

³²P radionuclidic Labeling in the Study of Agrochemical Efficiency of Some Extra-radicular Fertilizers Based on Proteins and Amino Acids

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This paper presents a study on the possible use of ³²P radionuclidic labeling of some unconventional fertilizers based on proteins and amino acids, in order to determine their agrochemical and ecological efficiency. Optimal conditions for ³²P radionuclidic labeling, foliar treatment procedures of the plants and methods for biological material conditioning were established for four types of extra-radicular fertilizers. The quantities of phosphorous translocated from the leaves (the application place) to different plant organs (other leaves, stems and flowers) were determined by adequate radiochemical analytical methods. We found a significant translocation of ³²P from the leaves it was applied on, to the other plant organs. The ³²P translocated depends on the labeled fertilizer's nature and, for a given fertilizer the foliar application efficiency is influenced by some factors like the pH of the fertilizer solution, relative humidity, leaves age etc.

Key words: radionuclidic labeling, extra-radicular fertilizers, agrochemical efficiency, radiochemical analytical methods

Applying of nutritive elements as diluted solution by foliar spraying became a usual practice for many crops; nutrients absorption and translocation from foliar surface to different plant organs being an unquestionable reality [1-3]. Additional, foliar stimulating fertilizing becomes interesting from practical point of view due to economical factors (high costs of soil fertilization) and high requirements on massive chemical intervention in the environment, justified by the danger of chemical contamination with some fertilizer components. High economic efficiency of foliar fertilization also comes from even three times larger crops than in case of soil fertilization. On the other way, foliar fertilization can be considered a useful method of environment protection against chemical pollution [4, 5].

Nutrient absorption mechanisms through leaves are not very different than radicular ones. In the leaves take place nutritive elements absorption and translocation processes; element translocation being considered as a chain of re-absorption processes from one cell to another.

The chemical elements are not absorbed as atoms and molecules but mainly as ions. Only as an exception, some substances like natural or synthetic chelates are absorbed into the plant as undissociated molecules [6, 7].

The main way for the ions and other compounds to enter the plant is the diffusion from the leaf surface into the plant. Urea passes through the cuticular membrane ten times faster than other substances or ions facilitating the penetration of other ions so it can be considered as a permeability promoter [4, 8].

The isotopic labeling, mainly the radionuclidic one, highlights the labeled elements translocation from the entering place to the growing peaks, roots, stems seeds and fruits [1, 2, 9-11]. This radiochemical investigation method led to several important conclusions:

- the absorption and transport of nutritive elements applied as diluted solutions on plant cuticles are of metabolic nature;

- cations penetrate faster than the anions the cuticle, the epidermis and the layers beneath;

- nutrient permeability is higher on the upper side of the leaf than on the inferior side;

- chelation considerably increases the penetration and translocation processes for a lot of ions with low mobility in plants (Ca; Fe; Mn; Zn; Cu, etc.).

Foliar nutrients application depends of some factors like:

- the pH value of the applied solution;

- antagonist phenomena between nutritive elements;

- the characteristics of surfactants and additives playing a role in the adhesion of foliar fertilizers diluted solutions applied on leaves surface;

- leaves age (young leaves absorb faster the nutritive elements than the older ones);

- air relative humidity;

- chelation agents effects.

Radionuclidic labeling allows a quantitative evaluation of nutrient penetration and translocation in plants by measuring the so called specific absorption. This term is defined as the nutritive element quantity absorbed on the foliar surface unit in a given period of time, in normal growth conditions of light, temperature and oxygen concentration.

Experimental part

We selected the ³²P radionuclide for our fertilization study because of the wide use of the element phosphorous in the agriculture and its convenient radionuclidic features: pure beta emitter with the maximum β^- radiation energy $E_{\max} = 1.7$ MeV and a half life of 14.3 days.

The radioactivity of each sample was measured with a gas flow proportional counter, connected to an alpha/beta portable monitor (MAP-2, IFIN-HH). The ³²P radioisotope used for the labeling of fertilizers was purchased from , Hungary, as ³²P-orthophosphate in diluted hydrochloric acid solution (pH 2-3), carrier free, with radioactive concentration of 370 MBq/mL. The activity values for all

Table 1
COMPOSITION AND PH VALUES OF THE FERTILIZER SOLUTIONS

Fertilizer solution nr.	Composition	pH value
1	PK + Micro + H*	6.70
2	N-(NH ₄) ₂ SO ₄ + PK + Micro + H*	7.27
3	N-NH ₄ NO ₃ + PK + Micro + H*	7.62
4	N-(NH ₂) ₂ CO + PK + Micro + H*	7.60

samples were registered in counts/s, being converted later into Bq using a conversion factor of 3.714 Bq/imp. This conversion factor was calculated by comparative measuring of samples from the ³²P labeled fertilizer solutions with the portable alpha/beta monitor and by liquid scintillator method [12-14]. In order to allow the comparison between the results of radioactivity measurements performed at different moments in time, all the values obtained were calculated for a reference time - the samples prelevation moment (the plant harvesting moment). This correlation was necessary taking into account the relatively short half time of ³²P (14.3 days).

