Folia Microbiol. **26**, 24–28 (1981)

# Dynamics of Oxidation of Inorganic Sulphur Compounds in Upper Soil Horizons of Spruce Forests*

A. LETTL, O. LANGKRAMER and V. LOCHMAN

*Forestry and Game Management Institute, 255 01 Zbraslav-Strnady,*
*Czechoslovakia*

*Received May 20, 1979*

**ABSTRACT.** Dynamics of oxidation of inorganic sulphur compounds to sulphate by the soil of spruce forests was investigated. Sulphide, sulphite and thiosulphate are oxidized to sulphate at a maximal rate at the beginning of the reaction, oxidation of elemental sulphur exhibits a lag phase. Linear relationships between the amounts of the produced sulphate and concentrations of substrates in the soil could be detected. On the basis of this finding a method for comparison of the oxidative activity of various soils was proposed.

Most sulphur in the soil is bound in the form of organic compounds, from which it is only difficult to release in the form of incompletely oxidized inorganic compounds (Freney 1961). These compounds are unstable in the soil: according to actual conditions they are either oxidized all the way to sulphate, stable only under aerobic conditions, or can be reduced up to the sulphide ion, stable only under anaerobiosis (Starkey 1966). Thus, inorganic sulphur plays the most important role in the sulphur cycle.

In upper soil horizons of the spruce forests aerobic conditions predominate. The aerobic part of the sulphur cycle can be characterized as follows:

$$S^{2-} \rightarrow S^0 \rightarrow S_2O_3^{2-} \rightarrow SO_3^{2-} \rightarrow SO_4^{2-}$$ (Pochon and de Barjac 1958).

The biological nature of these processes is generally recognized, in spite of the fact that abiotic mechanisms might be partially involved. An important role was attributed to the genus *Thiobacillus* (Starkey 1966).

So far, the dynamics of sulphate production from individual compounds has been studied mainly in connection with fertilization of the soil by elemental sulphuur with no attempts at quantification. The oxidative activity of forest soils has not yet attracted pronounced attention.

It is the aim of the present communication to investigate the dynamics of the production of sulphate from inorganic compounds of the oxidative part of the sulphur cycle in the soil sample. Thus, a basis for a possible comparison of the oxidative activity in various soils should be obtained.

## MATERIALS AND METHODS

*Soils.* A sample of the humus horizon from the spruce forest in the neighborhood of Zbraslav, samples of the fermentative (F) and humus (H) horizon and horizon A

---

* 1st communication of the series Ecology of Thiobacilli.

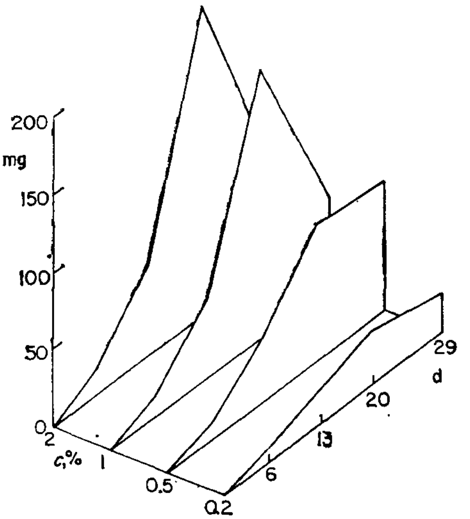

FIG. 1. Production of sulphate (mg) by oxidation of elemental sulphur (concentration c, %) in a 20 g sample of the soil from the H horizon.

