CHAPTER IV

METHOD OF EXPERIMENT AND ANALYSIS

When the tritiated water falls on the vegetation it will be absorbed by the leaves and stems of the plant leaf through the cuticle and also via the stomata, and once within the leaf will be involved in the transpirational stream and swept out of the plant with the large flux of water transpired each day, or it will be involved in photosynthesis, and fixed in organic compounds which may be translocated to other parts of the plant.

In an ecological study, the exposure of growing local vegetation to a single tritiated water application was made to study both soil and plant behaviour. Tritium was applied to the experimental plot as tritiated water, by hand, with compressed air garden sprayer. Plant uptake of tririated water continued throughout the growing season from the single application of tritiated water, because of the long half-time of tritium in the soil. The experimental plots are daily watered with stable water. Then the plants and soil samples are taken at different intervals of time. These samples are then analysed to obtain tritium concentration for further interpretation.

IV.1 Field Experiment

IV.1-1 Experimental Plot

The plot was selected at Kasetsart University. The whole cultivated area for this research at the site was, 4 m x 30 m (120 m^2). This is divided into 5 plots according to the species of local vegetation selected for experimentation.

IV.1-2 Species of Vegetation Used

Five species of vegetation are used for this experiment, namely,

Tomato	Lycopersicon esculentum Linn.
Common Kale	Brassica Oleracea Linn. Var. acephala DC.
Radish	Raphanus sativus Linn. Var. radicula Pers.
Cowpea	Vigna sinensis Savi
Shallot	Allium ascalonicum Linn.

These local vegetation are selected with the aim of their rather short life cycle which is of about two months duration, and it is easy to trace them in order to obtain the result of behaviour of tritium in ecological system.

IV.1-3 Tritiated Water Activity Applied

Tritium was applied to the experimental plot as tritiated water. Approximately, 2,250 ml of

tritiated water was applied to a 1.32 m^2 plot of Radish. The total amount of tritium applied to the plot was 22,500 μCi or approximately 1,704.55 μCi per square meter. The specific activity of the tritiated water applied was 10 μCi per ml or 1.0×10^7 pCi per ml. The data relating to other vegetation mentioned earlier are shown in Table IV-1.

TABLE IV-1

Vegetation Plots and Application of Tritiated Water (HTO)

Species	Applied area m^2	Applied time(sec)	Amount of HTO		Activity of HTO	
			ml	ml/m^2	μCi	μCi/m^2
Radish	1.32	211	2,250	1,704.55	22,500	17,045.45
Common Kale	1.2	178	1,880	1,566.66	18,800	15,666.67
Tomato	1.6	180	1,900	1,187.50	19,000	11,875.00
Shallot	1.3	90	980	753.85	9,800	7,538.46
Cowpea	3.48	306	3,130	899.43	31,300	8,994.25

Specific activity of tritiated water is 10 μCi per ml.