During the experiment, we used four different fertilizers, having the composition and pH values presented in table 1. These fertilizer solutions had 0.456 g/L P₂O₅ average content, corresponding to 4.56 x 10⁻⁴ g/mL P₂O₅ average concentration.

For radionuclidal labeling of the fertilizer solutions we added 171 μL ³²P-orthophosphate in diluted hydrochloric

acid solution (pH 2-3), carrier free to 50 mL of each fertilizer solution. The activity of each vial containing ³²P labeled fertilizer solution was 1.25 MBq. The labeling was performed under natural condition of temperature and humidity. The average radioactive concentration of the ³²P labeled fertilizer solutions was 25053 kBq/mL. We used four pots with two sun flower plants each, applying one ³²P labeled fertilizer solution on both plants of every pot. We applied 250 mL labeled fertilizer solution on three leaves of every plant for three consecutive days. So each plant received 56329 kBq (on all three treated leaves).

After three days from the last application, we harvested samples from each plant as follows:

- the three leaves used for fertilizer solution application;
- the three pair leaves of the treated ones (placed opposite to the treated leaves);
- the stem (10 cm of the stem, places immediately beneath the flower);
- the flower.

The denominations for each sample are presented in table 2. The mass of every sample was determined by weighing with an analytical balance (after adequate breaking up and homogenizing), the radioactivity of each sample was measured and the specific activity was calculated. The specific activity of the samples is presented in table 3.

Results and discussions

In order to ease the interpretation of radioactivity measurements for the investigated biological samples, we adopted the denomination of the samples as introduced in table 2.

Pot no.	Plant no.	³² P labeled fertilizer solution	Denomination for the leaves treated with ³² P fertilizer solution	Denomination for the pair leaves to the treated ones	Stem samples denomination	Flower sample denomination
Pot 1	Plant 1 A	Fertilizer 1	1AF1-P	1AF1	1AT	1AI
			1AF2-P	1AF2		
			1AF3-P	1AF3		
	Plant 1 B	Fertilizer 1	1BF1-P	1BF1	1BT	1BI
			1BF2-P	1BF2		
			1BF3-P	1BF3		
Pot 2	Plant 2 A	Fertilizer 2	2AF1-P	2AF1	2AT	2AI
			2AF2-P	2AF2		
			2AF3-P	2AF3		
	Plant 2 B	Fertilizer 2	2BF1-P	2BF1	2BT	2BI
			2BF1-P	2BF1		
			2BF1-P	2BF1		
Pot 3	Plant 3 A	Fertilizer 3	3AF1-P	3AF1	3AT	3AI
			3AF2-P	3AF2		
			3AF3-P	3AF3		
	Plant 3 B	Fertilizer 3	3BF1-P	3BF1	3BT	3BI
			3BF2-P	3BF2		
			3BF3-P	3BF3		
Pot 4	Plant 4 A	Fertilizer 4	4AF1-P	4AF1	4AT	4AI
			4AF2-P	4AF2		
			4AF3-P	4AF3		
	Plant 4 B	Fertilizer 4	4BF1-P	4BF1	4BT	4BI
			4BF2-P	4BF2		
			4BF3-P	4BF3		

Table 2
DENOMINATION FOR THE BIOLOGICAL SAMPLES

Using the samples denomination presented in table 2, the results of radioactivity measurements are presented in table 3, specifying the mass (g), the activity (Bq) and the calculated specific activity (as radioactivity to mass ratio) of each biological sample.

