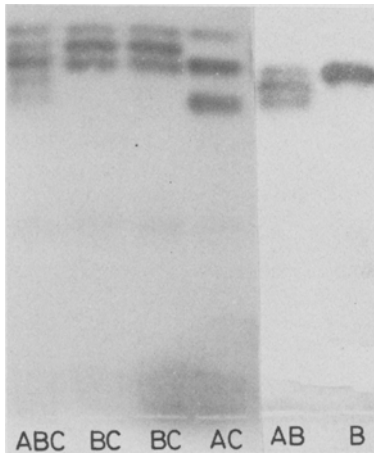


the other hand, seeds produced by crossing soybean plants differing in IDH-phenotype (e.g. AB×BC or AB×AC) have an IDH-phenotype with 5 bands (IDH-ABC, figure). Furthermore, preliminary data for a parental cross of AB×AC yield approximately $\frac{1}{4}$ AB: $\frac{1}{2}$ BC: $\frac{1}{4}$ AC in the F₂ progeny. These findings and the occurrence of a single



Isocitrate dehydrogenase electrophoretic patterns in the cultivated soybean *Glycine max*. Horizontal electrophoresis using 12% hydrolyzed starch in citrate-phosphate buffer pH 6.8 was carried out at 80 V and 4°C for 17 h. The agar-overlay technique was used for staining the enzyme.

IDH-band in seeds with IDH-B phenotype and 3 bands in seeds with IDH-AB, BC or AC phenotypes are consistent with the hypothesis of an *Idh* gene duplication. The present findings in 12 varieties of cultivated soybean cannot be explained in terms of multiple alleles of a single *Idh*-locus as, for example, in the case of *Pinus rigida*⁷. On the other hand, the results concur with those found for the spadefoot toads (*Scaphiopus* spp.) which exhibit gene duplication at the fastermoving *Idh*-locus⁸. Gene duplications have been reported in a number of organisms and are represented in plants by alcohol dehydrogenase and phosphoglucosomerase in diploid species of *Clarkia*^{9,10}. The present finding of isocitrate dehydrogenase gene duplication in the cultivated soybean could facilitate the study of the evolution of the genus *Glycine*. This gene-enzyme system also serves as a useful marker for assessing the extent of natural cross-pollination, for cultivar identification and for evaluating the homogeneity of soybean cultivars.

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Measuring cellulose decomposition using Benchkote-paper, for the estimation of soil pollution with copper¹

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Summary. Cellulose decomposition in paddy soil polluted by copper was determined by the use of polyethylene-backed absorbent paper (Benchkote) as a source of cellulose. It was shown that the paper is a suitable material for measuring the rate of cellulose decomposition. Correlations were found between copper content in the soil, the growth of rice plants and the cellulose decomposition rate.

A variety of soil pollutants influence microbial activities in farm land. These activities are usually assayed by chemical methods under laboratory conditions. However, the methods require much labor, and, moreover, the experimental results do not always correspond to the activity under field conditions. It is necessary to search for some convenient and useful methods for the estimation of microbial activity in the field. In order to assay cellulase activity in soil, calico strips, cotton wool and other materials have been used as a source of cellulose^{2,3}. These are in fact

useful for the experiments. However, there is the possibility of loss when they are removed from the soil. In this experiment, polyethylene-backed absorbent paper (Benchkote) made by Whatman Ltd was used as a source of cellulose, and the decomposition of the cellulose in paddy soil was determined, to estimate the inhibitory effect of soil pollutants on cellulase activity. **Materials and methods.** The experiments were carried out in a paddy field, under rice plant cultivation, located within easy reach of Matsue city. The field had been irrigated with

Table 1. Growth of rice plants and cellulose decomposition in paddy soil polluted by copper mine drainage

Point	Copper content in soil	Length of rice plants	Number of tillers	Remaining amount of cellulose	
				16 days	23 days
A	144.0*	48.4**	18.5**	54.9***	12.3
B	154.0	61.3	28.5	60.4	11.8
C	154.0	47.6	27.3	76.7	19.0
D	177.5	57.6	22.3	53.9	7.9
E	204.5	16.0	4.8	64.6	33.7
F	255.0	11.5	4.8	86.5	42.0
G	272.0	22.9	6.3	72.5	44.9

* µg/g dry soil. Copper was extracted with 0.1 N HCl; ** length (cm) and number: The average of 4 hills around the point; *** percent.

