

## **Diverse Distribution Study Of C-14 Through Out The Major Crops Of Punjab (Pakistan) Using Biological Oxidizer And Liquid Scintillation Counter**

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### **Abstract**

In this study the assay of labeled pesticide treated plants (Wheat and Maize), the major crops of Punjab was performed using biological oxidizers Soxhlet extraction operators and scintillation counting assembly. The data received after the treatment the wheat and maize plants with C14 the measured radioactivity showed that greater content was accumulated in the wheat root (0.15 $\mu$ Ci), medium in leaves (0.10 $\mu$ Ci) and least in stem (0.07 $\mu$ Ci). While in case of Maize the order of accumulation was found to be greater in leaves (0.14 $\mu$ Ci), medium in root (0.12 $\mu$ Ci) and least in stem portion (0.08 $\mu$ Ci) respectively. Data also revealed that Maize showed more absorption capability of pesticides as compared to Wheat which is more probability due to large fat contents and high transpiration rate (Higher be the nuclear area of cross section more be the probability of absorption). It is strongly recommended that use of pesticide Maize should be minimized to overcome the hazardous effects on humans, animals, plants and eco-system disturbance.

**Keywords:**

Malathion, C14 labeled Wheat, Maize plants, Biological oxidizers, scintillation counter, maize more absorptive than wheat, reduced pesticide spray, health hazards, protective measures

**Introduction**

Radiation affects the growth rate and germination percentage of crop seeds and also genetic variations can also be made through irradiation process. In recent years, ionizing radiation has been used successfully to bring about genetic variations in different crop species and is considered as valuable tool for the improvement of different crop plants. The effects of radiation on various crop plants have been reported by different authors throughout the scientific world. Present work is a humble attempt in this regard (Abidi and Nizami, 1995; Abbas *et al.*, 2006; Adriyano *et al.*, 2006).

Malathion is an organophosphate parasymphomimetic, which binds irreversibly to cholinesterase. Malathion is an insecticide of relatively low human toxicity. In the former USSR it was known as carbophos, in New Zealand and Australia as maldison and in South Africa as mercaptothion. However, Malathion breaks down into malaoxon, which is 60 times more toxic than Malathion. For this reason, if Malathion is used or somehow enters an indoor environment, as it breaks down into malaoxon, it can seriously and chronically poison the occupants living or working in this environment. Malathion present in untreated water is converted to malaoxon during the chlorination phase of water treatment, so Malathion should not be used in waters that may be used as a source for drinking water, or any upstream waters. In 1976, numerous malaria workers in Pakistan were poisoned by isomalathion, a common impurity in Malathion, which is capable of inhibiting carboxyesterase enzymes in those exposed to it; the original toxicity evaluation for

Malathion had not anticipated isomalathion co exposure (Halstead and Renni 2006; Howard and Pelc 1953; Marshall and Wright 1998; Mumma and Hamilton 2003; Macias *et al*, 2006; Nishikawa *et al* 1994; Ahmad, and Bhutta 2005).

