in the pure metallic form and 5.0 g of Dy in the form of Dy_2O_3 (5.9 g of the compound) were dissolved together in concentrated nitric acid. After dissolution, the acidic In and Dy stock solution was neutralized by the addition of concentrated amonium hydroxide. Distilled water was added to make a total volume of 105 ml, one ml of which was used for the preparation of standards. The remaining 104 ml were introduced into Oak Creek (the stream) at a constant rate of 0.96 ml/min by use of a persistaltic pump manufactured by Holter Company (Bridgeport, PA) for 1.8 hr. All chemicals used were reagent grade or better.

Indium and Dysprosium Experiment With DTPA

Five g of In pure metal and 4.25 g of Dy in the form of Dy_2O_3 (5.0 g of the compound) were dissolved together in concentrated nitric acid and neutralized as in the In and Dy experiment without DTPA. In this experiment, 96 ml of DTPA approximately 34% water (Matheson, Coleman and Bell Manufacturing Chemists), were added to make a final volume of 150 ml. The contents were stirred in order to facilitate the formation of In-DTPA and Dy-DTPA (chelates). One ml of the In-DTPA and Dy-DTPA stock solution was used for preparation of standards. The remaining 149 ml were introduced into Oak Creek at a constant rate of 1.18 ml/min by the persistaltic pump for 2.1 hr. All chemicals used were reagent grade or better.

Standard Preparation

For the In and Dy experiment, duplicate samples of 1.19 ppm and 0.238 ppm (0.5 mℓ each) were prepared by the dilution method from the stock solution to work as standards (see Appendix A for presentation of the calculations). For the In-DTPA and Dy-DTPA experiment, duplicate samples of 0.167 ppm and 0.0667 ppm (0.5 mℓ each), were prepared by the dilution method from the stock solution to work as standards (see Appendix B for presentation of the calculations). Two standards from the National Bureau of Standards, fly ash and orchard leaves, weighing 0.1015 g and 0.1094 g, respectively, were used for testing the sensitivity of INAA technique. The standards were prepared in a clean room, and encapsulated into clean 2/5-dram polyethylene vials. These small vials were heat sealed and then placed into larger 2-dram polyethylene vials which were also heat sealed before activation with thermal neutrons.

Sampling and Sample Preparation

Duplicate samples of Oak Creek water, alder roots, mud, and organic detritus were taken from the experimental area, before the administration of the In and Dy tracers into the stream, in order to establish the background levels of native In and Dy. For the non-chelated In and Dy experiment, the root and water samples were taken from the site of experiment 1, located 8 m downstream from the point of introduction, as well as from the three distant locations (Stations I, II, and III), which were located 60 m, 100 m, and 200 m respectively, downstream from the site of experiment 1 (Fig. 2-2). For the chelated In and Dy experiment, the root and water samples were taken from the site of experiment 2 located 8 m downstream from the point of introduction (Fig. 2-2).

Root samples were obtained by cutting small portions of the new growth, free floating alder roots over one cm in length, which naturally grow in large masses in the stream water. The wet root sample weights ranged between 0.30 and 1.63 g. The sampling time intervals varied according to the dilution of the stable tracers used at the sampling sites, which, in turn, depended upon the speed of stream flow. Root samples were dried under a heat lamp, drying decreased the root weights by a factor of approximately 16. The dry weight of the samples ranged between 0.02 and 0.10 g. All water samples were 0.5 ml and were always obtained just before alder roots samples were taken.

All samples of roots and water of the two experiments were doubly encapsulated in the same manner as the background and standards.

Instrumental Neutron Activation Analysis (INAA)

To achieve the detection sensitivity of the tracers and ease of analysis and sample handling, INAA was chosen as the method for detecting In and Dy tracer concentration in the parts per million (ppm) range. General references on the INAA sample handling methodology are available (Green 1968a, b; Bowen and Gibbons 1963; and Laul et al. 1977).

All samples, including background samples and standards, were activated by thermal neutrons at the Oregon State University Research reactor. All samples and standards were irradiated for 4 min at a flux of 10^{12} neutrons-cm^{-2}-sec^{-1}. Activation was carried out by the use of Pneumatic Transfer System (rabbit) which was employed for the rapid movement of the samples into and out of the reactor core. The activated samples were then transferred to the counting room for instrumental analysis after the samples had fulfilled the safety regulations and were released by the health physicist.

Data Acquisition, Calculation, and Analysis

Data were collected by a lithium-drifted germanium detector [Ge(Li) or "jelly" detector] manufactured by Canberra Industries. This detector has the advantage of high resolution for the analysis of the characteristic gamma ray spectrum of the activated In and Dy tracers. The Ge(Li) detector was coupled with a multichannel analyzer, and was connected with a computer for data analysis.

The 0.417 MeV photopeak was utilized in the analysis of ^{116}In, and the 0.361 MeV photopeak was used in the analysis of ^{165}Dy. Known standards of ^{60}Co, ^{137}Cs, and ^{22}Na from the National Bureau of Standards were used to calibrate the Ge(Li) detector. All data were analyzed by the use of a Hewlett-Packard-65 (HP-65) programmable calculator, (activation analysis abundance program). The program can be obtained from the Radiation Center, Oregon State University, Corvallis, Oregon 97331. Time zero in these analysis was set to the time when the first drop of stock solution hit the water surface of Oak Creek.

Laboratory and Radiation Safety

The preparation of standards and stock solutions were made under laboratory hoods, protective laboratory coats and gloves were used during such work. The radiation safety regulations and procedures (Regulatory Guide 1975), as well as the security protocol at the Radiation Center at Oregon State University were followed during the entire project. The evolution of radioactive argon (^{41}Ar) and radioactive chlorine (^{38}Cl) from the pneumatic system were noticed and precautions were made to reduce personnel exposure. Gloves were changed after any contamination by radioactivity was detected and double checks were made with the use of portable survey meters. The transfer of activated samples from the pneumatic transfer room to the counting room was done in special lead boxes designed particularly for such a purpose (see Appendix C for safety regulations).

CHAPTER THREE

RESULTS

Instrumental neutron activation analysis (INAA) of the background samples of water, alder roots, and stream sediments (a mixture of organic and inorganic sediments) indicated that the occurrance of both In and Dy were below the limits of detection in the experimental sites.

Alder roots were found to sorb both In and Dy, whether the stable tracers were administered in the ionic or in the chelated form (Tables 3-1, 3-2, 3-3, and 3-4). In alder roots, the concentration of In was less than In-DTPA by a factor of 1.9 after 1.8 hr of tracer administration, when additions of the tracer was stopped (Fig. 3-1). The concentration of sorbed In-DTPA in alder roots on a dry weight basis was 41 times the concentration of In-DTPA in the stream water after 1.8 hr of tracer administration (Fig. 3-2).

The alder root data of both In and In-DTPA experiments showed a gradual increase in the sorption of In by alder roots during the first 1.8 hr and then a decrease after the tracer was stopped (Fig. 3-1). The concentration of In-DTPA in alder roots began to decrease after 3.2 hr from the beginning of the tracer administration, (i.e., 1.2 hr after termination of the tracer administration (Fig. 3-2). The concentration of In in the stream water was 0.33 µg/ml after 3.0 hr from the beginning of tracer administration, whereas the

TABLE 3-1

Values obtained during the non-chelated In experiment. Indium in µg/ml in stream water and In in µg/g of alder root (dry wt.) as well as the ratio between In in roots/In stream water (coefficient of accumulation). Values determined by INAA, In administrated for 1.8 hr at the rate of 0.96 ml/min.

Sample Number	Sampling Time in hr	µg In/ml Stream Water	µg In/g of Root (dry wt.)	Coefficient of Accumulation
1	0.00	0.00	0.00	0.00
2	0.15	0.02	0.45	22.50
3	0.37	0.09	1.42	15.79
4	0.40	0.20	1.45	7.25
5	0.72	0.21	1.68	8.00
6	1.72	0.45	1.70	3.78
7	2.05	0.37	1.58	4.27
8	2.80	0.33	1.48	4.48
9	3.20	0.23	1.42	6.17
10	7.70	0.16	1.14	7.13
11	11.00	0.10	1.08	10.80
12	34.00	0.06	0.95	14.83
13	83.00	0.01	0.88	44.00
14	150.00	b.d.l.*	0.68	--
15	290.00	b.d.l.	0.58	--

* Below detection limits (less than 0.01 µg)

TABLE 3-2

Values obtained during the chelated In experiment (In-DTPA). Indium in µg/ml in stream water and In in µg/g of alder root (dry wt.) as well as the ratio of In in alder roots/In in stream water (coefficient of accumulation). Values determined by INAA, In-DTPA administrated for 2.1 hr at the rate of 1.18 ml/min.

Sample Number	Sampling Time in hr	µgIn/ml Stream Water	µgIn/g of Root (dry wt.)	Coefficient of Accumulation
1	0.00	0.00	0.00	0.00
2	0.15	0.20	0.58	2.90
3	0.17	0.21	0.88	4.19
4	0.58	0.10	2.60	26.00
5	1.50	0.05	3.25	65.00
6	2.30	0.02	3.00	150.00
7	5.00	0.01	1.40	140.00

TABLE 3-3

Values obtained during the non-chelated Dy experiment. Dysprosium in μg/ml of stream water and Dy in μg/g of alder root (dry wt.) as well as the ratio of Dy in roots/Dy in stream water (coefficient of accumulation). Values determined by INAA, Dy administrated for 1.8 hr at the rate of 0.96 ml/min.

Sample Number	Sampling Time in hr	μgDy/ml Stream Water	μgDy/g of Root (dry wt.)	Coefficient of Accumulation
1	0.00	0.00	0.00	0.00
2	0.15	0.07	1.30	18.57
3	0.37	0.10	7.42	74.20
4	0.40	0.12	10.10	84.17
5	0.72	0.09	11.90	132.22
6	1.72	0.09	26.30	292.22
7	2.05	0.07	28.00	400.00
8	2.80	0.05	28.70	717.00
9	3.20	0.03	26.10	870.00
10	7.70	0.02	23.50	1175.00
11	11.00	0.01	24.00	2400.00
12	35.00	b.d.l.*	19.80	--
13	83.00	b.d.l.	12.50	--
14	150.00	b.d.l.	8.90	--
15	290.00	b.d.l.	8.90	--

* Below detection limits (less than 0.01 μg)