polluted water from Homan-zan, a closed copper mine. The paper was cut into strips of 4×20 cm. For easier manipulation they were fixed to plastic plates. Each set, consisting of 5 strips, was buried lengthwise, keeping 5 cm of the top above the land surface. They were placed at several points in the field. At the end of the incubation period, the strips were removed and soil particles were washed away by showering. After the top of each strip was cut off at the land surface, the residue was dried for 12 h at 70°C , and the remaining cellulose of each paper was weighed, together with the polyethylene back. The weight

Table 2. Correlation coefficients between copper content in soil, growth of rice plants and amount of cellulose remaining on paper in the polluted paddy soil

	Length of rice plants	Number of tillers	Remaining amount of cellulose	
			16 days	23 days
Copper content in soil	-0.836*	-0.816*	0.615	0.919**
Length of rice plants	-	-	-0.665	-0.935**
Number of tillers	-	-	-0.431	-0.872*

* Significant at the 5% level; ** significant at the 1% level.

of cellulose per unit size of the paper had been measured prior to the incubation.

Results and discussion. The burying of the strips in the soil, the measurement of the growth of the rice plants, and the collection of soil samples from the points measured were carried out on July 3, 1979. The copper content in soil collected from the various points ranged from a minimum value of $144.0 \mu\text{g/g}$ dry soil to a maximum value of $272.0 \mu\text{g/g}$ dry soil as shown in table 1. There was a close correlation between the copper content and the growth of rice plants, as shown in table 2. The strips of Benchkote were removed 16 and 23 days after the start of the incubation. As shown in table 1, cellulose decomposition was clearly inhibited in soil polluted excessively by copper. In particular, the amount of cellulose remaining on the strip removed after 23 days was closely related to the copper content of the soil, as shown in table 2. Judging from the observation that the remaining cellulose is easily removed without loss and the remaining amount is closely related to the soil pollution level, it seems that the polyethylene-backed paper is very suitable as a source of cellulose.

- 1 Acknowledgment. Gratitude is extend to Dr M.H. Martin, Department of Botany, University of Bristol, England, for suggesting the use of Benchkote paper.
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Modifying effects of divalent ions on the sulfhydryl content of normal and tumorous beet root tissue under thermal and γ -irradiation stress

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Summary. Temperature and γ -irradiation stresses result in the loss of DTNB (5-5' Dithionitrobenzoic acid) - reactive sulfhydryl groups in the TCA (trichloroacetic acid) - insoluble protein of normal and tumorous beet root tissue. Metal ions like Ca^{2+} , Zn^{2+} and Pb^{2+} have been found to partially prevent the change of -SH quantity under stress conditions. An attempt has been made to correlate the observed loss of DTNB - reactive sulfhydryl content with the loss of membrane permeability under thermal and irradiation conditions.

Zn^{2+} and Ca^{2+} are known to stabilize membranes under various physico-chemical stresses^{2,3}. Divalent metal ions like Ca^{2+} , Zn^{2+} , Mg^{2+} and Pb^{2+} have already been shown to inhibit the heat-induced efflux of betacyanin in beet roots by stabilising membranes to thermal stress⁴⁻⁶. Ca^{2+} also causes an inhibition of the UV- and γ -irradiated efflux of betacyanin^{6,7}. Thermal and radiation resistance in the presence of metal ions seems to be due to the interaction of these ions with protein or lipid components of both. Bresciani et al.⁸ have demonstrated that some lipid-requiring enzymes like membrane ATPase, possessing -SH groups, are inactivated by radiation and hence the observed change in permeability. Rothstein⁹ suggested earlier the existence of a relationship between cation transport and membrane-bound -SH groups in yeast cells. The relationship between γ -irradiation induced K^+ loss and decrease in -SH groups found by Rink¹⁰ also lent support to such a possibility.

Alterations in the tissue -SH content due to neoplastic growth in animals are well documented¹¹ but little information is available on the -SH content in plant tumors and even less on the quantity of -SH under thermal and radiation stress. The present work was carried out to see the

changes in the -SH content in both normal and tumorous beet root tissue under thermal and radiation stress, which was found to cause damage to the membrane permeability. The extent of damage was monitored in terms of the efflux of betacyanin⁶, Na- and K-ions, and 260 and 280 nm absorbing materials (unpublished data of this laboratory already communicated). The present experiments were designed to make a comparative study on the role of divalent metal ions under similar circumstances.

Sulfhydryl and protein content in normal tissue, tumorous tissue and normal tissue adjacent to a tumor

	SH (nM mg^{-1} protein)	SH content nM g^{-1} fresh weight of tissue	Protein mg protein g^{-1} weight of tissue
Normal tissue	15.0	18.26	1.217
Tumor	4.17	8.67	2.0869
Normal tissue (adjacent to tumor)	6.57	6.268	0.954

The values are the mean determinations of 4 values.