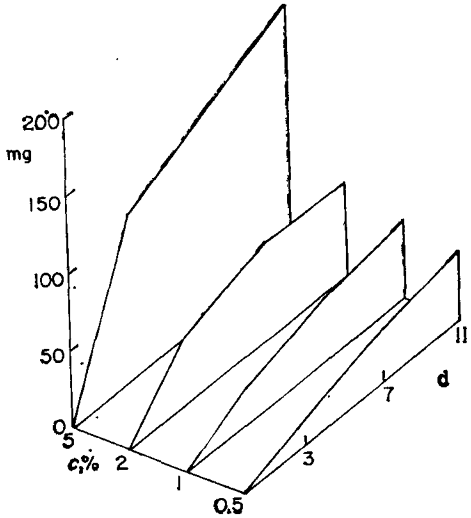

FIG. 2. Production of sulphate (mg) by oxidation of $Na_2S_2O_3.5H_2O$ (concentration c, %) in a 20 g sample of the soil from the H horizon.

from the spruce forest near the villages Šabina in the Sokolov region and Trstěnice near Mariánské Lázně. The soils were first separated through a sieve (mesh size 2 mm) and then used in the experiments.

The water content of the soils was determined from a mass decrease of 10 g fine soil after drying for 2 h at 105 °C (Klika *et al.* 1954).

The content of soluble sulphates in the soil was determined gravimetrically as $BaSO_4$ (Tomíček 1933) in combined extracts after extraction of 20 g of soil by three portions (200 + 100 + 100 mL) of 0.3 % HCl (Evans and Rost 1945).

Production of sulphonate was followed in 20 g soil samples placed in 500-mL bottles. The samples were supplemented with increasing amounts of sulphur compounds. Incubation proceeded at 25 °C, individual bottles were taken at suitable time intervals and the content of sulphate was determined in the soil samples. The difference between values determined before and after the incubation is taken as the amount of sulphate produced.

Compounds of the oxidative part of the sulphur cycle (except for polythionates that are difficult to obtain) served as substrates: sodium sulphide, elementary sulphur (pharmaceutical sulphur flowers), sodium sulphite and thiosulphate. These compounds (except for sulphur) were added in a 5 ml solution, sulphur was added as substance

## RESULTS

Two types of dynamics could be detected when investigating the course of oxidative reactions: oxidation of elemental sulphur exhibited a lag phase, the reaction started only after a longer incubation time (Fig. 1) and was induced by a pronounced proliferation of autotrophic oxidants of sulphur (thiobacilli), whereas the oxidation of sulphide, sulphite and thiosulphate (Fig. 2) proceeded at the highest rate at the very beginning and the production of sulphate gradually decelerated. In the first type of the reaction the sulphate content reached its maximum after a 21-d incubation,

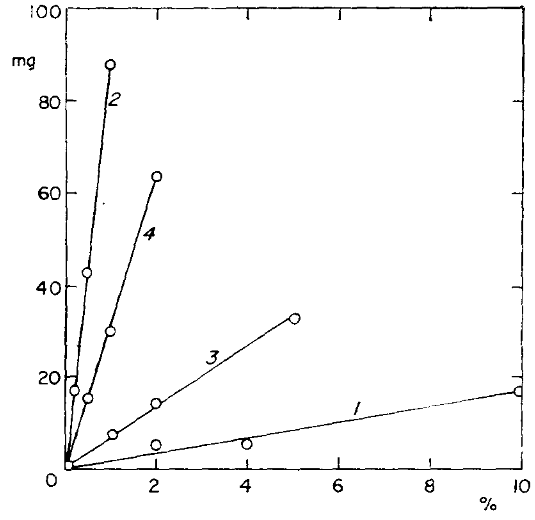

FIG. 3. Relationship between the amount of sulphate produced (mg) in a 20 g sample of the soil from the H horizon and concentration of substrate (%) in the soil: 1 $Na_2S.9H_2O$, 2 elemental sulphur, 3 $Na_2SO_3$, 4 $Na_2S_2O_3.5H_2O$.

in the second type the maximum was reached after 7 d. The amount of sulphate produced was always linearly proportional to substrate concentration in the soil, on the condition that the limiting concentration, above which the reaction is inhibited, was not exceeded (Fig. 3).