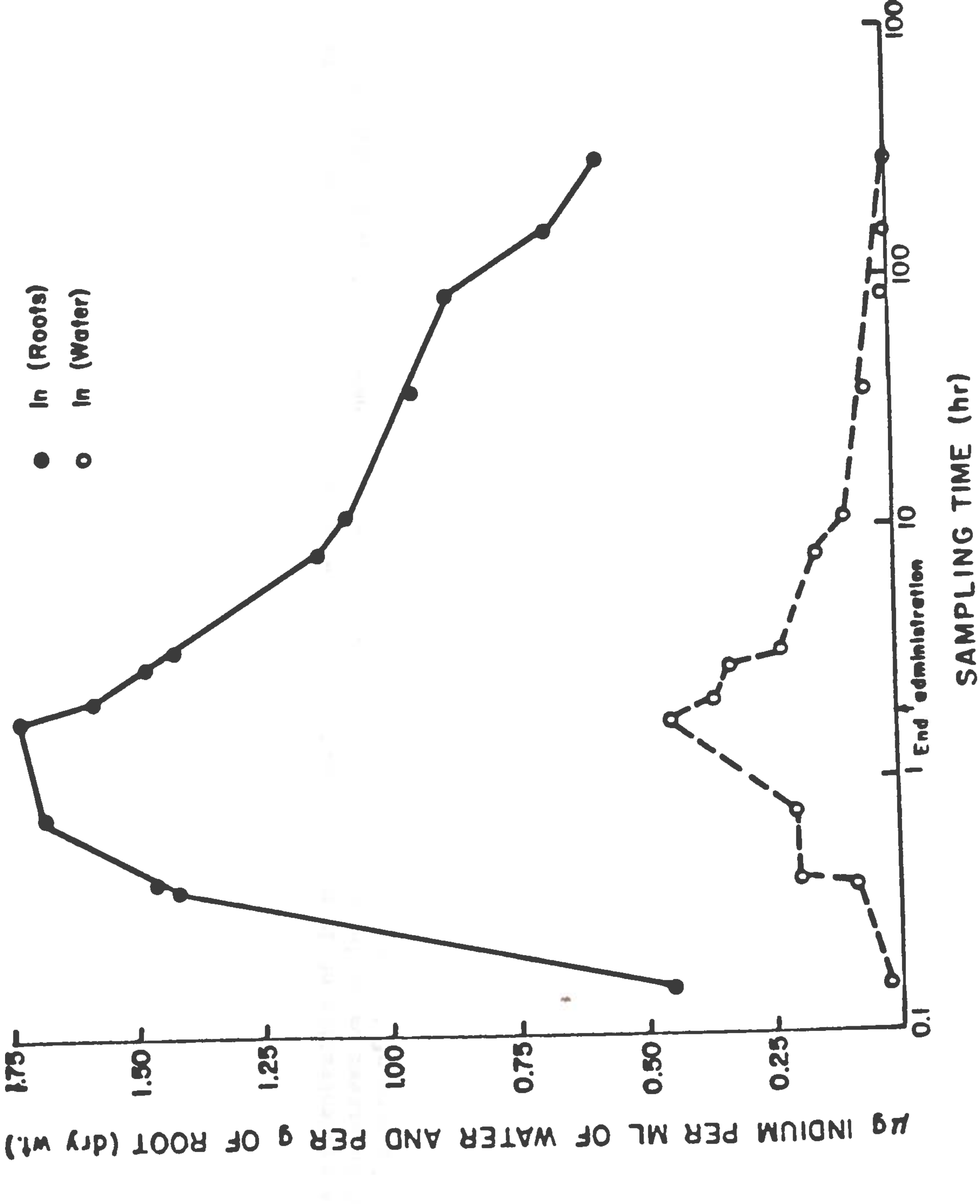
TABLE 3-4

Values obtained during the chelated Dy experiment (Dy-DTPA). Dysprosium in µg/ml of stream water and Dy in µg/g of alder root (dry wt.) as well as the ratio of Dy in roots/Dy in stream water (coefficient of accumulation). Values determined by INAA, Dy-DTPA administrated for 2.1 hr at the rate of 1.18 ml/min.

Sample Number	Sampling Time in hr	µgDy/ml Stream Water	µgDy/g of Root (dry wt.)	Coefficient of Accumulation
1	0.00	0.00	0.00	0.00
2	0.15	0.13	0.89	6.85
3	0.17	0.17	2.50	14.71
4	0.58	0.08	9.50	106.74
5	1.50	0.06	3.60	51.72
6	2.30	0.02	3.25	216.67
7	5.00	0.01	3.00	300.00

FIGURE 3-1

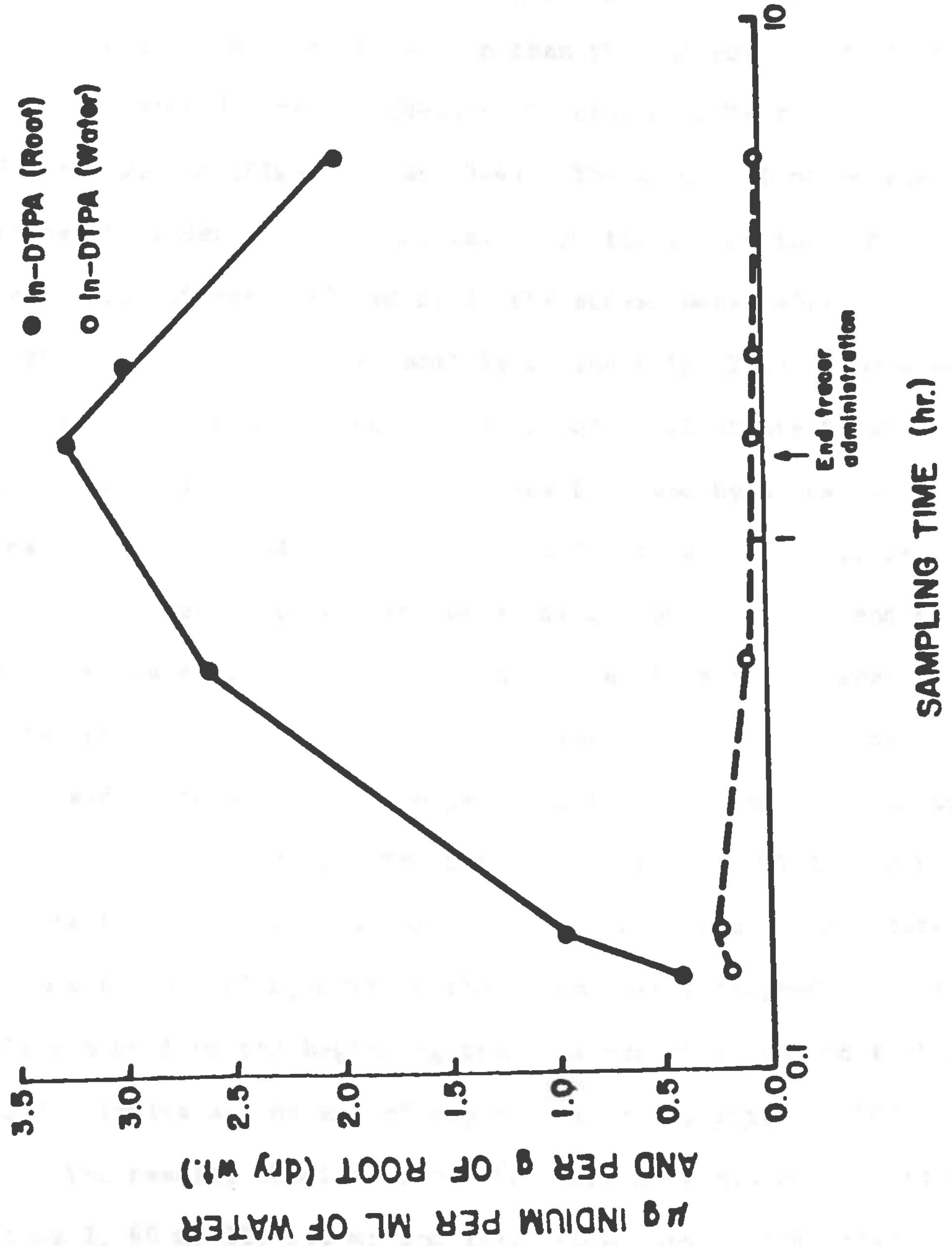
The concentration of In in *Alnus rubra* Bong. in μg/g of root (dry weight) and in stream water in μg/ml vs. log of time is shown. Indium administrated for 1.8 hr at the rate of 0.96 ml/min.



● In (Roots)
○ In (Water)
μg INDIUM PER ML OF WATER AND PER g OF ROOT (dry wt.)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
SAMPLING TIME (hr)
0.1
1
10
100
1000
End administration

FIGURE 3-2

The concentration of In metal as In-DTPA in *Alnus rubra* Bong. roots in µg/g of root (dry weight) and in stream water in µg/ml vs. log of time is shown. Indium-DTPA administrated for 2.1 hr at the rate of 1.18 ml/min.


● In-DTPA (Root)
o In-DTPA (Water)
End tracer administration
SAMPLING TIME (hr.)
0.1
1
10
μg INDIUM PER ML OF WATER AND PER g OF ROOT (dry wt.)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

concentration of the In-DTPA in the stream water was between 0.02 and 0.01 μg/ml after the same time of tracer administration.

The accumulation of non-chelated Dy by the alder roots was found to follow a different pattern than that of non-chelated In. Alder roots sorbed more non-chelated Dy than Dy-DTPA by a factor of 8 after 2.1 hr (Figs. 3-3 and 3-4). The amount of non-chelated Dy sorbed by alder roots was at least 300 times more than the concentration of non-chelated Dy in the stream water after 2.0 hr from the beginning of tracer administration (Fig. 3-3). There was a gradual increase in the concentration of Dy after the first 1.8 hr of tracer administration which then was followed by a gradual decrease up to the end of sample collection at 290 hr (Fig. 3-3). The Dy-DTPA alder root data and water data show a clear trend with a rapid decrease in Dy-DTPA sorption before the end of tracer administration. The Dy-DTPA concentration in alder roots was 3.0 μg/g of alder roots (dry wt.) after 5 hr from the beginning of tracer administration (Fig. 3-4). The concentration of Dy-DTPA in stream water started to decrease after only 0.60 hr of tracer administration. The concentration of Dy-DTPA in the stream water dropped to 0.01 μg/ml after 5 hr from the beginning tracer administration and to below detection limits at the end of experiment (i.e., after 28 hr).

The results obtained from the three more distant locations, Stations I, 60 m; II, 100 m; and III, 200 m, downstream, (Tables 3-5 and 3-6) show a similar pattern of In and Dy over a 200 m distance from the site of Experiment 1. The concentrations of In in the stream water as well as in alder roots in the three Stations I, II, and III,

FIGURE 3-3

The concentration of Dy in *Alnus rubra* Bong. roots in μg/g of root (dry weight) vs. log of time is shown on the left axis. The concentration of Dy in stream water in μg/ml is shown on the right axis. Dysprosium administrated for 1.8 hr at the rate of 0.96 ml/min.

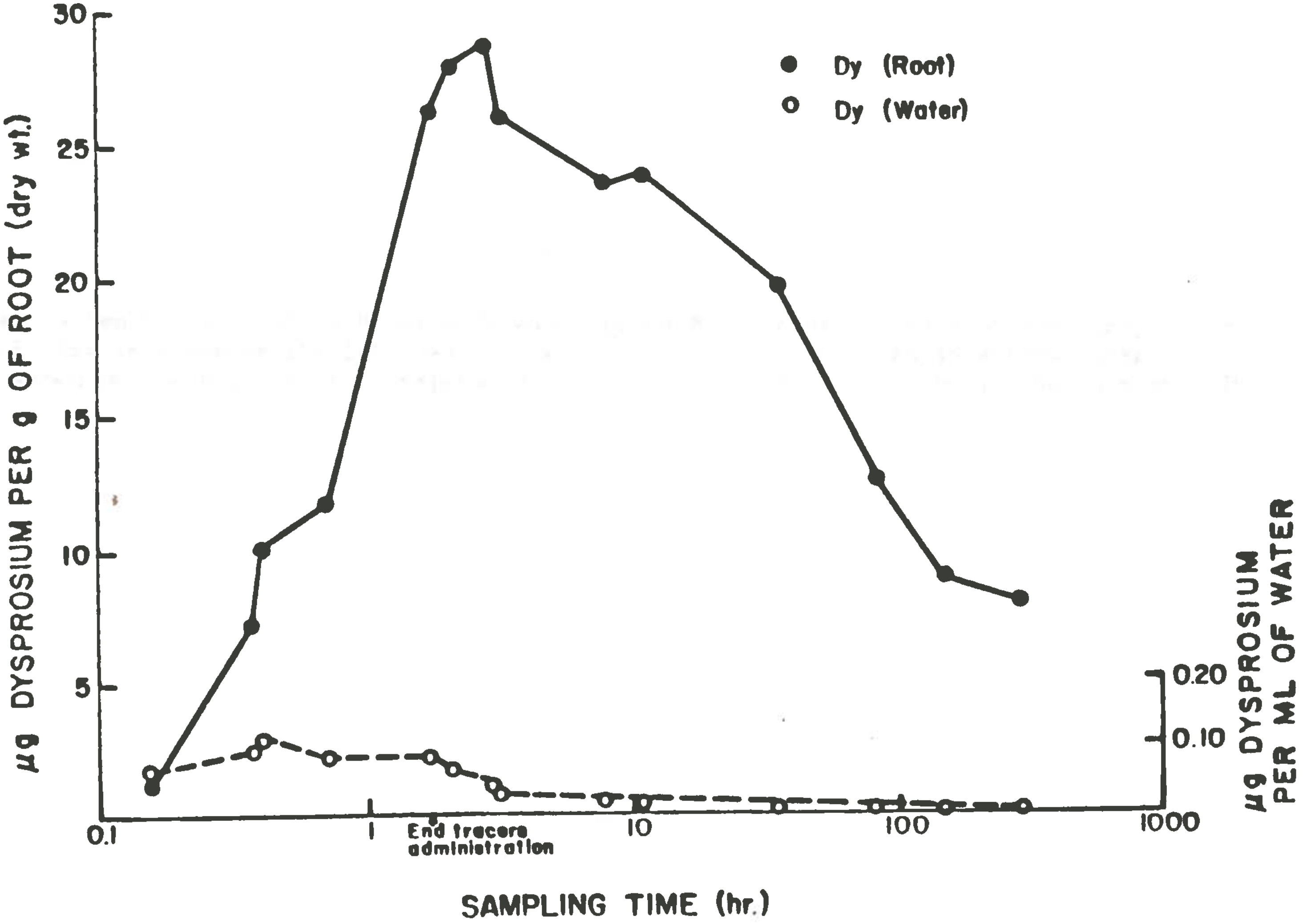

● Dy (Root)
○ Dy (Water)
μg DYSPROSIUM PER g OF ROOT (dry wt.)
30
25
20
15
10
5
μg DYSPROSIUM PER ML OF WATER
0.20
0.10
0.1
1
10
100
1000
End tracers administration
SAMPLING TIME (hr.)