Sample name	Mass (g)	Activity (Bq)	Specific activity (Bq/g)
1AF1-P	1.1391	12625.25	11083.53
1AF2-P	1.2073	21705.57	17978.61
1AF3-P	0.9113	20302.77	22278.91
1AF1	1.0773	17.65	16.38
1AF2	1.1590	16.12	13.91
1AF3	0.7910	21.49	27.17
1AI	3.2989	68.99	20.91
1AT	2.0190	154.76	76.65
Total (plant 1A)	11.6029	54912.60	
1BF1-P	0.9464	13530.44	14296.75
1BF2-P	0.9740	16956.89	17409.54
1BF3-P	0.8412	16587.23	19718.53
1BF1	0.9341	18.19	19.47
1BF2	1.1620	24.79	21.33
1BF3	0.8899	16.62	18.68
1BI	2.8635	249	86.96
1BT	1.9595	156.28	79.76
Total (plant 1B)	10.5706	47539.44	
2AF1-P	1.3797	15810	11459.01
2AF2-P	1.1529	16648.84	14440.84
2AF3-P	0.8731	11810.11	13526.64
2AF1	1.6183	15.62	9.65
2AF2	1.5637	18.72	11.97
2AF3	0.7567	22.10	29.21
2AI	4.1410	726.55	175.45
2AT	1.8507	326.34	176.33
Total (plant 2A)	13.3361	45378.28	
2BF1-P	1.9571	17449.76	8916.13
2BF1-P	1.5342	23264.77	15164.11
2BF1-P	1.3978	17027.97	12181.98
2BF1	1.9960	16.15	8.09
2BF1	2.0555	17.54	8.53
2BF1	1.2057	17.83	14.79
2BI	6.1313	565.31	92.20
2BT	2.3475	415.55	177.02
Total (plant 2B)	18.6251	58774.88	
3AF1-P	1.0407	15241.29	14645.23
3AF2-P	1.1237	18577.70	16532.62
3AF3-P	0.8238	19748.28	23972.18
3AF1	1.1729	15.07	12.85
3AF2	1.2625	15.44	12.23
3AF3	0.8832	23.51	26.62
3AI	3.7661	118.96	31.59
3AT	1.5163	108.49	71.55
Total (plant 3A)	11.5892	53848.74	
3BF1-P	1.2661	16082	12702
3BF2-P	1.1460	18336	16000
3BF3-P	0.7968	14246.06	17879.09
3BF1	1.1498	16.82	14.62
3BF2	0.9073	14.87	16.39
3BF3	0.4884	15.70	32.15
3BI	4.5423	280.86	61.83
3BT	1.8934	245.72	129.78
Total (plant 3B)	12.1901	49238.03	
4AF1-P	3.1632	21696.09	6858.91
4AF2-P	1.9844	20023.15	10090.28
4AF3-P	1.1205	21966.23	19603.95
4AF1	2.7765	15.02	5.41
4AF2	2.3318	15.05	6.45
4AF3	1.3716	14.42	10.51
4AI	10.0638	235.88	23.44
4AT	2.5832	167.33	64.78
Total (plant 4A)	25.395	64228.93	
4BF1-P	4.1515	17113.28	4122.19
4BF2-P	3.0040	19734.06	6569.26
4BF3-P	1.9098	20046.85	10496.83
4BF1	3.5699	17.72	4.96
4BF2	2.8877	17.79	6.16
4BF3	1.3257	17.20	12.97
4BI	12.4288	407.63	32.80
4BT	2.8934	265.28	91.68
Total (plant 4B)	32.1708	57619.81	

Table 3
MASSES, ACTIVITIES AND SPECIFIC
ACTIVITIES OF THE BIOLOGICAL SAMPLES

Table 4
TOTAL TRANSLOCATED SPECIFIC ACTIVITIES REPARTITION IN DIFFERENT PLANT ORGANS, TRANSLOCATED SPECIFIC ACTIVITY CORRECTED WITH THE MASS CORRECTION FACTOR

Pot no. / fertilizer no.	Translocation organ	Mass (g)	Translocated activity		Translocated specific activity (Bq/g)	Mass correction factor	Translocated specific activity (corrected value) (Bq/g)
			(Bq)	(%)			
1/1	Leaves	6.0133	114.9	15.5	19.1	1	19.1
	Flower	6.1624	318	42.7	51.6	1	51.6
	Stem	3.9785	311	41.8	78.2	1	78.2
	All plant	16.1542	743.9	100	46.1	1	46.1
2/2	Leaves	9.1959	108	5	11.7	1.47	17.3
	Flower	10.2723	1291.9	60.3	125.8	1.47	184.9
	Stem	4.1982	741.9	34.6	176.7	1.47	259.8
	All plant	23.6664	2141.7	100	90.5	1.47	133.0
3/3	Leaves	5.8641	101.4	11.9	17.29	1.09	18.9
	Flower	8.308	399.8	46.7	48.1	1.09	52.5
	Stem	3.4097	354.2	41.4	103.9	1.09	113.3
	All plant	17.5822	855.4	100	48.7	1.09	53
4/4	Leaves	14.2632	97.2	8.3	6.8	2.61	17.8
	Flower	22.4926	643.5	54.8	28.6	2.61	74.7
	Stem	5.4766	432.6	36.9	79	2.61	206.2
	All plant	42.2324	1173	100	27.8	2.61	72.5