On the basis of these findings it was possible to take the ratio of 0.2 mol sulphur to 1 kg wet soil evaluation of the oxidative activity of spruce forest soils. Thus, for 20 g soil for portions 961 mg $Na_2S.9H_2O$ (*i.e.* 4.8 %), 128 mg elemental sulphur (0.64 %), 504 mg $Na_2SO_3$ (2.52 %) and 496 mg $Na_2S_2O_3.5H_2O$ (2.48 %) were used. The incubation time was 21 d and 7 d for the oxidation of elemental sulphur and other compounds, respectively. The amount of sulphate produced in 20 g wet soil was then recalculated per 1 kg of dry soil. The reliability of these reactions performed in parallel with a single soil sample is evaluated in Table I.

The usefulness of the proposed procedures was verified by comparing the activities of horizons F, H and A taken on July 13, 1977, in the neighborhood of Trstěnice and Šabina. The results are summarized in Table II.

TABLE I. Reliability of analytical methods

| Parameters | Determination | | Oxidation[b] | | | |
|---|---|---|---|---|---|---|
| | $SO_4^{2-}$ as $BaSO_4$[a] | $SO_4^{2-}$ in soil[b] | $S^{2-}$ | $S^0$ | $S_2O_3^{2-}$ | $SO_3^{2-}$ |
| Number of determinations | 10 | 5 | 10 | 10 | 10 | 10 |
| Aritmetic mean | 99.8 | 335 | 1 946 | 4 834 | 3 327 | 4 514 |
| Variation range | 1.5 | 44.3 | 1 094 | 710 | 393 | 1 376 |
| Standard deviation | 0.6 | 18.4 | 344 | 282 | 146 | 519 |
| Variation coefficient, % | 0.6 | 5.5 | 17.6 | 5.8 | 4.4 | 11.5 |

[a] mg $SO_4^{2-}$.
[b] mg $SO^{2-}$ per kg dry weight.

**Table II.** The oxidative activity of samples of the spruce forest soil[a] from the localities of Trstěnice and Šabina toward inorganic sulphur compounds

| Locality | Trstěnice | | | Šabina | | |
|---|---|---|---|---|---|---|
| Horizon | F | H | A | F | H | A |
| Water content, mass % | 29.0 | 48.8 | 16.6 | 20.9 | 50.4 | 15.9 |
| $SO_4^{2-}$ content in soil (mg $SO_4^{2-}$ per 1 kg dry soil) | 183.1 | 175.8 | 175.8 | 240.2 | 201.6 | 190.2 |
| Oxidation of | | | | | | |
| $S^{2-}$ | 1 176 | 2 900 | 827 | 1 479 | 2 429 | 476 |
| $S^0$ | 810 | 1 582 | 420 | 752 | 1 431 | 428 |
| $SO_3^{2-}$ | 422 | 957 | 0 | 278 | 363 | 220 |
| $S_2O_3^{2-}$ | 3 761 | 4 678 | 2 272 | 2 699 | 3 558 | 1 902 |

[a] mg $SO_4^{2-}$ produced by oxidation of the substrates, referred to kg dry soil.

## DISCUSSION

Water (Needham and Hauge 1952), a mixture of acetic acid and sodium acetate (Pramer and Schmidt 1965) and diluted HCl (Evans and Rost 1945) were proposed for the extraction of soluble sulphates from the soil. In spite of the fact that the results are identical, the last procedure is advantageous due to fastest filtration. According to our experience the above procedure can detect 95 % of soluble sulphate in the soil.

More modern methods used to determine sulphates utilize expensive or single-purpose apparatuses, or use reagents that can be obtained only with difficulty (for a review see Holz 1973). The rapid nephelometric method appeared to be attractive (Butters and Chenery 1959). However, it was found that absorbance of colloid $BaSO_4$ (irrespective of the linear relationship between absorbance and concentration) is highly influenced by composition of the solution. This basic insufficiency could not be removed in various modifications suggested (Massoumi and Cornfield 1963). Therefore, the gravimetric determination was chosen due to its reliability and simplicity (Tomíček 1933). However, non-physiologically high doses of substrates had to be used to be able to weigh the sulphate to be determined.