FIGURE 3-4

The concentration of Dy metal as Dy-DTPA in *Alnus rubra* Bong. roots in μg/g of root (dry weight) vs. log of time is shown on the left axis. The concentration of Dy-DTPA in stream water in μg/ml is shown on the right axis. Dysprosium-DTPA administrated for 2.1 hr at the rate of 1.18 ml/min.

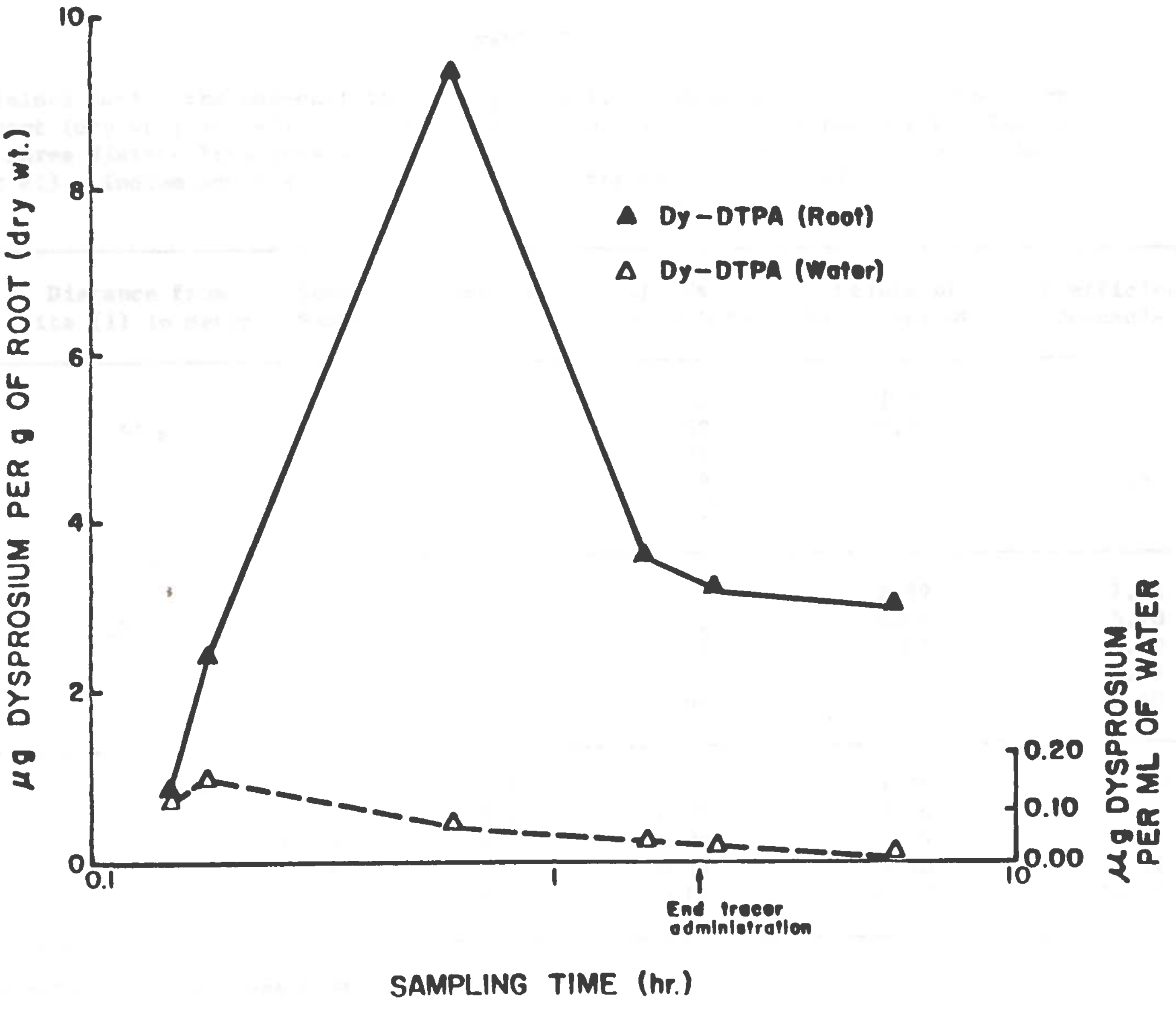

μg DYSPROSIUM PER g OF ROOT (dry wt.)
10
8
6
4
2
0
0.1
1
10
End tracer administration
SAMPLING TIME (hr.)
μg DYSPROSIUM PER ML OF WATER
0.20
0.10
0.00
▲ Dy-DTPA (Root)
△ Dy-DTPA (Water)

TABLE 3-5

Values obtained during the non-chelated In experiment. Indium in μg/ml of stream water and In in μg/g of alder root (dry wt.) as well as the ratio of In in roots/In in stream water. Values determined by INAA from three distant locations along Oak Creek in the direction of flow (below the site of experiment #1). Indium administrated for 1.8 hr at the rate of 0.96 ml/min.

Location	Distance from Site (1) in Meter	Sample Number	Sampling Time in hr	μgIn/ml Stream Water	μgIn/g of Root (dry wt.)	Coefficient of Accumulation
Station I	60 m	1	2.0	0.44	1.42	3.22
		2	2.7	0.39	0.98	2.51
		3	3.0	0.32	1.24	3.90
		4	5.0	0.19	1.03	5.42
		5	28.0	b.d.l.*	0.83	—
Station II	100 m	1	2.0	0.39	2.89	7.41
		2	2.7	0.33	1.88	5.70
		3	3.0	0.29	0.87	3.00
		4	5.0	0.31	1.27	6.05
		5	28.0	0.05	0.88	17.60
Station III	200 m	1	2.0	0.34	1.28	3.70
		2	2.7	0.29	0.89	3.07
		3	3.0	0.26	0.94	3.61
		4	5.0	0.18	1.30	7.22
		5	28.0	0.04	0.59	14.75

* Below detection limits (less than 0.01 μg)

TABLE 3-6

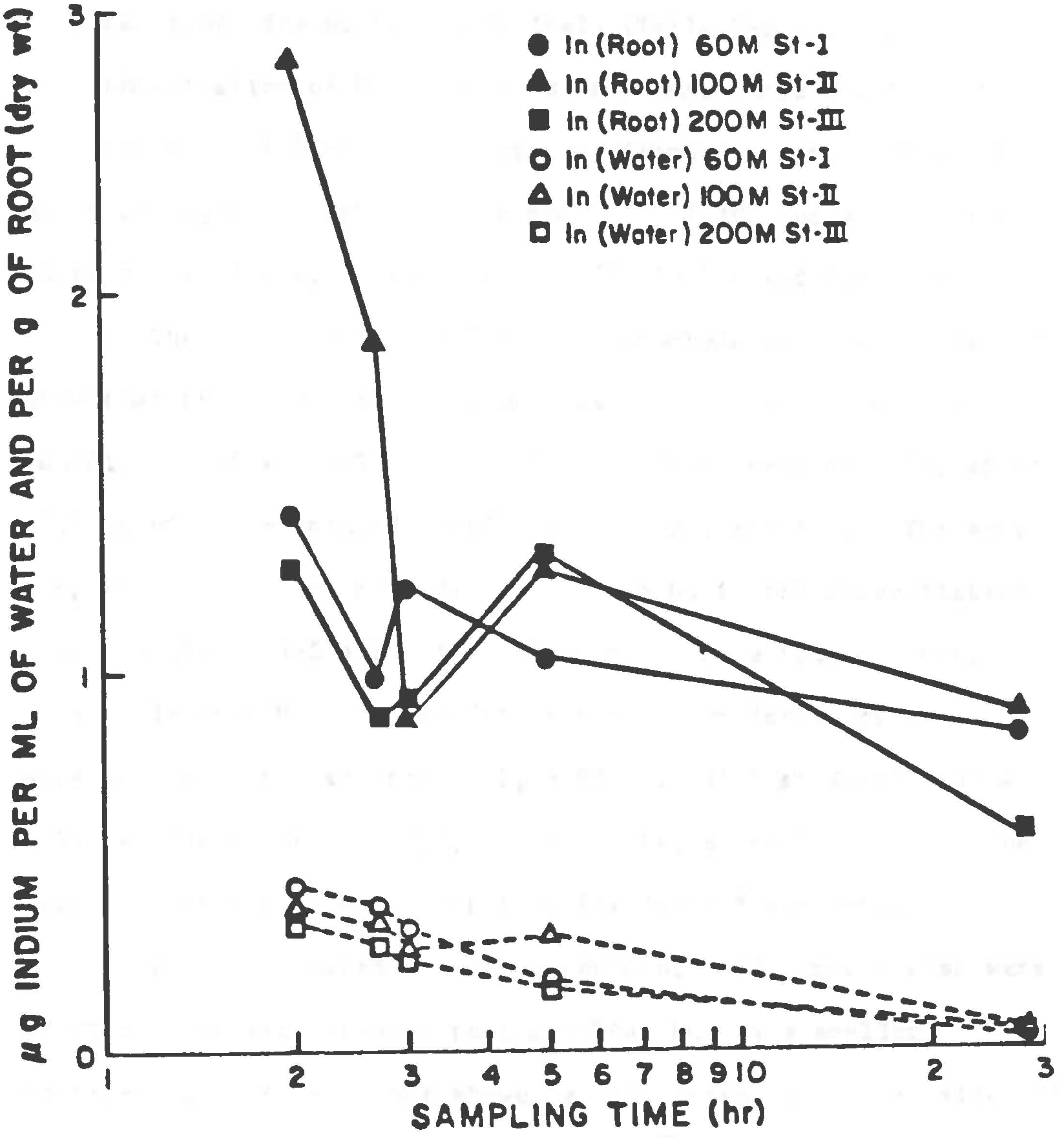
Values obtained during the non-chelated Dy experiment. Dysprosium in μg/ml of stream water and Dy in μg/g of alder root (dry wt.) as well as the ratio of Dy in the roots/Dy in stream water. Values determined by INAA from three distant locations along the Oak Creek in the direction of flow (below the site of experiment #1). Dysprosium administrated for 1.8 hr at the rate of 0.96 ml/min.

Location	Distance from Site (1) in Meter	Sample Number	Sampling Time in hr	μgDy/ml Stream Water	μgDy/g of Root (dry wt.)	Coefficient of Accumulation
Station I	60 m	1	2.0	0.097	10.62	109.48
		2	2.7	0.073	8.71	119.32
		3	3.0	0.059	5.88	99.66
		4	5.0	0.041	4.80	117.07
		5	28.0	b.d.l.*	3.21	--
Station II	100 m	1	2.0	0.074	18.41	248.78
		2	2.7	0.061	12.61	206.72
		3	3.0	0.041	7.31	178.29
		4	5.0	0.031	4.23	135.48
		5	28.0	b.d.l.	2.57	--
Station III	200 m	1	2.0	0.061	16.32	267.21
		2	2.7	0.048	6.65	137.50
		3	3.0	0.034	5.65	165.88
		4	5.0	0.030	3.55	118.33
		5	28.0	b.d.l.	5.11	--

* Below detection limits (less than 0.01 μg)

FIGURE 3-5

The concentration of In in *Alnus rubra* Bong. roots in μg/g of root (dry weight) and in stream water in μg/ml vs. log of time from three distant locations; Stations I, II, and III. Data were obtained from the In and Dy non-chelated experiment. Sampling was started at time = 2.0 hr.