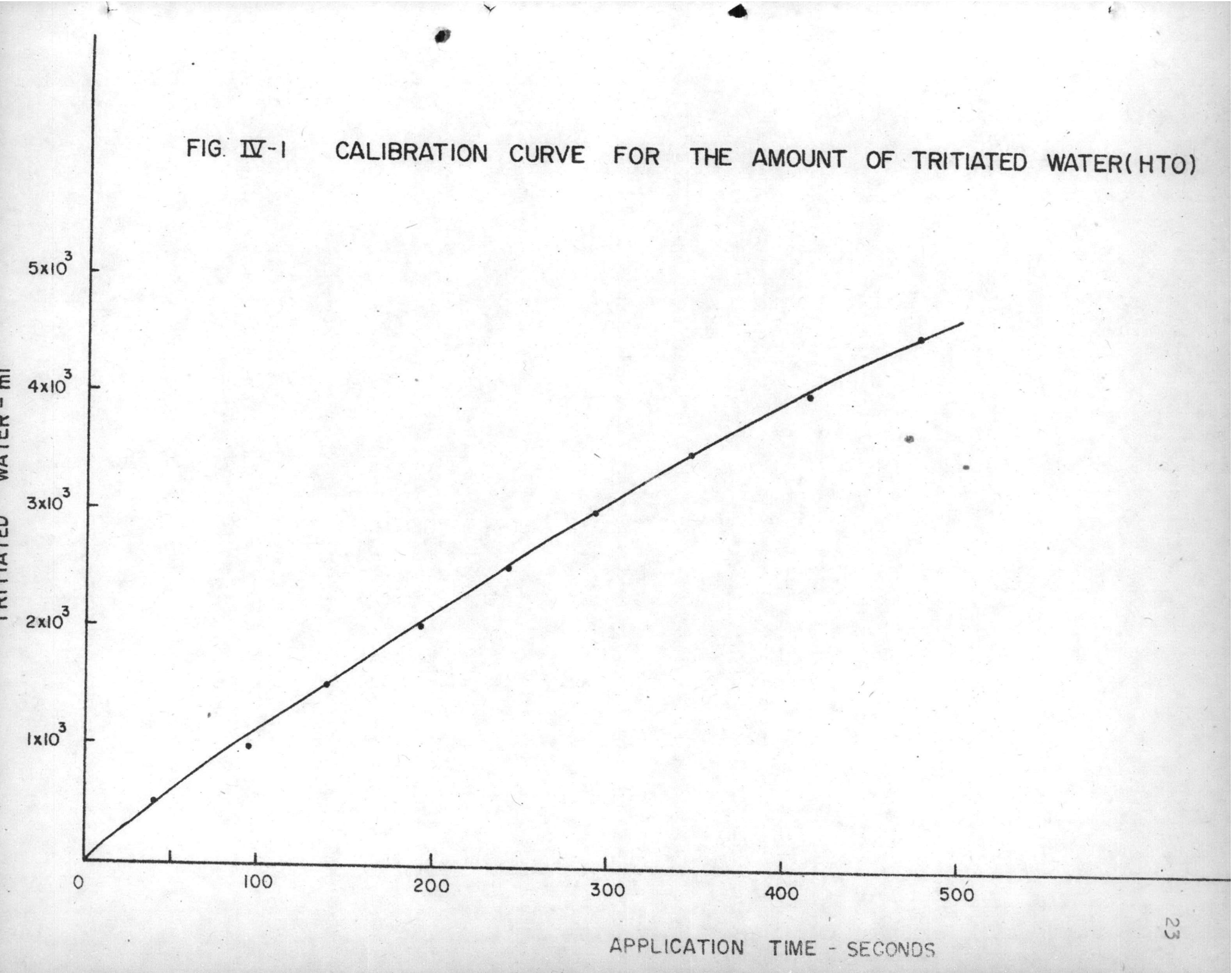
The volume of tritiated water sprayed to each plot can be calculated by the calibration curve between the volume of tritiated water versus the spraying time as shown in Figure IV-1.

During the whole period of experiment no rain was observed. The temperature was about 30-33°C and humidity of the air ranged between 50 to 80 per cent over the experimental plots.

IV.2 Method of Analysis

The samples are taken one hour after the application of tritiated water. Further removal of samples is done periodically. Only the eatable portions of the vegetation are taken as samples, such as Radish. Here the root which has modified into a stem, is taken since it is the only eatable part of the plant. As for Common Kale and Shallot, the whole parts are taken as samples. For Cowpea only the bean is taken for experimentation. Tomato leaf and Tomato are separately taken for analysis, since in this instance it is intended to have some information on the leaf-fruit relation. These samples are then collected and are immediately wrapped in separate polythene bags and are frozen to prevent loss of tritium, then they are transferred to cold freezer chamber for storage until analysis.

FIG. IV-1 CALIBRATION CURVE FOR THE AMOUNT OF TRITIATED WATER(HTO)



The soil samples are taken by using soil auger after digging to the depth of 30 cm. The soil removed from the core of the auger is also kept in polythene and immediately frozen and stored for further analysis.

IV.2-1 Laboratory Analysis

At the laboratory, the samples are prepared for analysis. A small piece is cut from each sample. The purpose is to extract the tissue water from the plants with more efficiency and shorter duration of time of extraction. Care must be taken into account, no contamination between the samples is to occur. They are then transferred into round-bottomed flasks. They are weighed and transferred to the vacuum freeze-dry apparatus. The weight of samples ranges between 9 to 18 g and 50 to 80 g of the soil samples.

The vacuum freeze-dry apparatus is maintained at the pressure of 20 to 30 microns throughout the experiment and can serve only six samples at each run. The samples are obviously dry; the time taken for each run of experiment is about 8 to 10 hours. The samples are then disconnected from the vacuum freeze-dry apparatus, they are weighed again; the tissue water content ranges between 89 to 94 per cent. As for

the soil samples, the water content from surface to the depth of 25 cm is about 17 per cent. The dry samples are kept well in air-tight plastic bottles for further analysis of tissue-bound tritium.

The tissue water are condensed inmto the tube, then the tube is disconnected from the apparatus and kept in the vials for further tritium concentration analysis.