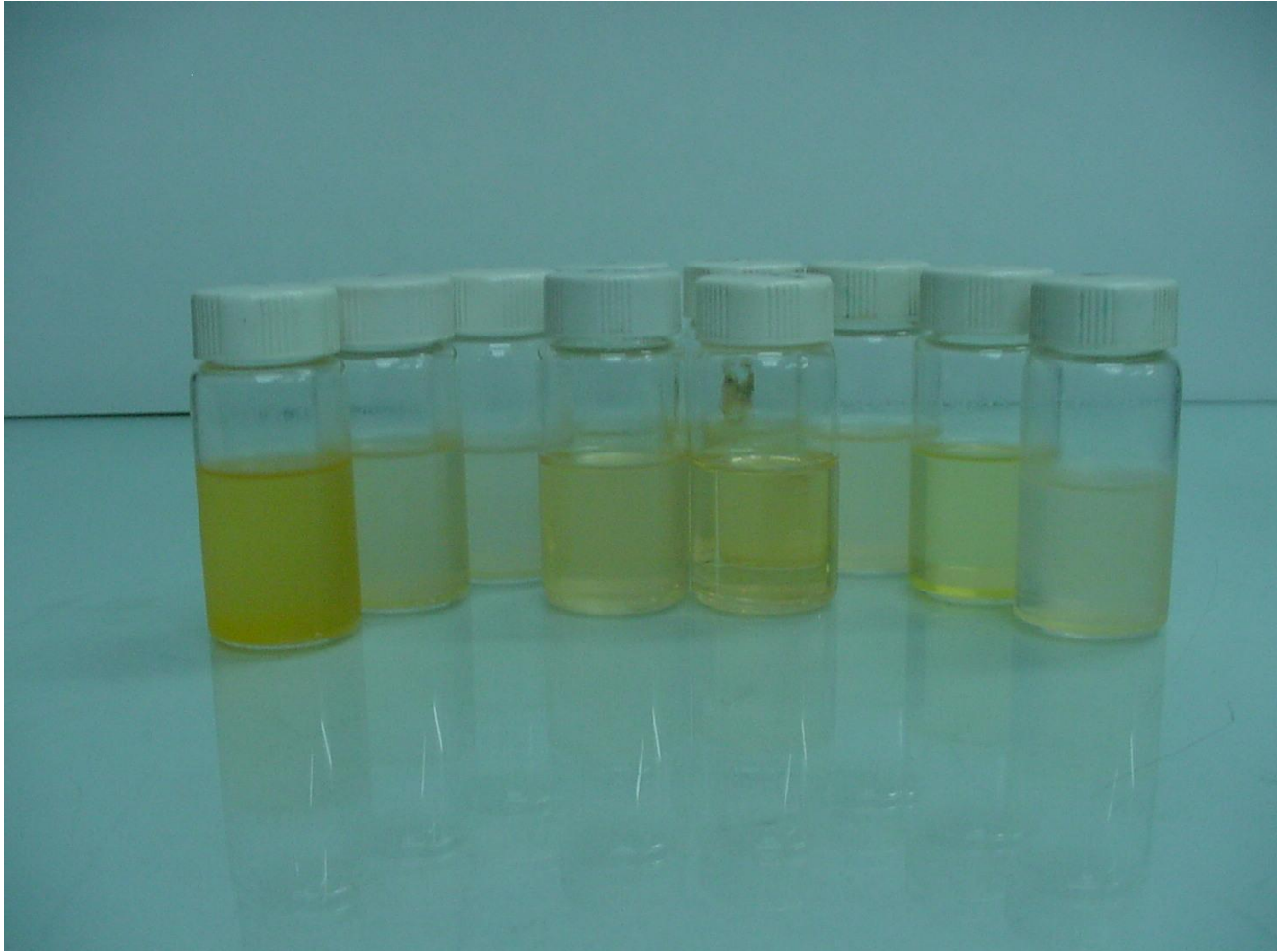
### **Materials and Methods**

Wheat (*Triticum aestivum* L.) and Maize (*Zea mays* L.) were taken as test plants in this study. Seeds of Wheat and Maize were germinated in the clay pots by taking 500 g of soil in each pot. Before the germination of seeds a pesticide labeled  $C^{14}$  (Malathion) of activity  $0.4\mu Ci$  was mixed in the soil. Both the pots were placed in the laboratory. After 15 days plants were uprooted gently. The healthy plants were weighed. The weight of the healthy plant of Maize was 500 mg and that of Wheat plant was 3600mg. The parts (roots, stems and leaves) of the both plants were separated and were dried in the oven at  $50^{\circ}C$  for 24 hours and weighed. The weight of the Wheat roots, stem and leaves was 530mg, 850mg and 960mg, respectively. Similarly the weight of Maize roots, stem and leaves was 150mg, 130mg and 120mg, respectively. The weight of the each sample of soil was taken as 500mg. The samples were put into cone and formed a pallet. Then these samples were combusted in the Harry Biological oxidizer Model Ox-600 and  $C^{14}$  was trapped by the trap solution (Ethanol amine+ Methanol). The trap solution was taken in the 20ml glass scintillation vial (Packard, USA) and mixed with scintillation cocktail (Ahmad, 2005). Scintillation solution was prepared by dissolving 4g of PPO (2, 5-diphenyloxazole) and 0.1g of POPOP [1, 4-bis- (5-phenyloxazolyl)-benzene] Packard USA in 1 liter of Toluene (analytical grade) Merck Germany (Laboratory training manual, IAEA, 1991). The vials were subjected to counting  $C^{14}$  activity using a liquid scintillation counter (Nuclear Enterprises Model LSC1) shown in Figure 1 & 2 (Nilanjani 2006; Tarpely and Victor, 1997; Ahmad and Bhutta, 2005). The block diagram of the scintillation counting assembly is shown in Fig 3.