μg INDIUM PER ML OF WATER AND PER g OF ROOT (dry wt)
SAMPLING TIME (hr)
0
1
2
3
1
2
3
4
5
6
7
8
9
10
2
3
● In (Root) 60M St-I
▲ In (Root) 100M St-II
■ In (Root) 200M St-III
○ In (Water) 60M St-I
△ In (Water) 100M St-II
□ In (Water) 200M St-III

at 3.0 hr after the beginning of tracer administration were 0.32, 0.29, and 0.26 μg/ml of stream water and 1.24, 0.87, and 0.94 μg/g of alder root (dry wt.), respectively (Table 3-5 and Fig. 3-5). The concentration of Dy in water at the same three stations after the same time (3.0 hr) of tracer administration were 0.059, 0.041, and 0.034 μg/ml of stream water and 5.80, 7.30, and 5.64 μg/g of alder roots (dry wt.), respectively (Table 3-6 and Fig. 3-6).

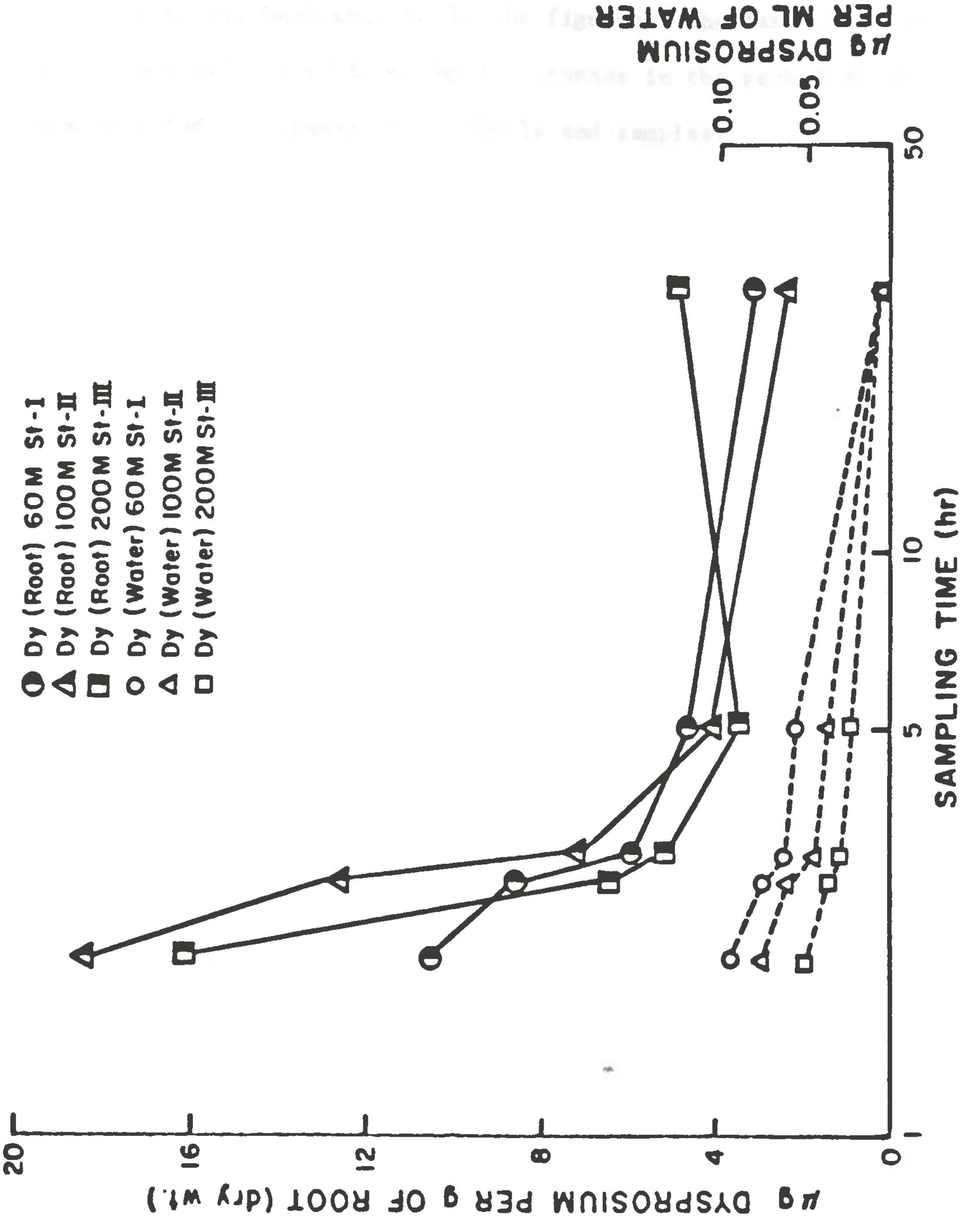
The alder root data from the non-chelated tracer experiments show that the alder roots respond less to In than Dy, with a coefficient of accumulation of 10.8 vs. 2400, respectively, after 11.0 hr of tracer administration (Tables 3-1 and 3-3). The same sorption pattern was recorded for In and Dy in the three distant stations (Figs. 3-5 and 3-6). Alder roots were found to respond less to In than Dy. The coefficient of accumulation of In vs. Dy were 5.42 vs. 160, at Station I, 6.05 vs. 135.5 at Station II and 7.22 vs. 118 at Station III, respectively, after 5.0 hr from the beginning of tracer administration (Tables 3-5 and 3-6).

In the chelated tracers experiment, alder roots also were found to sorb less In-DTPA than Dy-DTPA, but by a smaller differential. Alder roots showed a coefficient of accumulation of 150 vs. 300 respectively after 5 hr from the beginning of tracer administration (Tables 3-2 and 3-4).

A comparison of the data obtained from the two experiments (Tables 3-1, 3-2, 3-3, and 3-4) indicates that there is at least an 8 fold difference in the sorption pattern of Dy over In, whether the tracer elements are chelated or non-chelated.

FIGURE 3-6

The concentration of Dy in *Alnus rubra* Bong. roots in μg/g of root (dry weight) vs. log of time are shown on the left axis. The concentration of Dy in stream water in μg/ml are shown on the right axis, from three distant locations; Stations I, II, and III. Data were obtained from the In and Dy non-chelated experiment. Sample collection start at time = 2.0 hr.


◐ Dy (Root) 60M St-I
△ Dy (Root) 100M St-II
◧ Dy (Root) 200M St-III
○ Dy (Water) 60M St-I
△ Dy (Water) 100M St-II
□ Dy (Water) 200M St-III
μg DYSPROSIUM PER g OF ROOT (dry wt.)
0
4
8
12
16
20
μg DYSPROSIUM PER ML OF WATER
0.05
0.10
SAMPLING TIME (hr)
1
5
10
50

For the sake of simplicity, error bars for counting statistics have been omitted in the figures. The maximum error was 7% and originated from the differences in the peak area of the selected photopeaks of standards and samples.

CHAPTER FOUR

DISCUSSION

The term, root, is meant to encompass the root structure itself, together with its attendant microflora and microfauna. No attempt was made to separate the root from the aufwuchs associated with the root mass.

A problem arose during the counting and analysis of the activated samples in experiment 1 due to the different half-lives of the two radioactive tracers ^{165}Dy and ^{116}In. Dysprosium-165 has a half-life of 141 min and ^{116}In of 54.2 min. While waiting for the ^{165}Dy gamma ray intensities to decay tq a measurable quantity, the ^{116}In decayed at a differentially faster rate, resulting in a lessening of In detection sensitivity. A 15% reduction of Dy in experiment 2 (to 4.25 g) eliminated this problem.

The growth of alder roots, and the resulting increase in volume, was visually noticable during the sampling period. Over 5 days of sampling, the root volume was estimated to have increased nearly 25 percent.

The sorption of stable tracers by plant roots depends mainly on the availability of the tracer anions or cations in the root zone. The sorption rates of the tracers In and Dy in the experiments, which were carried out in a natural free-flowing creek, may be affected by the environmental conditions of the stream, such as pH, temperature, and dilution by stream flow. Although In and Dy currently are not considered to be biologically required nutrients, Moore (1972) reported that micronutrient uptake by plant roots grown in soil is directly related to the quantity of micronutrients required by plants. The low sorption of non-chelated In seen in Fig. 3-1 might be a result of $In(OH)_3$ formation which may then co-precipitate with other low level hydroxides such as Ca^{+2}, Mg^{+2}, Mn^{+2}, Ba^{+2}, and Fe^{+2} that may be present in the stream water. Dilution by stream flow appears to enhance the formation of insoluble trace hydroxides which may eliminate the availability of ions to be sorbed by plant roots (Hanson 1970). Lindsay (1972) who worked with inorganic phase equilibria in soil reported that acidity of the medium and changes in the oxidation reduction potential of metals affects their solubility in the tested soil.

The low sorption of non-chelated In may be due to the formation of insoluble $In(OH)_3$, and to the possibility that In co-precipitates with available hydroxides to form a colloidal suspension which will inhibit sorption rate of In by plant roots. The sorption of more In-DTPA than In by alder roots, as seen in Fig. 3-2, may be caused by the DTPA ligand working as a barrier to the formation of the insoluble $In(OH)_3$ and/or that the In-DTPA complex has a high stability at pH of 7.1 ± 0.2. Alvarez and Arriaga (1978) reported that at pH above 3.5, In formed insoluble hydroxides. In addition, they reported that the formation and stability of In-EDTA, In-DTPA, and In-EDTMP were related to the pH of the medium. Loveland (1978) working at the W-llamette River in Oregon reported that chelation with DTPA greatly improves the solution stability of In and Dy tracers over that of the simple ionic forms. Metal chelates of amino carboxylic acids such as EDTA and DTPA possess a greater thermodynamic stability constant than other chelating agents (Schwarzenbach et al. 1954; Reilly et al. 1959). Chick (1979) in laboratory tests on time dependence of tracer stability reported that chelation with DTPA provides high stability over ionic form. However, non-chelated In and Dy suffers substantial losses after a period of 8 days, with approximately 38 to 70% of original In and Dy tracers remaining in the solution respectively. Lindsay (1974) reported that the DTPA chelates of Ca^{+2}-, Mg^{+2}-, Mn^{+2}-, Ba^{+2}-, and Fe^{+2}- were likely to be more stable at pH 7.5. Harder and Chaberek (1959) reported that Dy possesses a high solubility with DTPA compared to other rare earths. Loveland (1978) stated that Dy-DTPA offers the best tracer possibility since its solution stability is superior to the more widely used In-chelate of DTPA. According to Hanson (1970) losses of metal chelate tracers may be attributed to an exchange reaction with metal species present in creek water during the experiment period; or sorption reactions with solid phases, including sediment and sample container; and/or a biodegradation of chelates. Wallace et al. (1978) reported chelating agents facilitated the movement of metal through roots into plants. Dekock and Mitchell (1957) reported that terrestrial plants were found to accumulate much more of the elements In, gallium, and chronium when they were added as water soluble organic complexes than the non-chelated form. This contrasts with the findings reported here for sorption of In-DTPA vs. In by alder roots growing in flowing water (Figs. 3-2 and 3-1). The lower sorption of In-DTPA and Dy-DTPA in general may be affected by many factors that influence the stability of chelates. These included the ligand properties of the type and number of donor groups, size, and number of rings formed, and both steric and resonance effects of substitution on the donor groups. The metal properties, such as ionic potential and ligand field stabilization, also effect the chelate formation, as well as the effects of the competing reactions and pH of the medium (Martell and Calvin, 1952).