IV.2-2 Counting Technique

Tritium concentration of the samples can be obtained by using standard liquid scintillation counting technique. Take one ml of tissue water, then add 10 ml of scintillator for counting. The efficiency of the liquid scintillation system is about 16 per cent. The resulting data were corrected for counting efficiency and were expressed as DPM (Disintegration per minute) per ml of water as shown in Tables IV-2 to IV-10.

TABLE IV-2

Tritium Activities in Plant Tissue of Tomato at Different Times after a Single Surface Application of Tritiated Water

Sample No.	Tritium Concentration in Plant Tissue			Days after Application
	cpm	dpm	pCi/ml	
TF_2-4	10,881	68,006	30,633	5
TF_2-5	9,767	61,043	27,496	7
TF_2-6	11,777	73,603	33,155	8
TF_2-7	10,467	65,418	29,467	9
TF_2-8	10,060	62,875	28,322	12
TF_2-9	10,231	63,943	28,803	13
TF_2-10	6,804	42,525	19,155	14
TF_2-11	7,122	44,512	20,050	15
TF_2-12	6,556	40,975	18,457	16
TF_2-13	7,270	55,437	20,467	19
TF_2-14	6,141	38,381	17,288	23
TF_2-15	5,422	33,887	15,264	27
TF_2-16	3,511	21,943	9,884	30

TABLE IV-3

Tritium Activities in Plant Tissue of Tomato Leaf at different Times of a single Surface Application of Tritiated Water

Sample No.	Tritium Concentration in Plant Tissue			Days after Application
	cpm	dpm	pCi/ml	
T_2-1	117,428	733,931	330,599	-
T_2-2	23,457	146,606	66,038	1
T_2-3	14,046	87,787	39,543	2
T_2-4	8,062	50,387	22,696	5
T_2-5	7,489	46,806	21,083	7
T_2-6	9,381	58,631	26,410	8
T_2-7	10,600	66,250	29,842	9
T_2-8	7,933	49,581	22,333	12
T_2-9	8,171	51,068	23,003	13
T_2-10	7,449	46,556	20,971	14
T_2-11	19,662	122,887	55,354	15
T_2-12	8,900	55,625	25,056	16
T_2-13	5,018	31,362	14,127	19
T_2-14	5,313	33,206	14,957	23
T_2-15	12,956	80,975	36,475	27
T_2-16	7,934	49,587	22,336	30

TABLE IV-4

Tritium Activities in Plant Tissue of Common Kale at different Times after a Single Surface Application of Tritiated Water

Sample No.	Tritium Concentration in Plant Tissue			Days after Application
	cpm	dpm	pCi/ml	
K_2-1	90,123	563,268	253,724	-
K_2-2	37,717	235,731	106,185	1
K_2-3	21,353	133,456	60,119	2
K_2-4	8,239	51,493	23,195	5
K_2-5	7,814	48,837	21,998	7
K_2-6	10,471	65,443	29,478	8
K_2-7	10,078	62,987	28,372	9
K_2-8	7,497	46,856	21,106	12
K_2-9	6,335	39,593	17,834	13
K_2-10	6,106	38,162	17,190	14
K_2-11	7,421	46,381	20,892	15
K_2-12	7,832	48,950	22,049	16
K_2-13	6,396	39,974	18,006	19
K_2-14	4,762	29,762	13,406	23
K_2-15	3,085	19,281	8,685	27
K_2-16	3,368	21,050	9,081	30

TABLE IV-5

Tritium Activities in Plant Tissue of Radish at different Times after a single Surface Application of Tritiated Water

Sample No.	Tritium Concentration in Plant Tissue			Days after Application
	cpm	dpm	pCi/ml	
R_2-5	9,293	58,081	26,162	7
R_2-6	9,730	60,812	27,392	8
R_2-7	8,927	55,793	25,131	9
R_2-8	7,412	46,325	20,867	12
R_2-9	6,954	43,462	19,577	13
R_2-10	6,216	38,850	17,500	14
R_2-11	9,420	58,875	26,520	15
R_2-12	7,659	47,868	21,562	16
R_2-13	7,753	48,456	21,827	19
R_2-14	7,944	49,650	22,365	23
R_2-15	3,777	23,606	10,633	27
R_2-16	4,547	28,418	12,800	30

TABLE IV-6

Tritium Activities in Plant Tissue of Cowpea at different Times after a Single Surface Application of Tritiated Water