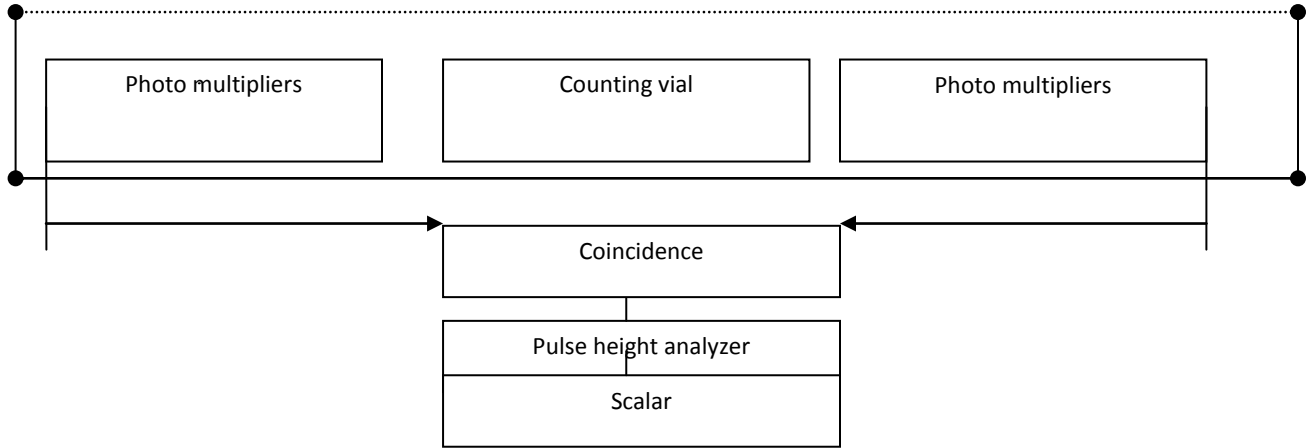


**Fig. 1 Liquid scintillation counter**





**Fig. 2 Liquid scintillation vials**



**Fig. 3. Block diagram of Liquid Scintillation Counter**

### **Internal Standardization and instrument optimization**

The counting is determined by counting the sample ( $C_s$ ) and then adding a known amount of activity ( $D_i$ ) and recounting ( $C_{s+i}$ ). The added radioactive standard material must be compatible with the sample Scintillator system and its absolute activity  $D_i$  must be accurately known. The counting efficiency  $\epsilon$  for the quenched sample is

$$\epsilon = C_{s+i} / D_i$$

The activity of quenched sample is thus

$$D_s = C_s / \epsilon$$

Counting of the sample and recounting after addition of standard must be performed using the same instrument for gain and window setting (Khan, 2003; Kazmi and Zada 2003).

## **Results and Discussion**

Radiation and radioactive substances are natural and permanent features of the environment, and thus the risks associated with radiation exposure can only be restricted, not eliminated entirely. Additionally the man made radiation is wide spread. Sources of radiation are essential to modern health care. Disposal medical supplies sterilized by intense radiation have been central to combating disease.

Radiology is a vital diagnostic tool and radiotherapy is commonly part of the treatment of malignancies. The use of nuclear energy and application of its by-products i.e., radiation and radioactive substances, continue to increase around the world. Nuclear techniques are in growing use in industry, agricultural, medicine and many fields of research, benefiting hundreds of millions of people and giving employment to millions of people in the related occupations.