With a stream flow of approximately 0.1 m^3/min and with the addition of 5 g of tracer over a time period of more than 100 min, calculation of the administered tracer concentration in the vicinity of the roots would be just under 0.5 μg of the tracer/ml (see Appendix D for calculations). This would assume thorough mixing and no uptake between the point of tracer administration and the roots, which were 8 m downstream. As may be seen in Fig. 3-1, when there was a small amount of sorption of non-chelated In there was a correspondingly higher amount of non-chelated In in the water. In fact, the observed amount of non-chelated In in the water approaches the calculated value (0.45 μgIn/ml vs. 0.458 μgIn/ml, respectively) after the end of administration. And when nearly twice as much In-DTPA is sorbed by the roots (Fig. 3-2), the observed amount of In-DTPA in the water falls below the calculated values (less than 0.2 μgIn-DTPA/ml vs. 0.391 μgIn-DTPA/ml, respectively). In contrast, the curves in Fig. 3-3 describe a significantly higher sorption of non-chelated Dy by the roots and approximately the same amount of Dy-DTPA sorption (Fig. 3-4), as was witnessed by both In and In-DTPA root sorption rates. However, the concentration of Dy in the stream water, in both the non-chelated Dy and Dy-DTPA experiments, was found to be less than one-half (less than 0.2 μgDy/ml) of the Dy that would be expected from calculated values of 0.458 μgDy/ml after the end of administration. This also assumes no sorption of Dy between the point of tracer administration and root sampling station, 8 m downstream. The difference in the calculated values and the observed values might be assumed to be a reflection of the sorption taking place in the stream environment.

Although the sampling at the three stations (Stations I, II, and III, and Fig. 2-2) took place 2 hr or more after the beginning of tracer administration in the first experiment (experiment 1), the data reported in Tables 3-5 and 3-6 show an expected lower sorption of non-chelated In and non-chelated Dy by alder roots due to the sorption of In and Dy by the bottom sediments and periphyton, formation of insoluble hydroxides of In and Dy over the greater distance traveled, and dilution due to distance between the introduction point and the more distant sampling sites, 60, 100, and 200 m downstream (Fig. 2-2).

A comparison of the peak sorption values of non-chelated In (Figs. 3-1 and 3-5) revealed that sorption of In at Stations I and III was lower than the main station by a factor of 0.84 and 0.76 respectively. At Station II the sorption of In was higher by a factor of 1.7. However, the comparison of the peak sorption values of non-chelated Dy (Figs. 3-3 and 3-6) revealed that the sorption of

Dy at the three distant locations (Stations I, II, and III) was lower than at the main station by factors of 0.37, 0.64 and 0.57 respectively.

Although In and Dy show a similar pattern in the three stations, they were more highly sorbed by alder roots at Station II (Figs. 3-5 and 3-6). The higher sorption level at Station II might be due to lower water velocity since the stream does not have a uniform channel width.

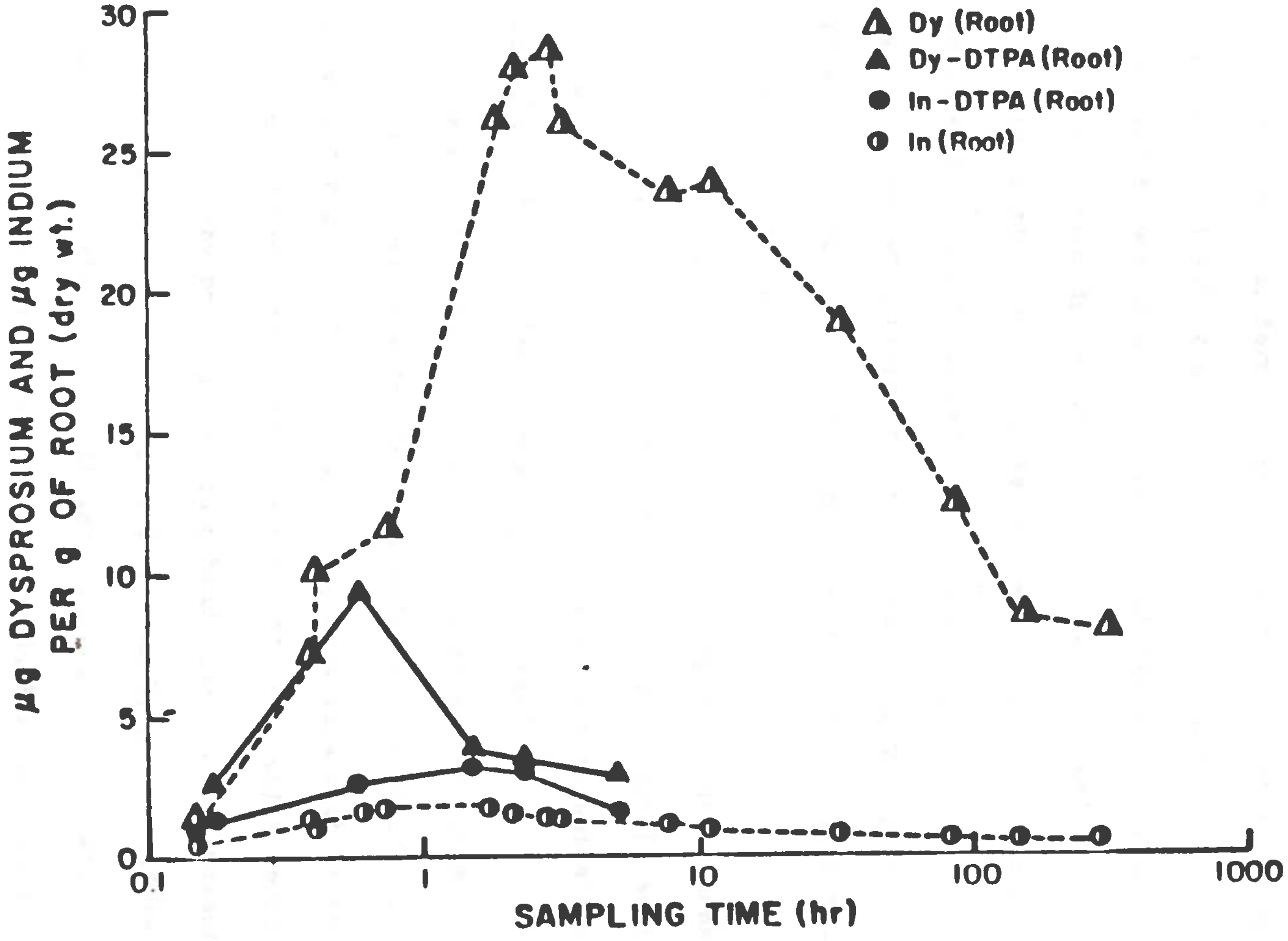
In general, at Stations I, II, and III, the sorption pattern of both In and Dy show a sharp decrease after the first 3 hr sampling (Figs. 3-5 and 3-6). This may be a result of water velocity, outwash of roots, and dilution with the stream flow. As stated earlier, dilution by stream flow appears to enhance the formation of insoluble trace hydroxides which may eliminate the availability of ions to be sorbed by plant roots, particularly at Stations I, II, and III (Hanson 1970). The In and Dy pattern then follow a gradual decrease up to the end of sampling which may be related to the low concentrations of the tracers in the water of the stream (Figs. 3-5 and 3-6), to the greater opportunity for sorption of the tracers by bottom sediments over greater distance, and to the increase in growth of the alder roots, particularly in samples after 24 hr. Loveland (1978) reported that sorption of In and Dy tracers by suspended particulates present in unfiltrated (natural) river water samples was negligible compared to sorption by bottom sediments. The same results were reported by Chick (1979) while he was working with stable tracers In and Dy in hydrologic studies.

For ease of comparison, only the alder root data from Figs. 3-1, 3-2, 3-3, and 3-4 are shown in Fig. 4-1. Although root sorption of chelated In and chelated Dy follows essentially the same pattern, In in the ionic form behaved significantly differently than Dy in the ionic form in alder roots. Ionic In sorption was lower than In-DTPA sorption by a factor of 1.8, ionic Dy sorption was higher than the sorption of Dy-DTPA by a factor of 3. However, the sorption of ionic Dy was higher than both In-DTPA and Dy-DTPA by a factor of 8 and 3 respectively.

The remarkable ability of the roots to distinguish between non-chelated In and Dy, even though the two tracers were at essentially the same molar concentration, suggests the possibility of a discrimination mechanism. One such mechanism could be based upon the difference in ionic radii. Studies have shown that roots of terrestrial plants to cause a fractionation of rare earth uptake and to behave according to the selective charge and size of the metal ion (Giordiano and Mortvedeth 1972). Laul _et al_. (1977) reported that the availability of rare earths in soluble form has a great effect on rare earth sorption by plant roots growing in soil. The greater sorption by roots of Dy introduced in water as Dy^{+3} than In introduced as In^{+3} may be due to the discrimination by alder roots and associated periphyton for elements with large ionic radii; the greater the radius, the greater the anion and cation formation, hence more sorption will occur. Dysprosium (Dy^{+3}) with an ionic radius of 1.25 Å was sorbed to alder roots 16 times more than In^{+3} with an ionic radius of 0.81 Å (Fig. 4-1). Curry (1976) reported that the

FIGURE 4-1

The concentrations of In, In-DTPA, Dy, and Dy-DTPA in *Alnus rubra* Bong. roots in μg/g of root (dry weight) vs. log of time. At time = 0.0, In and Dy tracer concentrations in roots were below the limits of detection.


Δ Dy (Root)
▲ Dy-DTPA (Root)
● In-DTPA (Root)
o In (Root)
µg DYSPROSIUM AND µg INDIUM PER g OF ROOT (dry wt.)
30
25
20
15
10
5
0
0.1
1
10
100
1000
SAMPLING TIME (hr)

rare earth lanthanum (ionic radius 1.15 Å) introduced into a stream microcosm in ionic form was sorbed by the roots of black willow in high amounts [24.1 μg/g root (dry wt.)]. In unpublished work, Knaus (1978) worked with the tracing of radioactive tracers in model stream microcosm in Louisiana, found that black willow roots sorp both radioactive rare earth lanthanum (^{140}La) and radioactive molybdenum (^{99}Mo) ionic radius 0.68 Å in high amounts (101 and 102 μg/g root dry wt., respectively). These findings agree with the present work for the sorption of rare earth Dy (ionic radius 1.25 Å) by red alder roots.

The results of the experiments reported here demonstrate the usefulness of In and Dy as tracers which can either be sorbed or not sorbed. Dysprosium, introduced in the ionic form was shown to be readily sorbed to alder roots. Other investigations of similar ions with larger ionic radii under similar stream conditions show that these other ionic species behave similarly. In addition, the higher Dy sorption points to the possibility that ions with large ionic radii would mimic the environmental pollutants which would readily sorb to periphyton. On the other hand, the low, but measurable sorption of non-chelated In, In-DTPA, and Dy-DTPA could be better used to trace the movement of water flow and water masses. Europium, a rare earth with a large ionic radius 1.18 Å, possess a high sensitivity like In and Dy (Hanson 1970) when used as a water tracer.

Since the ability of alder roots to maintain the tracers concentrations were shown clearly in Figs. 3-1, 3-2, 3-3, 3-4, 3-5, and 3-6, alder roots could serve as a bioaccumulator for pollutants in lotic systems. Alder roots as well as many bioaccumulators in nature, such as perennial leaves, lichens, mollusks, insect larvae, and the like, could give significantly varying results according to the chemical nature of the elements, especially in aquatic systems. Potential pollutants such as oil, chemical cargos, and dredge spoils might be "finger printed" by adding a series of stable tracers which when analyzed by neutron activation would give an unmistakable identification of the pollution source. However, a major lesson from the work presented here is that the casual use of mixed rare elements as a finger printing tool may be compromised by the selectivity of biological and other environmental monitors.

