

## EFFECTS OF HERBICIDES, LUCERNE MEAL, AND ZINC ON MICROBIAL ACTIVITY AND AGGREGATE STABILITY OF SOILS

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**A b s t r a c t.** In laboratory experiments aggregates (1-2 mm) of a loamy colluvial soil and a silty luvisol soil were treated with the herbicides Gramoxone and Goltix WG, the active agent of Goltix WG Metamitron, lucerne meal and zinc. Lucerne meal clearly enhanced the aggregate stability of both soils. At dosages 10 and 50 times those of the recommended application rate also Goltix WG slightly enhanced the stability of aggregates 1 - 2 mm in both soils in the first experiment. There were no differences between Goltix WG and its active agent Metamitron. The stabilization of aggregates can be attributed to an increased metabolism of easily degradable organic substances. It was assumed that by this process stabilizing metabolic products were formed. The recalcitrant herbicide Gramoxone and zinc destabilized soil aggregates by reducing microbial activity at dosages 10 times the recommended application rate (Gramoxone) and 240 mg kg<sup>-1</sup> (Zn), respectively. Aggregate stability was more reduced in the colluvial soil showing lower aggregate stability than in the loamy colluvial soil.

**K e y w o r d s:** aggregate stability, microbial activity, herbicides, zinc, lucerne meal

### INTRODUCTION

Soil microorganisms are directly involved in the development of soil structure [1,5,10]. The two main processes in biological improvement of aggregate stability are the adhesive effects of bacterial metabolic products and the physical entanglement of soil particles with living bacteria and fungal hyphae. The first effect is mainly attributed to polysaccharides and other biopolymers. The effectiveness of polysaccharides in aggregating soil particles

should be classified as transient because the polysaccharides themselves may be used as a carbon source by other organisms unless they are physically - protected.

With respect to the erodibility of soils, aggregate stability is of major importance. In this paper, we investigated the influence of two herbicides, zinc and lucerne meal on soil aggregate stability and microbial activity in laboratory experiments. Herbicides are applied directly to the soil, and may reduce microbial activity as well as aggregate stability, and thus may enhance erodibility of soils after application. Lucerne meal and zinc were used in order to show whether easily degradable organic substances enhanced aggregate stability of soils and whether reductions of microbial activity resulted in the destabilisation of soil aggregates.

### MATERIALS AND METHODS

#### Soils

We used a silty luvisol soil (L) and a loamy colluvial soil (K) sampled from the surface layer (0-25 cm) to study the effects of different treatments on microbial activity and aggregate stability, respectively. Neither soil had been treated with the herbicide formulations in recent years. Table 1 contains the most important abiotic properties. Aggregates of 1-2 mm size were obtained by sieving the moist soils.

**Table 1.** Physical and chemical properties of soils (Ap-horizons)

Parameter	Clay (%)	Silt (%)	Sand (%)	pH (CaCl <sub>2</sub> )	C <sub>org</sub> (%)	N <sub>t</sub> (mg/g)	CEC (cmol/z/kg)
Silty luvisol soil (L)	12	80	9	7.7	0.94	1.94	12.1
Loamy colluvial soil (K)	19	29	52	6.7	1.5	1.29	15.2

They were stored in loosely tightened plastic bags at 4 °C if not used at once.

### Experiment 1

Aggregates (1-2 mm) were treated with the herbicide solutions Goltix WG (BAYER, 70 % Metamitron = 4-Amino-3-methyl-6-phenyl-1,2,4, triazin-5-on; CAS No. 41394-05-2) and Gramoxone (ICI, 10 % Paraquat = 1,1'-Dimethyl-4,4'-bipyridilium-dikation; CAS No. 468-14-7) as follows:

A thin layer of 300 g field moist aggregates was spread on a plastic tray and sprayed with the herbicide solutions containing the appropriate herbicide concentrations (e.g., Gramoxone 10x = 0.08 ml kg<sup>-1</sup> soil; Goltix WG 10x = 0.133 mg kg<sup>-1</sup> soil; Goltix WG 50x = 0.667 mg kg<sup>-1</sup> soil). The controls were sprayed with distilled water. In order to ensure a homogenous distribution of the herbicide solutions or the water the aggregates were turned during this procedure. The water content of the soils was to 11 % in the colluvial soil (K) and 21 % in the luvisol soil (L) after the treatment. This was equivalent to 40 % (K) and 50 % (L) of the maximum water holding capacity, respectively.