Irradiation is used around the world to preserve foodstuffs and reduce wastage, and sterilization techniques have been used to eradicate disease carrying insects and pests. Industrial radiography is in routine use, for example to examine welds and detect cracks and help prevent the failure of engineered structures.

In this study, the assay of labeled pesticides treated plant was performed using Biological Oxidizer, Soxhlet Extraction Apparatus and Liquid Scintillation Counting techniques. The plants (wheat and maize) treated with malathion ( $0.4\mu\text{Ci}$ ) were harvested after 15 days and separated to different parts i.e. roots, stems and leaves. The soil of both pots was combusted and extracted using methanol as solvent and then noted the radioactivity by liquid scintillation counting. The data of maize plant (root, stem and leaves) and soil before and after harvesting the plant are given in Table1 and 3.

$^{14}\text{C}$ -labelled malathion ( $0.4\mu\text{Ci}$ ) was applied to soil and maize and wheat seeds were grown in the treated soil. The applied labeled pesticide was systemic in nature and believed that it may distribute and translocate to all parts of plants with in short interval of time.

### **Radioactivity in maize plant and Soil**

Table 1 summarizes the total DPM and activity found in different parts of plant. A major portion of the total activity was restricted in leaves of maize (34%) of the applied activity but least was found in stem part (20%). Our results are in accordance with the earlier reported investigations.

Root of maize plant was combusted using Biological Oxidizer and the  $^{14}\text{CO}_2$  was trapped in trapping solution and mixed with Scintillator. The activity was determined using scintillation counter and found reasonable activity in this part of plant (29.75%). From the data it is evident that the activity translocated to every part of plant with in 15 days. Our results were agreed with (Harhash *et al.*, 2007). They conducted the fate of labeled fungicide in bean plants with high application rate (2mCi/g).

The soil of maize treated plant was assayed for radioactivity before and after the plant harvest. From the data it is clear that some portion of the labeled pesticide remained in soil after 15 days. It is evident that labeled compound showed strong interactions with soil fractions like humus. For mass balance the soil was combusted and extracted in methanol. After careful observations, it was found that substantial portion of the activity was retained in soil (31.72%). The data are shown in Table 1 Our results are in agreement with (Zhang *et al.*, 1989). The data of graphical presentation and activity in maize parts and soil is given in Table 2 and illustrated in graph 1 respectively.



**Table 1 Assay of radioactivity of maize by liquid scintillation counter**

Sr . #	Sample of Maize	CPM			Average CPM	DPM	95% Efficiency	Total DPM	Activity ( $\mu$ Ci)
		1	2	3					
1.	<b>Root</b>	37	38	36	374.333	456.851	529.6816	264840.799	0.11929
		5	6	2	3	3		9	8
2.	<b>Stem</b>	24	25	26	251.666	307.144	356.1083	178054.144	0.08020
		0	5	0	7			2	5
3.	<b>Leaves</b>	46	48	41	455.333	555.707	644.2966	322148.292	0.14511
		5	6	5	3			7	2
4.	<b>Soil before plantation</b>	98	97	96	972.333	1186.67	1375.851	687925.746	0.30987
		0	2	5	3	4		6	6
5.	<b>Soil after plantation</b>	36	41	42	398.333	486.141	563.6416	281820.797	0.12694
		5	0	0	3	9		8	6

**Table 2 Fate of C<sup>14</sup> radioactivity in maize and soil**

<i>Sr. #</i>	<b>Sample Name</b>	<b>Activity (μ Ci)</b>
1.	Root	0.12
2.	Stem	0.08
3.	Leaves	0.14
4.	Soil before plantation	0.31
5.	Soil after plantation	0.12

**Table 3 Assay of radioactivity of wheat by liquid scintillation counter**

<i>Sr. #</i>	Sample of Wheat	CPM			Average CPM	DPM	95% Efficiency	Total DPM	Activity (μ Ci)
		1	2	3					
1.	Root	485	490	488	487.6667	595.1678	690.0482	345024.1232	0.155416
2.	Stem	245	235	260	246.6667	301.0418	349.0333	174516.6447	0.078611