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APPENDIX A

Calculation of In and Dy Non-Chelated Experimental Standards

The amounts of In and Dy used to prepare the In and Dy stock solution were 5.00 g each of the element. The concentration of In or Dy in µg/ml (ppm) in the stock solution that was introduced into the stream was 4.76×10^4 µg/ml:

$$\frac{5.00 \text{ g of the element}}{105 \text{ ml}} = 0.0476 \text{ g/ml or } 4.76 \times 10^4 \text{ µg of the element/ml}$$

The preparation of standards was carried out as follows:

a) A dilution of 200 times was obtained by mixing 0.5 ml from the stock solution in 99.5 ml of distilled water (DW). The standard concentration became:

$$\frac{4.76 \times 10^4 \text{ µg/ml}}{200} = 2.38 \times 10^2 \text{ µg/ml} \tag{1}$$

b) Another dilution of 100 times was obtained by mixing 1.0 ml from the above standard stock solution plus 99 ml DW. The new concentration became:

$$\frac{2.38 \times 10^2 \text{ µg/ml}}{100} = 2.38 \text{ µg/ml} \tag{2}$$

c) Replicate standards were prepared as follows:

<u>Standard number-1</u>: This standard with a concentration of 1.19 ppm of each In and Dy was prepared by measuring 0.5 ml of the stock solution number (2), which has a concentration of 2.38 μg/ml of each In and Dy and then doubly encapsulated for INAA.

<u>Standard number-2</u>: This standard with a concentration of 0.238 ppm of each In and Dy was prepared by measuring 0.1 ml of the stock solution number (2), with the addition of 0.4 ml DW to make the volume of 0.5 ml and then doubly encapsulated for INAA.

APPENDIX B

Calculation of In-DTPA and Dy-DTPA Experimental Standards

The expected reaction between the tracers and the chelating agent is summarized below:

$$\underset{\text{(metal ions)}}{M} + \underset{\text{(chelating molecule)}}{L} \longrightarrow \underset{\text{(metal chelates)}}{ML}$$

The reaction expressed in moles:

$$\text{one mole of } (M) + \text{one mole of } (L) \xrightarrow[\text{form}]{\text{will}} \text{one mole of } (ML)$$

The elemental amounts of In and Dy used to prepare the In-DTPA and Dy-DTPA stock solution were 5.00 g and 4.25 g, respectively. These amounts were found to be equivalent to 0.044 and 0.026 moles, respectively.

Calculations based on mole basis for the reaction equivalence of the tracers used to the selected chelating agent:

$$\frac{\text{weight in grams}}{\text{atomic weight of element}} = \text{(number of moles) or (molecular weight of the compound)}$$

$$\frac{5.00 \text{ g}}{114.8} = 0.044 \text{ moles of In}$$

and

$$\frac{4.25 \text{ g}}{162.5} = 0.026 \text{ moles of Dy}$$

The amount or the volume of DTPA needed to prepare the In and Dy stable tracer chelates must be equivalent to at least 0.07 moles of DTPA pure form, or 35.3 g of DTPA.

Calculations of the weight of DTPA needed:

$$\frac{\text{weight in grams}}{\text{molecular weight in grams}} = \text{number of moles}$$

$$\frac{\text{wt. in g}}{503.26} = 0.07 \text{ moles}$$

$\therefore$ The weight needed = 35.5 g DTPA (pure form).

The DTPA used is present in commercial form as approximately 34% in water by weight. Therefore, grams of commercial DTPA 34% in water will be used:

For every 100 g of commercial DTPA there are 34 g of pure DTPA.

$$\frac{35.3 \text{ g pure DTPA} \times 100}{34} = 103.8 \text{ g of commercial DTPA}$$

$$\text{The specific gravity (density) of DTPA} = \frac{\text{wt. in grams}}{\text{volume in ml}}$$

$$\therefore \text{density} = \frac{1000 \text{ g}}{750 \text{ ml}} = 1.33 \text{ g/ml}$$

Hence the volume needed to prepare In and Dy chelates from commercial DTPA is:

$$\frac{103.8 \text{ g}}{1.33 \text{ g/ml}} = 78.05 \text{ ml}$$

A volume of 96 ml of commercial DTPA was used to give an excess amount of the chelating agent.

The concentration of chelated In and Dy in μg/ml (ppm) in the original stock solution of 150 ml (the solution that introduced to the stream) were calculated as follows:

$$\text{The concentration of chelated In} = \frac{\text{wt. in grams}}{\text{volume in ml}} = \frac{5\ \text{g}}{150\ \text{ml}} .$$

$$= 3.33 \times 10^{4}\ \mu\text{g/ml}$$

$$\text{The concentration of chelated Dy} = \frac{\text{wt. in grams}}{\text{volume in ml}} = \frac{4.25\ \text{g}}{150\ \text{ml}}$$

$$= 2.83 \times 10^{4}\ \mu\text{g/ml}$$

The In-DTPA and Dy-DTPA standard preparations were carried out as follows:

a) 1.0 ml from the original stock solution was diluted with 99 ml DW.

$$\frac{3.33 \times 10^{4}\ \mu\text{g/ml}}{100\ \text{ml}} = 3.3 \times 10^{2}\ \mu\text{g/ml} \qquad \text{(In)}$$

$$\frac{2.83 \times 10^{4}\ \mu\text{g/ml}}{100\ \text{ml}} = 2.83 \times 10^{2}\ \mu\text{g/ml} \qquad \text{(Dy)} \qquad (1)$$

b) A second dilution of 100 times was obtained by mixing 1.0 ml from the above stock solution + 99 ml DW the concentration became:

3.33 μg/ml (In) and 2.83 μg/ml (Dy) (2)

c) A third dilution of 10 times was obtained by mixing 1.0 ml from stock solution (2) of 3.33 μgIn/ml and 2.83 μgDy/ml + 9 ml DW:

0.333 μg/ml (In) and 0.283 μg/ml (Dy) (3)

d) Replicate standards were prepared as follows:

<u>Standard number-1</u>: This standard with concentration of 0.167 μgIn/ml and 0.142 μgDy/ml was prepared by measuring 0.5 ml of the stock solution number (3) which then doubly encapsulated for INAA.

<u>Standard number-2</u>: A standard with concentrations of 0.667 μgIn/ml and 0.0566 μgDy/ml was prepared by measuring 0.2 ml of the stock solution (3). The addition of 0.3 ml DW, made a uniform volume of 0.5 ml and then doubly encapsulated for INAA.

APPENDIX C

Oregon State University Radiation Center Safety Procedures

I. Rules for Working in Laboratories Where Radioactive Materials are Used.

1. Coats and other personal belongings, including books (except those required for work), should not normally be brought into the laboratory.
2. Eating, drinking, smoking, and the application of cosmetics in the laboratory are not permitted.
3. Protective clothing (e.g., lab coat, gloves, safety glasses, etc.) should be worn in the laboratory as appropriate, but not taken into counting rooms or other areas where radioactive materials are not used.
4. Pipetting liquids of any type by mouth or the performance of any similar operation by mouth suction is not permitted.
5. Operations with loose radioactive materials, evaporation of radioactive liquids, or any other process which could release airborned radioactivity must be carried out in a properly filtered hood.
6. No person should work with radioactive material if there any breaks in the skin on the hands. unless gloves are worn. All such breaks should be reported to the person responsible for the laboratory operation before work starts.

7. Radioactive liquid wastes should be poured into the labelled liquid radioactive waste containers provided (NOT DOWN THE DRAINS).

8. Radioactive solid wastes and other contaminated items should be placed in the special solid radioactive waste containers provided.

9. All wounds, spills, and other emergencies should be immediately reported to the health physicist and the person responsible for the laboratory operation.

10. Personnel will check their hands, feet, and clothing for radioactive contamination before leaving the laboratory. Normally, instructions are posted near each personnel check-out station calling attention to this requirement, and normally a red line will be present on the floor across the doorway to the area. If contamination is suspected, the individual should immediately notify the Health Physicist, the Radiation Specialist, or the Reactor Operations staff by calling the reactor control room (ext. 36) or by having the building receptionist page these individuals. The person responsible for the work in the laboratory should also be notified. If at all possible, individuals should stay within or nearby the contaminated laboratory area to avoid unnecessary spreading of any contamination. However, occupancy of the contaminated area is secondary to minimizing the potential

for ingestion, inhalation, or external contamination with loose surface, airborne, or liquid radioactive materials, or exposures to unshielded radiation sources. Evacuation of the laboratory is recommended if these risks can be reduced. If contamination is suspected after normal working hours, contact one of the individuals on the emergency call list displayed in the Radiation Center receptionist's office (A-100). If possible, someone other than the person suspected of being contaminated should make the phone call in order to minimize the potential for spreading contamination throughout the building.

11. Initial decontamination efforts by laboratory personnel should normally be limited to techniques that will contain radioactive material, provided they do not increase their risk of significant radiation exposure or contamination in the process.

II. Radiation Safety Program

In accordance with the Rules and Regulations of the OSU Radiation Safety Committee, radiation surveys of laboratories, radiation protection of personnel, maintaining records of receipts, shipments, disposal and inventory of radioactive materials, and any other pertinent radiation protection matters are the direct responsibility of the authorized Program Director. Significant assistance on these matters will be provided by the Radiation Center Health Physicist, either as part of the routine radiation protection program or at the special request of a Program Director. In addition, the Radiation Center Health Physicist will conduct independent radiation surveys of all laboratories at the Radiation Center and will manage the overall personnel monitoring program. Requests for film badges and/or pocket dosimeters, or area monitors, should be directed to the Radiation Center Health Physicist.

III. Radiation Survey Instruments

In addition to the radiation monitoring instruments possessed by individual Program Directors, a wide variety of radiation survey instruments are available from the Radiation Center Health Physicist. Repair and calibration of all available instruments will be the responsibility of the Health Physicist.

IV. Leak Testing of Sealed Radiation Sources

Leak testing of sealed radiation sources located in the Radiation Center will be coordinated by the Health Physicist. The Health Physicist will also maintain the master file of leak test records for sources located at the Radiation Center.

V. Possession, Transfer, and Responsibility for Radioactive Materials

A. All transfers of radioactive materials must be made in accordance with applicable regulations of the Oregon Energy

Facility Siting Council, Oregon Regulations for the Control of sources of Ionizing Radiation Including Radioactive Materials (Radiation Control Section, Oregon Health Division), OSU Radiation Safety Manual, and applicable regulations of the U. S. Department of Transportation, USNRC and U. S. Postal Service.

B. All transfers of radioactive materials to the Radiation Center from any other location must also be conducted in accordance with applicable regulations of the agencies listed in paragraph (5A). Advance notice of pending "transfers to" the Radiation Center must be provided to the Radiation Center Health Physicist by the responsible Program Director. This notice is essential if we are to meet regulatory requirements imposed on incoming shipments of radioactive materials.

C. Records of transfers of radioactive material from the Radiation Center will be kept by the Radiation Center Health Physicist and also provided to the recipient, the Program and the OSU Radiation Safety Committee.

VI. Radioactive Waste Disposal

Radioactive waste disposal will be coordinated by the Radiation Center Health Physicist. Appropriate containers for all of the various types of radioactive wastes are located throughout the Radiation Center. As a standard policy, liquid radioactive wastes will not be disposed down laboratory drains.

In rare cases, such as exceptionally large volumes of liquids with extremely low concentrations of radioactivity, controlled disposal down lab drains will be considered. In each case, concentrations of radioactivity must be determined in advance to be at the lowest readily achievable level below appropriate maximum permissible concentrations described in the "Oregon Regulations for the Control of Sources of Ionizing Radiation Including Radioactive Materials," or as applicable, regulations of the USNRC. Programs possibly requiring the disposal of radioactive liquids down laboratory drains should include this potential requirement as part of the request for program authorization in the description submitted (as outlined) to the OSU Radiation Safety Committee. Disposal of radioactive liquids down laboratory drains after approval must be under the supervision of the Radiation Center Health Physicist. No organic liquids (solvents) may be disposed down lab drains.

APPENDIX D

Calculations of In and Dy Chelated and Non-Chelated Within the Stream Water

The following assumptions were made:

(i) Stream flow rate of 0.1 $m^3/min = 6 \times 10^6$ ml/hr.

(ii) Five g of each In and Dy were introduced into the stream in mixture for 1.8 hr in experiment 1. The same amount of In (5.0 g) and 4.25 g of Dy were introduced into the stream in mixture for 2.1 hr in experiment 2.