We used herbicide dosages 10 times (Goltix WG x 10, Metamitron x 10, Gramoxone x 10) and 50 times (Goltix WG x 50) higher than recommended. The doses were calculated on the assumption that the herbicides are distributed in the field throughout the upper 50 mm soil layer having a bulk density of 1.5 Mg m<sup>-3</sup>. 10 times higher concentrations may occur under field conditions if we assume a penetration only into the uppermost 5 mm soil layer immediately after the application of herbicides.

Basal respiration, dehydrogenase activity, and aggregate stability of the soils were measured at intervals during the 35 days incubation period.

### Experiment 2

In the second experiment, the soil aggregates were treated with Goltix WG and its active ingredient Metamitron. This was done in order to get information about the effect the formulation of this herbicide. Moreover, the effect of lucerne meal and zinc were tested. Zinc (240 mg kg<sup>-1</sup>) was added by spraying a ZnCl<sub>2</sub>-solution to the aggregates. Lucerne meal was added by gently mixing with the soil aggregates. The controls and the lucerne meal treated aggregates were sprayed with distilled water to adjust them to the same water content as the Metamitron, Goltix WG, and zinc treated samples.

Besides the basal respiration and dehydrogenase activity, we measured the Ninhydrin-N in fumigated and unfumigated samples in order to detect herbicide effects on microbial biomass. The applied dosages also amounted to 10 times those of the recommended application rates.

### Microbiological analyses

#### Basal respiration

Basal respiration was measured by trapping CO<sub>2</sub> in 0.1 M NaOH and measuring electrical conductivity as proposed by Nordgren [7].

#### Dehydrogenase activity (DHA)

DHA was determined with the electron acceptor 2,3,5-triphenyltetrazolium chloride (TTC; first experiment) and 2-p-Iodophenyl-3-p-nitrophenyl-5-phenyltetrazoliumchloride (INT; second experiment) according to the methods proposed by Thalmann [9] and Spothelfer-Magaña and Thalmann [8], respectively.

### *Microbial biomass*

Microbial biomass was estimated via determination of Ninhydrin-N using the Fumigation-extraction method [3].

### *Aggregate stability measurements*

Stability of aggregates 1-2 mm was determined by wet-sieving according to the method proposed by Murer *et al.* [6].

### *Statistical procedure*

All data were expressed as means of four replicates, and were treated statistically using the SPSS-statistics package by analysis of variance and the LSD test ( $P < 0.05$ ). Results of  $\text{CO}_2$ -measurements in the 2nd experiment were not treated statistically because only two replicates were measured.

## RESULTS

### **Experiment 1**

#### *Microbiological analyses*

Goltix WG increased the basal respiration in both soils significantly (Fig. 1a L & K). As expected the higher dosage (Goltix WG 50x) was more efficient than the lower (Goltix WG 10x). Gramoxone caused higher respiration rates only during the first day of the experiment in the luvisol soil (L). As a result of the depletion of the substrate, respiration declined in both soils and in all treatments during the experiment.

Dehydrogenase activity in the herbicide treated soils and the controls is shown in Fig. 1b. In the controls of the colluvial soil, the dehydrogenase activity declined from 143 to 105  $\mu\text{g TPF g}^{-1}$  dry weight soil probably because of the lack of substrate. Gramoxone reduced dehydrogenase activity of this soil significantly from the 1st to the 22nd day, but was ineffective in altering the activity of the luvisol soil throughout the experiment. Goltix WG 10x caused negative effects on the dehydrogenase activity of the luvisol soil on the 14th, 28th and 34th day and Goltix WG 50x was inhibitory on the 1st, 28th and 34th day (Fig. 1b).

Significant reductions were also obtained in the colluvial soil (K) after the addition of Goltix WG. The 10-fold dosage had less effect on the dehydrogenase activity than the 50-fold dosage, which reduced the activity until the end of the experiment.