Sample No.	Tritium Concentration in Plant Tissue			Days after Application
	cpm	dpm	pCi/ml	
AB_2-4	8,535	53,343	24,027	5
AB_2-5	7,532	47,075	21,204	7
AB_2-6	6,990	43,687	19,678	8
AB_2-7	6,689	41,812	18,834	9
AB_2-8	6,928	43,300	19,504	12
AB_2-9	7,651	47,818	21,539	13
AB_2-10	7,765	48,531	21,860	14
AB_2-11	7,660	47,900	21,576	15
AB_2-12	7,721	48,256	21,736	16
AB_2-13	6,384	39,900	17,972	19
AB_2-14	5,254	32,837	14,791	23
AB_2-15	4,816	30,105	13,560	27
AB_2-16	5,487	34,293	15,447	30

TABLE IV-7

Tritium Activities in Plant Tissue of Shallot at different Times after a Single Surface Application of Tritiated Water

Sample No.	Tritium Concentration in Plant Tissue			Days after
	cpm	dpm	pCi/ml	Application
SH_2-1	10,416	65,100	29,324	7
SH_2-2	11,460	71,630	32,265	8
SH_2-3	10,731	67,074	30,213	9
SH_2-4	8,494	53,089	23,913	12
SH_2-5	8,246	51,539	23,215	13
SH_2-6	6,778	42,367	19,085	14
SH_2-7	7,627	47,669	21,472	15
SH_2-8	6,746	52,167	18,994	16
SH_2-9	4,924	30,779	13,864	19
SH_2-10	2,970	18,566	8,363	23
SH_2-11	3,788	23,675	10,664	27
SH_2-12	3,876	24,225	10,912	30

TABLE IV-8

Tritium Activities of Soil-Water at Depth 0-5 cm at different times after a Single Surface Application of Tritiated Water

Sample No.	Tritium Concentration of Soil Water			Days after
	cpm	dpm	pCi/ml	Application
S_1-1	14,193	88,706	39,957	1
S_1-2	11,940	74,631	33,617	2
S_1-3	10,169	63,562	28,631	5
S_1-4	9,384	58,650	26,418	7
S_1-5	8,366	52,293	23,555	8
S_1-6	7,612	47,580	21,429	9
S_1-7	7,283	55,523	20,505	12
S_1-8	6,932	43,331	19,518	13
S_1-9	6,324	39,525	17,804	14
S_1-10	5,841	36,510	16,445	15
S_1-11	5,358	33,490	15,085	16
S_1-12	5,012	31,325	14,110	19
S_1-13	3,858	24,118	10,863	23
S_1-14	3,443	21,520	9,693	27
S_1-15	3,128	19,550	8,806	30

TABLE IV-9

Tritium Activities of Soil-Water at Depth 5-15 cm at Different Times after a Single Surface Application of Tritiated Water

Sample No.	Tritium Concentration of Soil-Water			Days after Application
	cpm	dpm	pCi/ml	
S_2-1	7,524	47,031	34,698	1
S_2-2	9,240	57,750	26,013	2
S_2-3	10,321	64,510	29,058	5
S_2-4	9,701	60,370	27,331	7
S_2-5	8,984	56,150	25,292	8
S_2-6	8,804	55,031	24,788	9
S_2-7	8,016	50,105	22,569	12
S_2-8	7,281	45,510	20,500	13
S_2-9	6,960	43,500	19,594	14
S_2-10	6,644	41,530	18,707	15
S_2-11	6,800	42,500	19,144	16
S_2-12	6,000	37,505	16,894	19
S_2-13	5,000	31,250	14,076	23
S_2-14	4,344	27,150	12,229	27
S_2-15	3,869	24,185	10,894	30

TABLE IV-10

Tritium Activities of Soil-Water at Depth 15-25 cm at Different Times after a Single Surface Application of Tritiated Water

Sample No.	Tritium Concentration of Soil-Water			Days after Application
	cpm	dpm	pCi/ml	
S_3-1	4,884	30,528	13,750	1
S_3-2	5,204	32,525	14,650	2
S_3-3	6,744	42,150	18,986	5
S_3-4	7,720	48,250	21,734	7
S_3-5	7,880	49,250	22,184	8
S_3-6	8,244	51,530	23,211	9
S_3-7	7,680	48,000	21,621	12
S_3-8	7,120	44,505	20,047	13
S_3-9	7,201	45,010	20,274	14
S_3-10	6,798	42,490	19,135	15
S_3-11	6,495	40,595	18,286	16
S_3-12	6,014	37,590	16,923	19
S_3-13	5,201	32,510	14,644	23
S_3-14	4,591	28,695	12,925	27
S_3-15	4,190	26,190	11,797	30