(iii) A thorough mixing of the tracers in the stream water was assumed.

The concentration of In or Dy in the stream water at time of 1.8 hr (the end of introduction of tracer) in experiment 1 is calculated as follows:

Concentration of In or Dy in the stream water at time = 1.8 hr

$$= \frac{\text{Total amount of In or Dy introduced for 1.8 hr}}{\text{Volume of stream water for 1.8 hr}} \qquad \text{(A)}$$

$$= \frac{4.95 \times 10^6 \ \mu g \text{ In or Dy*}}{1.8 \times 6 \times 10^6 \text{ ml}} = 0.458 \ \mu g \text{ In or Dy/ml} \qquad (1)$$

---

* 0.05×10^6 μg of metal used in the preparation of standards.

The concentration of In or Dy in the stream water after 11 hr:

$$= \frac{4.95 \times 10^6 \ \mu g \text{ In or Dy*}}{11 \times 6 \times 10^6 \text{ ml}} = 0.075 \ \mu g \text{ In or Dy/ml} \qquad (2)$$

The concentration of In or Dy that were added to the stream (experiment 2) for 2.1 hr were calculated as follows:

Stream flow in ml for 2.1 hr = $2.1 \times 6 \times 10^6$ ml = 1.27×10^7 ml

Total amount of In introduced to the stream for the same time found to be 4.97×10^6 μgIn**.

Total amount of In introduced to the stream for the same time found to be 4.22×10^6 μgDy.

From (A):

The concentration of In in the stream water after 2.1 hr of tracer administration:

$$\frac{4.97 \times 10^6 \ \mu g\text{In}}{1.27 \times 10^7 \text{ ml}} = 0.391 \ \mu g\text{In/ml}$$

---

* 0.05×10^6 μg of metal used in the preparation of standards.

** 0.03×10^6 μg of metal used in the preparation of standards.

The concentration of Dy in the stream water after 2.1 hr of tracer administration:

$$\frac{4.22 \times 10^6 \ \mu gDy}{1.27 \times 10^7 \ ml} = 0.332 \ \mu gDy/ml \qquad (3)$$

Calculation of In and Dy concentration in stream water after 5 hr:

$$\text{The concentration of In} = \frac{4.97 \times 10^6 \ \mu gIn}{5 \times 6 \times 10^6 \ ml} = 0.166 \ \mu gIn/ml$$

$$\text{The concentration of Dy} = \frac{4.22 \times 10^6 \ \mu gDy}{5 \times 6 \times 10^6 \ ml} = 0.144 \ \mu gDy/ml \qquad (4)$$

VITA

Abuel-gassem Hamouda El-Fawaris was born in Regaba, Wadi El-Ajall, S.P.L.A. Jamahiriya (Libya) on December 11, 1950, the son of Hamouda Ali El-Fawaris and Mabrouka Abdel-Kariem. Following graduation from Sebha secondary school, Sebha, Libya in 1969, he entered Faculty of Science, El-Fatah University (Tripoli University) Tripoli, Libya. He received the degree of Bachelor of Science from El-Fatah University in June 1974 and presently holds a job with the Atomic Energy Agency of Libya. He entered Faculty of Science graduate program in September 1976, and received the degree of Diploma in Ecology from El-Fatah University in June, 1977. In the same year he was awarded fellowship from International Atomic Energy Agency (IAEA) Vienna, Austria in conjunction with National Academy of Science, Washington, DC for advanced training in the field of radiation detection and measurement in the Nuclear Science Center, Louisiana State University. He is currently a candidate for the Master of Science degree in Botany.

EXAMINATION AND THESIS REPORT

Candidate: Abuel-gassem Hamouda El-Fawaris

Major Field: Botany

Title of Thesis: Red alder (Alnus rubra Bong.) as a bioaccumulator of indium and dysprosium with and without DTPA treatment as measured by neutron activation analysis.

Approved:

Stephen W. Hugile, Cochairman
Major Professor and Chairman

Ronald Kraus, Co-Chairman

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EXAMINING COMMITTEE:

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Monday, April 21, 1980

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A BIOMONITOR OF TRACE HEAVY METALS: INDIUM AND DYSPROSIUM IN RED ALDER ROOTS (*ALNUS RUBRA* BONG.)

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Knaus R. M. and El-Fawaris A. H. *A biomonitor of trace heavy metals: indium and dysprosium in red alder roots* (Alnus rubra. *Bong.*). Environmental and Experimental Botany **21,** 217–223, 1981.—Bioaccumulation of indium (In) and dysprosium (Dy) by the roots of red alder (*Alnus rubra* Bong.) was tested in a small free-flowing stream in Oregon. Mixed solutions of the two elements, In and Dy, were continuously introduced into the stream water as non-radioactive tracers for 1.8 hr and 2.1 hr in two separate experiments. In the first experiment, the two elements were added in a non-chelated form; in the second experiment, upstream from the first, indium–DTPA [(carboxymethylimino)bis(ethylene-dinitrilo) tetraacetic acid pentasodium salt] and dysprosium–DTPA were used as tracers. Instrumental neutron activation analysis was used to determine the quantity of each stable tracer sorbed to the alder roots and the amount remaining in the stream water. Non-chelated In was sorbed by roots up to a concentration of 1.7 μg In/g of root (dry wt), whereas the In–DTPA sorption rate was higher by a factor of approximately 2. Non-chelated Dy was sorbed by roots up to a concentration of 29 μg Dy/g of root (dry wt), while the sorption of Dy–DTPA was less by a factor of 8.

INTRODUCTION

As more uses of heretofore unimportant or curious elements are discovered, a need exists for a methodology to monitor these types of elements in the environment. A study was carried out to demonstrate a possible method for monitoring two biologically conservative, technologically concentrated elements in a free-flowing stream.

Red alder (*Alnus rubra* Bong.) was used in this study to point to the possibility of using terrestrial plant roots as biomonitors. Throughout its range from Northern California to Alaska, where red alder grow on streambanks, we have observed its roots growing directly into the stream water. Terms like hydrophyte, hydrophile, hygrophyte, rheophile, emergent, aquatic, etc., do not accurately describe this phenomenon in a tree normally associated with a strictly terrestrial habitat. In southwestern United States a plant with a growing habit similar to the red alder is the French tamarisk (*Tamarix gallica* L.) and throughout the Mississippi River drainage system, the black willow (*Salix nigra* Marsh.) grows in dense stands with some of its roots growing in the river water. The roots from these trees create a large, natural surface area for possible sorption of river pollutants. These plants can be called rheophytes (a new term meaning water-current-plants). The sorptive potential of the roots of rheophytes makes them good choices for biomonitors of stream pollutants.

Indium (In), one of the elements studied in this work, has become an important commodity in modern industry and technology.$^{(5, 16)}$ Indium combines with group V elements to form inter-metallic semi-conductors which are widely used in infrared detectors. It is important in the production of magneto-resistors and transistors, solar energy conversion devices, and coatings for bearings to improve corrosion resistance. In addition to these applications, In has been used as a stable environmental tracer of air dispersion(15) and water flow.(9, 13, 18)

Dysprosium (Dy), the second element studied here, is a rare-earth element belonging to sesquioxides, (R_2O_3), and is fairly well distributed in nature, though at extremely low concentrations. Reports in the literature of uses of rare earths (other than Dy) either singly or as a class are common. However, references in the literature to technological uses for Dy specifically, occur only when the element is employed as a stable tracer or in highly specialized laser devices. Bibliographies of uses of rare-earth elements can be found in promotional articles of Moly Corp, Inc.(10, 11) and in SUNDERMAN and TOWNLEY.(17)

In preliminary work, KNAUS(6) found that Dy was quickly removed from water that was allowed to recirculate through an indoor laboratory stream microcosm of 800 l. capacity. Coefficients of accumulation for Dy in algae and snails (*Physa* sp.) in the microcosm ranged from 3000 to 60,000 during a period of from 4 to 55 days after a pulse introduction of 3.1 g of Dy. CURRY(2) reported that roots of *S. nigra* growing in a recycling outdoor stream microcosm (1200 l. capacity) sorbed the rare earth, lanthanum (La), up to a concentration of 24 μgLa/g of root (dry wt) 1.8 hr after the pulse introduction of 1 g of La. Using a stream microcosm operating in an open, free-flowing mode, KNAUS and CURRY(8) reported that *S. nigra* roots sorb manganese up to a concentration of 101 μgMn/g of root (dry wt) and that molybdenum(7) was sorbed up to a concentration of 110 μgMo/g of root (dry wt) in a free-flowing stream microcosm. These studies, involving Dy, La, Mn and Mo tracers, established the feasibility and the tracer methodology of the present work.

Personal communication with W. Loveland and his co-workers at Oregon State University suggested the plausibility of using In and Dy chelated with DTPA [(carboxymethylimino)-bis(ethylene-dinitrilo) tetraacetic acid penta-sodium salt] in natural stream flow studies. DTPA possesses a greater thermodynamic stability constant than other metal chelates.(12, 14)

The purpose of these studies is to suggest that rheophytes are useful as biomonitors of aquatic heavy metal pollutants under natural stream conditions. Additionally, we wished to determine if there was a significant difference in the root sorption of the tracers in two different chemical forms, chelated and non-chelated.

MATERIALS AND METHODS

The stream

The studies were carried out at Oak Creek, a perennial stream 6 miles west of Corvallis, Oregon. The first tracer experiment was conducted below the Sediment Transport Research Station located in McDonald Forest; the second experiment was conducted approximately 200 m above the research station. The heavily shaded stream-bed consists of a naturally winding channel, approx. 1–2 m wide, lined with coarse gravel and small rocks. The stream flow rate during the two experiments was approximately 0.1 m^3/min, the stream temperature was 18 ± 2°C, and the pH was 7.1 ± 0.2.

Red alder roots

The selected experimental organism, *A. rubra*, is the dominant tree species beside Oak Creek. Its abundant root growth makes it ideal for sampling. During the spring and summer growing season, a multitude of fast-growing, pink-colored roots is produced which are approximately 1 mm in width. They vary in length from 2-mm buds to 12-cm strands. Throughout this work the term, root, is meant to include the entire young root structure and its attendant microflora and microfauna. No attempt was made to separate the components of the root system.

Indium and Dy *experiment without DTPA*

Five grams of Dy in the form of Dy_2O_3 (5.9 g of the compound) and 5.0 g of In in the form of pure metal were dissolved together in concentrated nitric acid. After dissolution, the acidic solution was neutralized by addition of concentrated ammonium hydroxide. Distilled water was added to make a 105 ml total volume. One ml of this solution was used for preparation of standards. The remaining 104 ml were introduced to the natural stream at a constant rate of 0.96 ml/min for 1.82 hr by use of a peristaltic pump.

Indium and Dy *experiment with DTPA*

Five grams of In and 4.25 g of Dy were dissolved together in concentrated nitric acid. The chelation of the tracers was accomplished by the addition of 96 ml of DTPA, approximately 34% in water. After neutralization the volume was 150 ml. One ml of the stock solution was used for the preparation of standards, and the remaining 149 ml were introduced into the stream at a constant rate of 1.18 ml/min for 2.1 hr by use of the peristaltic pump.

In the chelated experiment, 4.25 g of Dy was used instead of the 5 g of Dy used in the experiment without DTPA. This was done to facilitate more efficient handling and analysis of activated root samples as a gamma ray detection problem arose in the non-chelated experiment due to the different half-lives of the two activated tracers.

Sample preparation and neutron activation

Samples of water, alder roots, mud and organic detritus were taken before the introduction of the stable tracers to establish background levels of native In and Dy. Root samples were taken during both of the experiments from large root masses that were cut and suspended in the stream water 8 m downstream from the point of tracer administration. Three more distant sampling stations were established during the non-chelated experiment. These stations had uncut alder roots and were used to test the validity of results obtained from the cut roots at the main sampling station. Stations I, II and III were located 60, 100 and 200 m, respectively, downstream from the point of tracer input.