### *Aggregate stability*

Aggregate stability measured by wet sieving was much greater in the luvisol soil than in the colluvial soil (Fig. 1c). It was enhanced by the addition of Goltix WG in the luvisol and the colluvial soil. The most distinct effects were obtained after addition of the 50-fold dosage to the luvisol soil. Gramoxone 10x significantly reduced the stability of aggregates 1-2 mm in the colluvial soil throughout the experiment (Fig. 1c L). No significant effects were obtained after addition of Gramoxone to the luvisol soil.

### **Experiment 2**

#### *Microbiological analyses*

Applications of lucerne meal and Goltix WG increased the basal respiration of both soils (Fig. 2a). In contrast, zinc reduced their respiration rates. The metal was more effective in the colluvial soil than in the luvisol soil.

The dehydrogenase activity of both soils was enhanced by amendments of lucerne meal while zinc reduced it (Fig. 2b). Goltix WG occasionally enhanced this process. This is difficult to explain especially since the biomass content did not increase in herbicide treated soils (Fig. 2c).

Ninhydrin-N which was used as a measure of microbial biomass (Fig. 2c). As expected, lucerne meal increased Ninhydrin-N whereas zinc reduced it. Goltix WG significantly reduced microbial biomass in the luvisol on the 1st day and in the colluvial soil during the first 14 days of the experiment.

Respiration rates, dehydrogenase activities, and Ninhydrin-N of Goltix WG and Metamitron (data not shown) treated variants did not differ significantly.