3.	Leaves	336	320	328	328	400.304 3	464.119 9	232059. 9707	0.10453 2
4.	Soil before plantati on	976	968	982	975.333 3	1190.33 6	1380.09 6	690048. 2463	0.31083 3
5.	Soil after plantati on	450	415	425	430	524.789 1	608.449 9	304224. 9617	0.13703 8

**Table 4 Fate of C<sup>14</sup> radioactivity in wheat and soil**

<b>Sr.#</b>	<b>Sample Name</b>	<b>Activity (<math>\mu</math> Ci)</b>
1.	Root	0.15
2.	Stem	0.07
3.	Leaves	0.10
4.	Soil before plantation	0.31
5.	Soil after plantation	0.13

The data of residue present in wheat plant (root, stem and leaves) and soil before and after harvesting the plant is given in Table 3, 4.

### **Radioactivity in wheat plant and Soil**

Table summarizes the total DPM and <sup>14</sup>C-activity found in different parts of plant. A major portion of the total activity was restricted in leaves of wheat (26.13%) of the applied activity but least was found in stem part (19.65%). Our results are in accordance with the

earlier reported investigations (Ahad *et al.*, 2000). Root of wheat plant was combusted using Biological Oxidizer and the  $^{14}\text{CO}_2$  was trapped in trapping solution and mixed with scintillator. The activity was determined using scintillation counter and found reasonable activity in this part of plant (38.85%). From the data it is evident that the activity was translocated to every part of plant with in 15 days. The results of (Agarwal and Rao 1998; Kiml *et al* 2004) also supported to our results. They concluded the fate of labeled fungicide in brinjal plants with high application rate (2.5mCi/g).

The soil of wheat treated plant was assayed for radioactivity before and after plant harvesting. From the data (Table 3 and 4) it is evident that some portion of the labeled pesticide remained in soil after 15 days. It is true that labeled compound showed strong interactions with soil fractions like humus. For mass balance, the soil was combusted and extracted in methanol. After careful observations, it was found that substantial portion of the activity was retained in soil (34.25%). The data are shown in Table 3. Our results are in agreement with Zhang *et al.*, 1989. The data of graphical presentation and activity in wheat parts and soil is given in Table 4 and illustrated in Graph 2 respectively.

## **Conclusions**

From the results it is evident that maize showed more absorption of labelled pesticide as compared to wheat. It may be due to its greater fat content and high transpiration rate. The labeled pesticide got conjugation with fatty acid molecule and persistent longer time that why the residue of pesticide is higher as compared to wheat crop. From the result it is recommended that use of pesticide on this crop may be minimized.

$\text{CO}_2$  is essential for photosynthesis because plants prepare Carbohydrates through its fixation. This study also shows that roots absorb the same amount of  $\text{CO}_2$  which is due to the application of  $\text{C}^{14}$  as an indicator. Absence of  $\text{CO}_2$  in the stem indicates that the



glucose in the roots splits into CO<sub>2</sub> and H<sub>2</sub>O. Some of CO<sub>2</sub> was absorbed by the roots and the remaining was up taken by the leaves.

This absorption and distribution may create health hazards for human beings and animals through the food chain, vegetables and fruits. For that purpose, survey was conducted and it was found that for the time being, there is no danger for human beings but it has mild symptoms in animals (Ahmad and Iram, 2005; Ahmad and Bhutta, 2005; Appleton, 1964; Rabidean, G.S. and L.W. Mericle. 1980; Scheunert, 1990; Schreiber, L. and J. Schonberr. 1992; Cohen, Y. and U. Gisi. 1993; ).