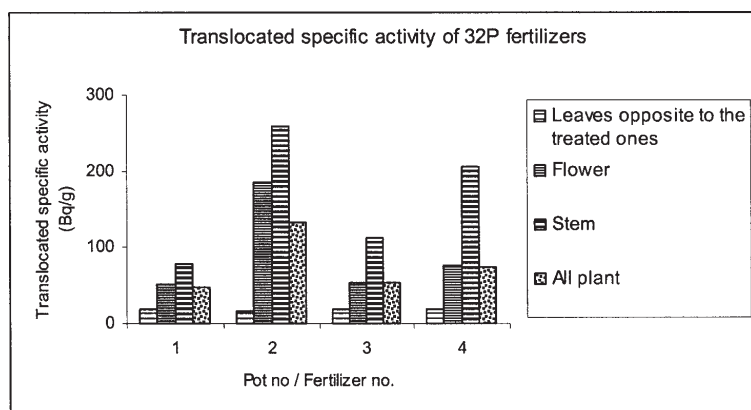


Fig. 1. Translocated specific activity of the ³²P labeled fertilizer solutions, corrected with the mass correction factor

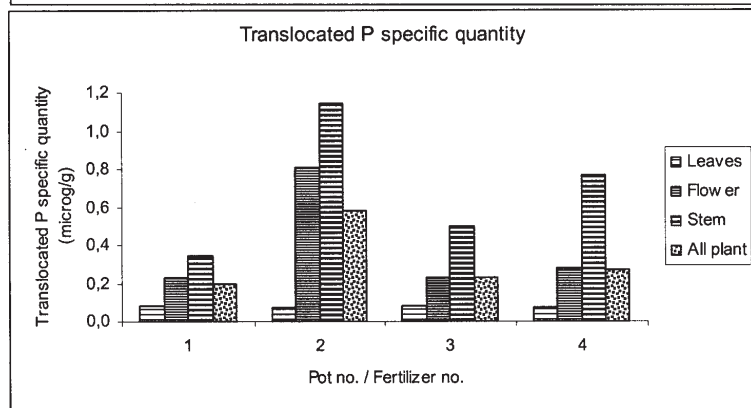


Fig. 2. Translocated phosphorous repartition in different plant organs for the four used fertilizer solutions

The plant mass, from 10 to 32 g, varies a lot between plants from different pots. Taking into account this mass variation and the fact that specific activity depends on the mass of the sample, in order to be able to compare the efficiency of the four fertilizer solutions, it is necessary to apply a "mass correction factor" to the calculated specific activity values. This factor was calculated dividing the total masses of the plant organs where the nutrient was translocated, with the total mass of the samples with the smallest mass (the total mass of the samples from pot no. 1)

In table 4, is presented the repartition between different plant organs of the total translocated specific activities, corrected with the mass correction factor.

The translocated specific activity of the ³²P labeled fertilizers, corrected with the mass correction factor is presented in the figure 1.

Every plant received a total quantity of 2.25 mL fertilizer solution, with 0.456 g/L P₂O₅ average content

corresponding to 45.6 x 10⁻⁵ g/mL P₂O₅, meaning 19.91 g/mL P. So the total phosphorous quantity applied on every plant was 447.98 µg P (³²P being carrier free, its mass can be neglected).

This way, we determined the translocated specific phosphorous quantity in different plant organs; this quantity is proportional to the correspondent specific activity and is presented in figure 2.

The results presented in table 4 allow the agrochemical efficiency estimation for every fertilizer. The agrochemical efficiency is the fertilizer capacity to be absorbed and translocated through the plant cuticles to different plant organs. From table 4, we deduced that the fertilizer solution no. 2 presents the higher agrochemical efficiency. For no. 2 fertilizer solution, the average specific activity translocated from the place of application to other plant organs was the higher (133 Bq/g), followed by fertilizer no. 4 (72.5 Bq/g), fertilizer no. 3 (53 Bq/g) and fertilizer no. 1 (46 Bq/g). The efficiency expressed by the specific quantity of phosphorous translocated from place of application to

different plant organs maintains the same decreasing order for the fertilizer solutions (fig. 2.). The higher value is registered for fertilizer no. 2 (0.58 $\mu\text{g/g}$), followed by no. 4 (0.27 $\mu\text{g/g}$), no. 3 (0.23 $\mu\text{g/g}$) and no. 1 (0.20 $\mu\text{g/g}$).