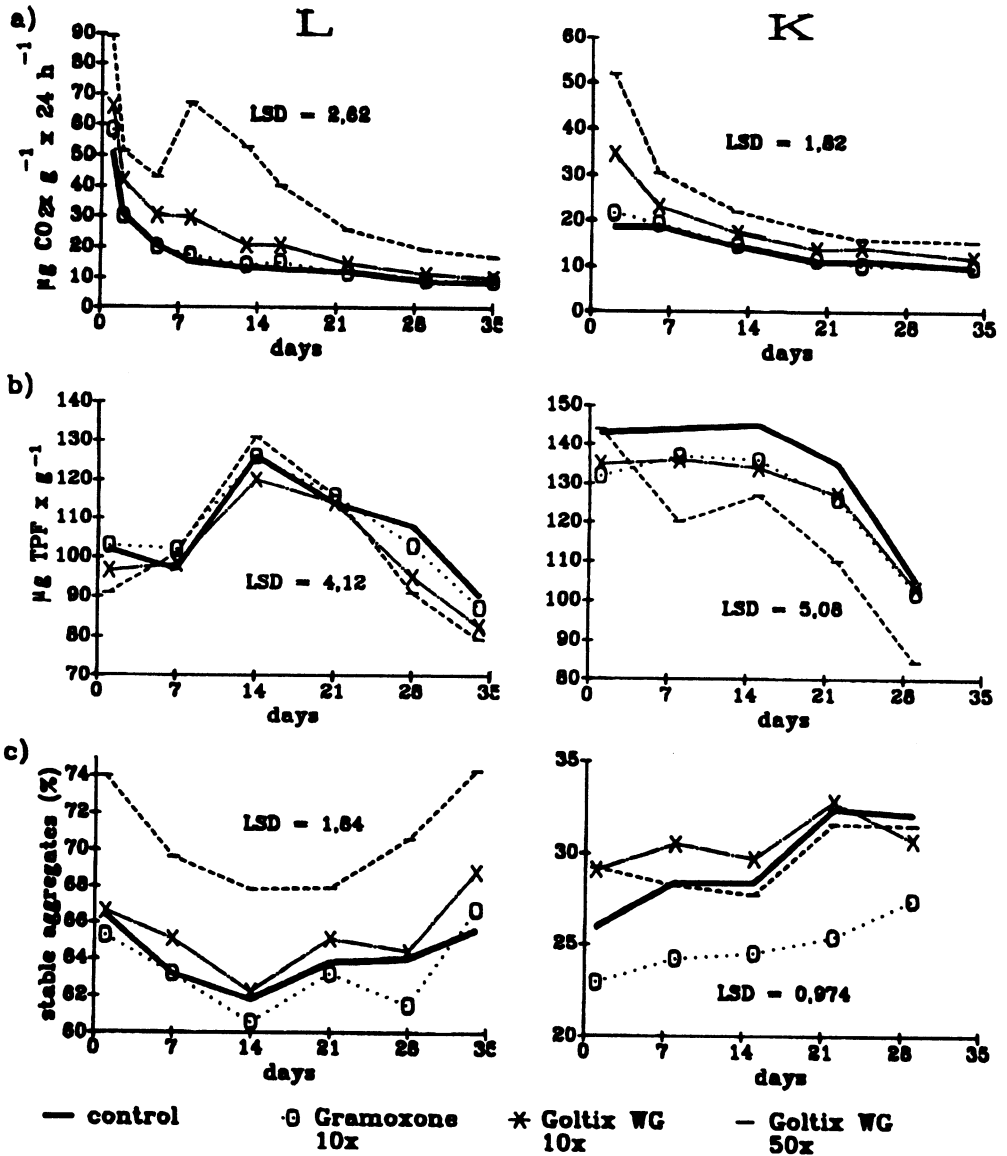


Fig. 1. Effects of Gramoxone and Goltix WG on respiration (a), dehydrogenase activity (b) and aggregate stability (c) of a silty luvisol soil (L) and a loamy colluvial soil (K). LSD - least significant difference.

*Aggregate stability*

The aggregate stability (1-2 mm) of the luvisol soil was lower than in the first experiment (compare Fig. 2d with Fig. 1c). This was probably due to different sampling dates. Samples for the first experiment were obtained in spring whereas samples for the second experiment were taken in autumn. Applications of Goltix WG significantly

increased aggregate stability of the luvisol only on the 14th day whereas lucerne meal additions increased it in both soils throughout the experiment (Fig. 2d). There were no significant differences between the effects of Goltix WG and its active agent Metamitron (data not shown). Zinc reduced the aggregate stability only of the colluvial soil.