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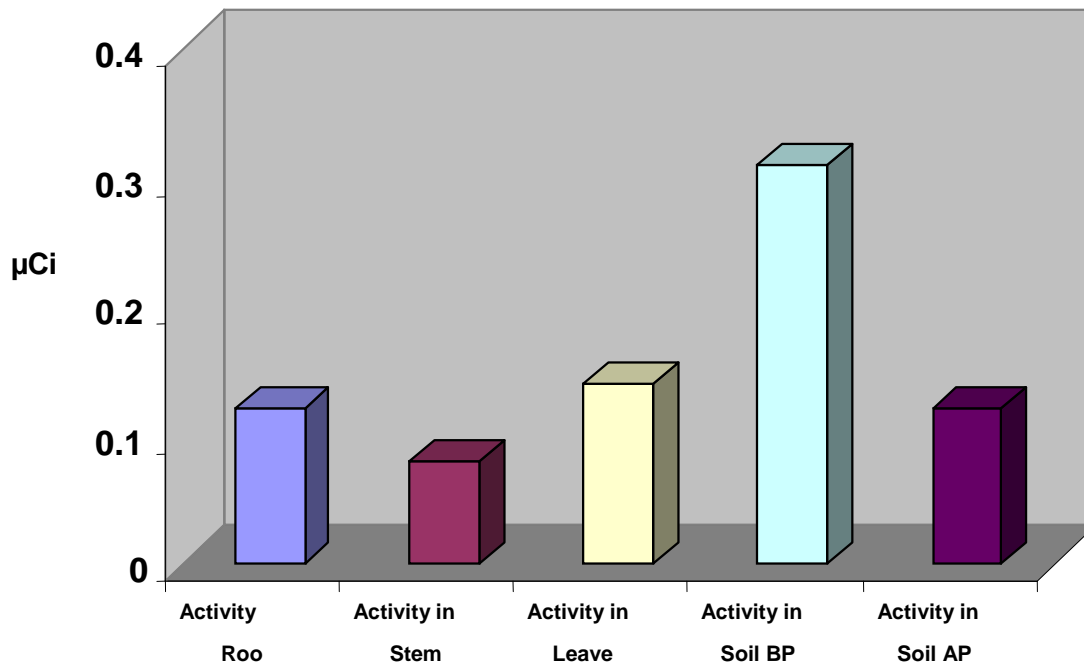
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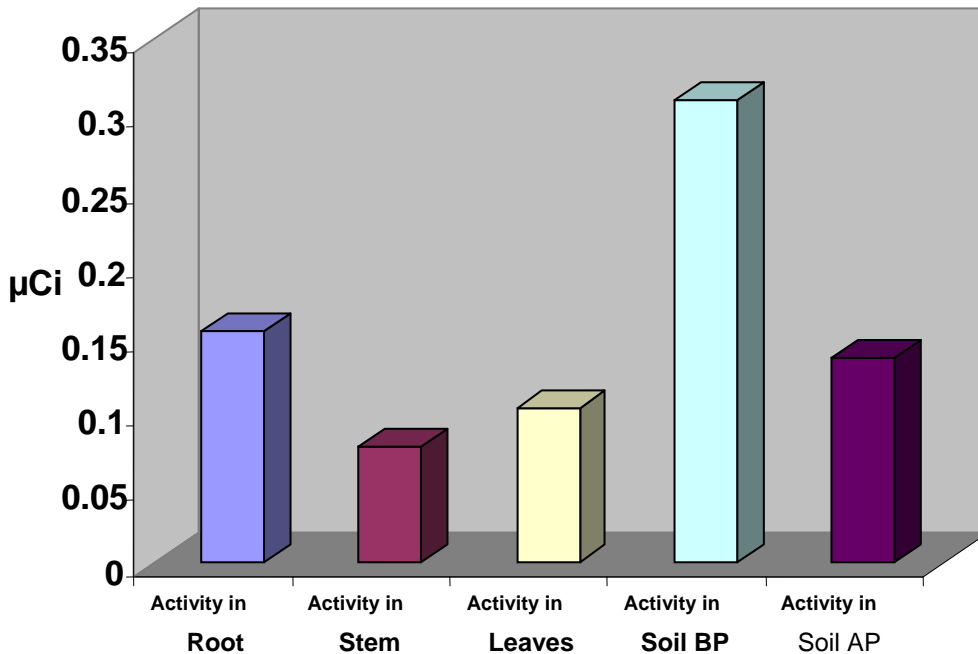
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**Fate of Radioactivity in Maize and Soil**



**Graph. 1 Fate of Radioactivity in Maize and Soil**

### Fate of Radioactivity in Wheat and Soil



**Graph. 2 Fate of Radioactivity in Wheat and Soil**

**AUTHOR,S PROFILE:**

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