Experimental water samples were taken at the site of the roots each time an experimental root sample was taken. About 0.02–0.10 g (dry wt) of root and 0.5 ml of water, including standards and background samples, were placed in clean 2/5-dram (1.2 × 2.5 cm) polyethylene vials and heat sealed. The 2/5-dram vials were then placed in 2-dram (1.6 × 5.5 cm) polyethylene vials which were also heat sealed to prepare doubly encapsulated containers suitable for activation with thermal neutrons. All standards and samples were irradiated at the Oregon State University Radiation Center in the TRIGA Reactor for 4 min each at a thermal neutron flux of 10^{12}-n/cm^2/sec. The pneumatic transfer system was employed for rapid movement of the samples to and from the reactor core. The activated samples were counted on a Ge(Li) detector system connected to a multichannel analyzer coupled with a computer for data analysis. General references on instrumental neutron activation analysis (INAA) methodology are available.(1, 3, 4)

RESULTS

Experimental results of the In and Dy administration are presented in Figs. 1 and 2; the chelated and non-chelated results are graphed as a function of time, with root and water data included in the same units to facilitate visual comparisons. The common unit is μg metal/g; root data are on a dry-weight basis. For the sake of simplicity, error bars for counting statistics have been omitted in the figures. The maximum relative error was ±7%.

Because the concentrations of native In and Dy were below the INAA lower limits of detection for all background samples, no correction was required for the experimental data.

Alder roots were found to sorb both In and Dy in both the chelated and non-chelated forms. The accumulation of In–DTPA in the roots was greater than non-chelated In by a factor of 1.9 after 1.8 hr of tracer administration (Fig. 1). For roots, the concentration of sorbed In-DTPA on a dry-weight basis was 41 times the concentration of In–DTPA tracer in the stream water at the end of the tracer

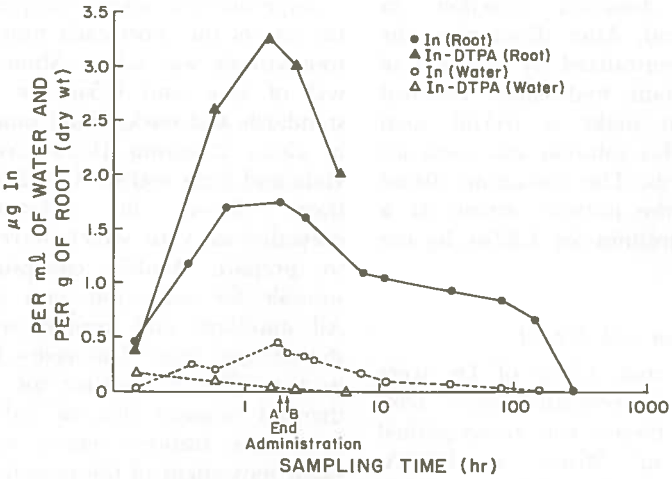

FIG. 1. The concentration of In and In–DTPA in *Alnus rubra* Bong. roots in µgIn/g of root (dry wt) and in stream water in µgIn/ml vs. log of time are shown. Slow, continuous In tracer administration was begun at 0.0 hr and ended at 1.82 hr (A). Slow, continuous In–DTPA tracer ended at 2.1 hr (B). At time = 0.0, In and In–DTPA concentrations in root and water were below limits of detection.

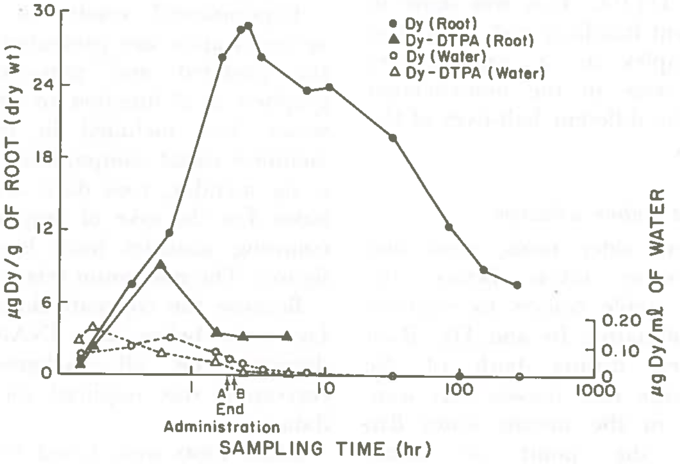

FIG. 2. The concentrations of Dy and Dy–DTPA in *Alnus rubra* Bong. roots in µgDy/g of root (dry wt) vs. log of time are shown on the left axis. The concentrations of Dy in stream water in µgDy/ml are shown on the right axis. Slow, continuous Dy tracer administration was begun at 0.0 hr and ended at 1.82 hr (A). Slow, continuous Dy–DTPA tracer ended at 2.1 hr (B). At time = 0.0, Dy and Dy–DTPA concentrations in root and water were below limits of detection.

administration (1.8 hr). Data for both In–DTPA and non-chelated In show a gradual increase in the sorption by alder roots during the first 1.8 hr. The non-chelated In concentration then decreased with time to the end of sample collection. The concentration of In–DTPA in stream water dropped to below the limits of detection after 7 hr, while the level of non-chelated In in water samples was 0.3 μgIn/ml after 3.2 hr and continued to show detectable amounts of In up to 120 hr after tracer administration.

The accumulation of non-chelated Dy by alder roots (Fig. 2) was found to be different from the In sorption patterns. The roots accumulated more non-chelated Dy than Dy–DTPA by a factor of 8 after 2.1 hr of tracer administration and remained high to 7 hr, when Dy–DTPA sampling stopped. The amount of non-chelated Dy sorbed by alder roots was 300 times more than the concentration of Dy tracer in the stream water near the end of tracer administration (2 hr). The non-chelated Dy concentrations in or on the alder roots gradually increased up to the end of tracer administration followed by a gradual decline to the end of the sampling at 150 hr. The non-chelated Dy concentrations in the stream water show a gradual increase in the first 0.6 hr, followed by a gradual decrease in concentration to below detection limits sometime after 10 hr. Chelated Dy accumulation by alder roots shows a rise in Dy–DTPA concentration early in the tracer administration phase followed by a decline in Dy–DTPA concentration to a relatively stable level up to the end of sampling (7 hr). A comparison of Figs. 1 and 2 leads to the observation that there is approximately an 8-fold increase in the sorption pattern of non-chelated Dy over In, whether or not In was chelated.

With a stream flow of approximately 0.1 m^3/min and with the addition of 5 g of tracer over a time period of more than 100 min, a rough calculation of the administered tracer concentration in the vicinity of the roots would be just under 0.5 μg of the tracer/ml. This would assume thorough mixing and no uptake between the point of tracer administration and the roots, 8 m downstream. As shown in Fig. 1, when there is a small amount of uptake of non-chelated In there is a correspondingly higher amount of non-chelated In in the water indicating the conservative nature of In. The observed amount of non-chelated In in the water approaches the calculated value (approximately 0.5 μg In/ml). When nearly twice as much In–DTPA is sorbed by the roots, the observed amount of chelated In in the water falls below the calculated values for water. In contrast, the curves in Fig. 2 describe a significantly higher sorption of non-chelated Dy by the roots and approximately the same amount of Dy–DTPA sorption as was witnessed by both of the In root sorption rates. However, the concentration of Dy in the stream water in both the non-chelated Dy and Dy–DTPA experiments was found to be less than one-half (less than 0.2 μg Dy/ml) of the Dy that would be expected from calculated values. This also assumes no sorption of Dy between the point of tracer administration and the root sampling station, 8 m downstream.

The data reported in Table 1 indicate that there was no difference in the accumulation of the two non-chelated tracers by alder roots which had been cut and suspended in the stream water, versus intact alder roots. Non-chelated In, at the main station (8 m downstream) was sorbed up to 1.57 μgIn/g of root (dry wt) 2 hr after the beginning of tracer administration. The sorption of In at Stations I, II and III was 1.42, 2.89 and 1.28 μgIn/g of root (dry wt), respectively, 2 hr after the beginning of tracer administration (Table 1). The sorption of non-chelated Dy at the main station was 27.5 μgDy/g of root (dry wt) 2 hr after the beginning of tracer administration. The sorption of Dy at Stations I, II and III was 10.6, 18.4 and 16.3 μgDy/g of root (dry wt), respectively, 2 hr after the beginning of tracer administration.

DISCUSSION

Industrial uses of In and of rare earths are not trivial, their annual productions being measured in hundreds of tons. A method for biomonitoring the impact to streams of the

Table 1. *Non-chelated* In *and* Dy *values from sampling stations: main* I, II *and* III; 8, 60, 100 *and* 200 m, *respectively, downstream from the point of tracer input*

	Sampling Station	Distance	Quantity measured	Sampling time (hr) 2.0	2.7	3.0	5.0	28.0
Indium	Main	8 m	μg/g alder root	1.57	1.48	1.45	1.29	1.12
			μg/ml stream water	0.37	0.34	0.38	0.20	0.10
	I	60 m	μg/g alder root	1.42	0.98	1.24	1.03	0.83
			μg/ml stream water	0.44	0.39	0.32	0.19	bld[a]
	II	100 m	μg/g alder root	2.89	1.88	0.87	1.27	0.88
			μg/ml stream water	0.39	0.33	0.29	0.31	0.05
	III	200 m	μg/g alder root	1.28	1.89	0.94	1.30	0.59
			μg/ml stream water	0.34	0.29	0.26	0.18	0.04
Dysprosium	Main	8 m	μg/g alder root	27.5	28.1	26.3	24.6	22.0
			μg/ml stream water	0.08	0.06	0.04	0.02	bld
	I	60 m	μg/g alder root	10.6	8.7	5.9	4.8	3.2
			μg/ml stream water	0.10	0.67	0.06	0.04	bld
	II	100 m	μg/g alder root	18.4	12.6	7.2	4.2	2.6
			μg/ml stream water	0.07	0.06	0.04	0.03	bld
	III	200 m	μg/g alder root	16.3	6.6	5.6	3.5	5.1
			μg/ml stream water	0.06	0.05	0.03	0.03	bld

All values are μg of metal per ml of water or per g of alder root (dry wt). Since sampling did not take place at exactly the same times at the main station as at stations I, II and III, values at the main station were calculated from Figs. 1 and 2. Alder roots at the main station were cut and suspended in the water, roots at stations I, II and III were uncut.

[a] Below the limits of detection.

waste from industrial uses of these metals does not exist. Intact red alder roots have been shown to sorb low concentrations of In and Dy (a representative of the rare earths) from running stream water to levels easily quantified by INAA.

From evidence presented in this work, non-chelated In appears to be the better stable tracer for the movement of water when compared to In in its chelated form, and to Dy in both the chelated and non-chelated forms. When water is sampled 200 m below the point of tracer input, non-chelated In remains approximately 5 times higher than non-chelated Dy values in identical samples. Since values for the chelated In in the roots were higher than those for non-chelated In in the root, the chelation of In would lessen the usefulness of In as a tracer of water, but would enhance its sorptive properties.

As would be expected, when a tracer was found to be at a high level in the roots, it was correspondingly low in the water. Conversely, when the tracer was low in the roots it was high in water. Non-chelated Dy in the roots was high when compared to chelated Dy and both forms of In. Consequently, the amount of non-chelated Dy remaining in the water was below the limits of detection at only 7 hr into the experiment. In setting up a monitoring program utilizing the sorptive properties of a biological monitor, such as a rheophytic plant, one would have to be concerned with the proximity of the sampling site to the pollution source, depending upon the species of element being monitored. For instance, with the high affinity of roots for non-chelated Dy, the sampling station would have to be close to the Dy source. On the other hand, sampling for non-chelated In, the root sampling site could be relatively more distant.

If Dy or In were chelated, the root sampling site could be an intermediate distance from the source.

In and Dy are becoming classic elements to use as stable tracers because they can be easily detected in extremely low concentrations by INAA. If they are to be used as simultaneous tracers as a finger-printing tool to identify sources of pollution such as ship cargoes or industrial effluents, allowances will have to be made for their differential sorption to biomonitors and consequent differential amounts left in the water. Chelation would tend to moderate the effect of the differential sorption.

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