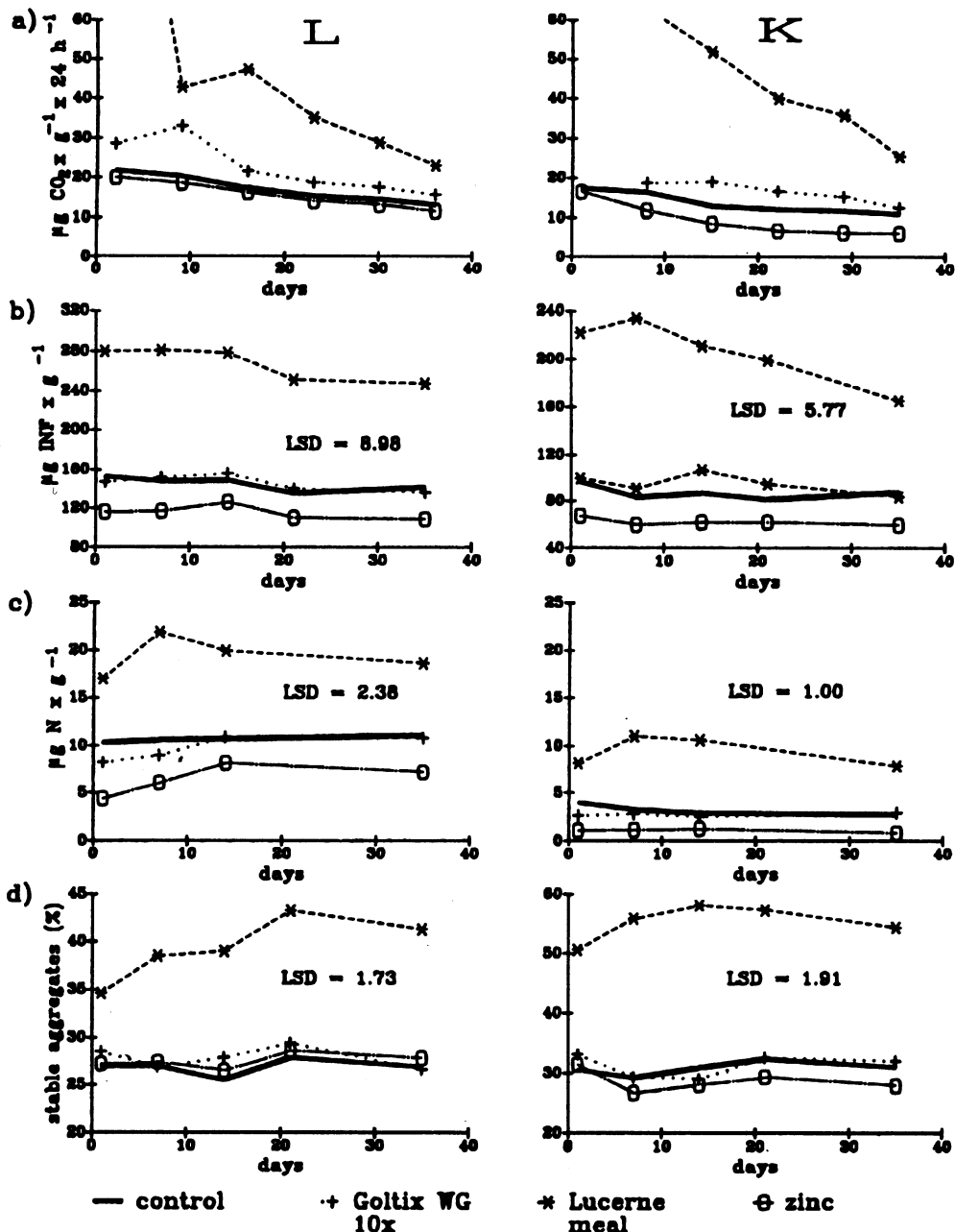


Fig. 2. Effects of Goltix WG, zinc and lucerne meal on respiration (a), dehydrogenase activity (b), ninhydrin-reactive nitrogen of microbial biomass (c), and aggregate stability of a silty luvisol soil (L) and a loamy colluvial soil (K). LSD - least significant difference.

DISCUSSION

Stimulated respiration during the first days of the 1st experiment in herbicide treated soils results from mineralization of freshly killed cells

by the surviving microflora. This effect has been shown in many experiments using different kinds of pollutants. Long-term enhancement of soil respiration cannot solely be

attributed to mineralization of dead microorganisms. It seems to be more likely that increased respiration rates were caused by biodegradation of the herbicide. This assumption is supported by many laboratory and field experiments which showed that Metamitron - the active ingredient of Goltix WG - is decomposed very rapidly with a half-life to approximately 10 days. In contrast to Goltix WG, Paraquat the active ingredient of Gramoxone is degraded very slowly mainly because of its adsorption by clay minerals [2].

The enhancement of aggregate stability by lucerne meal and Goltix WG can be attributed to an increased microbial decomposition of these substances. By this process, the production of stabilizing agents such as polysaccharides is assumed to be stimulated. It has been shown in many experiments that the addition of easily degradable organic materials enhances microbial activity as well as water stability of soil aggregates [4,10]. On the contrary recalcitrant herbicides like Gramoxone or heavy metals like zinc might reduce aggregate stability as a result of their detrimental effects on soil microorganisms. Destabilization of aggregates may be more pronounced in soils with relatively low aggregate stability. This assumption is supported by the fact that Gramoxone was more effective in reducing the amount of stable aggregates in the colluvial soil than in the luvisol soil in the first experiment. The greater effectiveness of zinc in reducing aggregate stability of the colluvial soil (second experiment) was probably due to the fact that the metal reduced the microbial activity of this soil to a larger degree than that of the luvisol soil.

All effects caused by Goltix WG can be mainly attributed to its active ingredient Metamitron because there were no significant differences between the effects of the two substances.

#### CONCLUSIONS

The experiments have shown that easily degradable organic compounds will enhance microbial activity as well as aggregate stability. In contrast, toxic substances will cause

contrary effects. The enhancement of aggregate stability is probably due to the formation of microbial biopolymers in the course of degradation of organic substances.

The effects caused by the two herbicides used in this investigation were negligible, although they were applied in high dosages. Nevertheless, it can be stated that the high additions of easily degradable herbicides like Goltix WG will increase microbial activity and subsequently also increase aggregate stability of soils for a short period.

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