The specific quantity of phosphorous translocated into the flower ($\mu\text{g/g}$) decreases in the following fertilizers order: no. 2 >> no. 4 > no. 3 ~ no. 1. The data presented in table 4, also show that, for all fertilizers, 40% -60% from the activity is translocated into the flower, followed by the translocation into the stem, and the lower quantity, 5 - 15 %, being translocated into the opposite leaves to the treated ones. Considering the specific activity corrected with the mass correction factor, the highest value was translocated into the stem.

Conclusions

The absorption and translocation processes were studied for four fertilizer solutions with different composition, applied as foliar treatments to sun flower plants. To track these processes we proceeded to ^{32}P radionuclidic labeling of the fertilizer solutions.

We noticed a significant translocation of ^{32}P , hence of the labeled fertilizer solution, from the treated leaves to the other plant organs (other leaves stem and flower).

The decreasing order of agrochemical efficiency of the four fertilizer solutions, expressed as the average specific activity value translocated from the applying place to the other plant organs, was established. The fertilizers efficiency expressed as the specific quantity phosphorous translocated together with the fertilizer or as specific quantity of phosphorous translocated into the flowers maintains the same decreasing order.

We also established that, for all four fertilizer solutions, 40 - 60 % of the phosphorous contained in absorbed fertilizer was translocated into the flowers and only 5 - 15 % in the leaves, other than the treated ones.

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References

1. ALAM, S.M., ANSARI, R., ATHAR KHAN, M., On line Journal of Biological Sciences, 1(3), 2001, p.82
2. HOSSAIN, M.B., RYU, K.S., PARK, J.S., KIM, G.T., The Journal of Agricultural Science, 40(1-2), 2007
3. DONG, S., CHENG, L., SCAGEL, C.F., FUCHIGAMI, L.H., Three Physiol., 22, 2002, p.1305
4. SOARE, M., BORLAN, Z., GAVRILUTA, I., ABRUDAN, A., BUDOI, G., Date privind mecanismul de actiune a ingrasamintelor complexe foliare de protectie a mediului si productiei vegetale, Seria AGRONOMIE, Universitatea Agronomica si de Medicina Veterinara, Iasi, Vol. 41, 1998
5. SOARE, M., BORLAN, Z., GAVRILUTA, I., BUDOI, G., SUTEU, G., Environmentally protective effect of foliar fertilization as disclosed and quantified by means of ^{32}P , CIEC, Ed. AGRIS, 2001
6. SOARE, M., STEFANESCU, D., BORLAN, Z., Analele ICPA, vol. LII, 1993, p.217
7. TOTTEN, W., MCCARTY, B., Foliar Feeding, Prism Business Media Inc. Dept. of Horticulture, Clemson University, Clemson, South Carolina, USA, 2004
8. SOARE, M., BORLAN, Z., STEFANESCU, D., BUDOI, G., GAVRILUTA, I., Absorption of mineral nutrients (N, P, K) in sunflower plants from complex foliar fertilizers (CFF) depending on the nitrogen chemical sources, N concentration and pH of the CFF diluted solutions, XXX th Annual Meeting of ESNA, Ed. Martin H. Gerzabek, 2000, p:152
9. *** Use of isotope and radiation methods in soil and water management and crop nutrition"; Training course, series nr.14, IAEA, 2001
10. CHAMEL, A., Foliar uptake of chemicals studied with whole plants and cuticles, in Plant Growth and Leaf Applied Chemicals, Newman, P.M., Ed., Crc. Press. Raton, 1988, p.27
11. NEDELCU, M., PODINA, C., CUPSA, D., OPREA, I.A., TEUSDEA, A., BURCA, I., Impact of Hazardous substances in/on soil, XXXVIIth, Annual Meeting of ESNA, 10-14, sept.2007, Dubna, Book of Abstracts, p.91
12. KESSLER, M. J., Liquid Scintillation Analysis, Publication 169-3052, Packard Instrument Co, 1989
13. GIBBS, J., EVERETT, L., MOORE, D., Sample Preparation for Liquid Scintillation Counting, Packard Instrument Co., Downers Grove, Illinois, SUA, 1978, p. 65
14. PENG, T., Sample Preparation in Liquid Scintillation Counting, Amersham Corporation, Arlington Heights, Illinois, SUA, 1977, p.48

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