It is known that the dynamics of sulphate production depends on the number of active organisms in the soil: the reaction begins immediately when the number is sufficient, otherwise only after a certain lag phase (Gleen and Quastel 1953). This finding may partially explain the prolonged oxidation of the elemental sulphur. In spite of the fact that a concentration of thiobacilli of about $10^4$/g corresponding to very active soils (Swaby and Fedel 1973) could be detected in the studied soil, an incommensurate size of the cells and sulphur particles plays apparently a role here; probably only a small number of cells can come into contact with a sulphur particle and the reaction may proceed only after their sufficient multiplication within the reach of the particle.

The extent of oxidation of elemental sulphur was found to depend on its concentration (Swaby and Vitolins 1968; Barrow 1971). In the experiments referred to here pronounced linear relationships between the amount of sulphate produced and concentrations of all substrates used could be detected, in agreement with data of other authors (*e.g.* Nor and Tabatabai 1977). These linear relationships can be considered as a basis for the analytical methods, as the amount of the product can be expected to be proportional to the oxidative activity of the soil under otherwise constant conditions. On the basis of this assumption conditions for comparison of the oxidative activity of different soils were determined; in parallel experiments the method was found to be sufficiently reliable.

The proposed procedure was verified by comparing samples of horizons F, H and A from two different spruce forests related ecologically. This single-step comparison (which does not make it possible to characterize the locality) revealed pronounced differences between activities of individual horizons. Significant differences between the two localities could also be detected.

## REFERENCES

BARROW N.J.: Slowly available sulphur fertilizers in southwestern Australia. I. Elemental sulphur. *Austral. J.Exp.Agr.Anim.Husb.* **11**, 211 (1971).

BUTTERS B., CHENERY E.M.: A rapid method for the determination of total sulphur in soils and plants. *Analyst* **84**, 239 (1959).

EVANS C.A., ROST C.O.: Total organic sulphur and humus sulphur of Minnesota soils. *Soil Sci.* **52**, 125 (1945).

FRENEY J.R.: Some observations on the nature of organic sulphur compounds in soil. *Austral.J.Agric.Res.* **12**, 424 (1961).

GLEEN H., QUASTEL J.H.: Sulphur metabolism in soil. *Appl.Microbiol.* **1**, 70 (1953).

HOLZ F.: Automatische photometrische Simultanbestimmung von Sulfat und Nitrat in Trink-, Fluss, und Dränwasser. *Landw.Forschung* **28**, Sonderheft I, 189 (1973).

KLIKA J., NOVÁK V., GREGOR A.: *Methods in Phytocoenology, Ecology, and Soil Science.* (In Czech) Publ. House Czech Acad. Sci., Prague 1954.

MASSOUMI A., CORNFIELD A.H.: A rapid method for determining sulfur in water extracts of soils. *Analyst* **88**, 321 (1963).

NEEDHAM J.W., HAUGE S.M.: Effect of sulfur fertilization on the vitamin content of alfalfa. *Soil Sci.* **74**, 365 (1952).

NOR Y.M., TABATABAI M.A.: Oxidation of elemental sulfur in soils. *Soil Sci.Soc.Am.J.* **41**, 736 (1977).

POCHON J., DE BARJAC H.: *Traité de microbiologie des sols*, p. 266. Dunod, Paris 1958.

PRAMER D., SCHMIDT E.L.: *Experimental Soil Microbiology.* Burgess, Minneapolis 1965.

STARKEY R.L.: Oxidation and reduction of sulphur compounds in soils. *Soil Sci.* **101**, 297 (1966).

SWABY R.J., FEDEL R.: Microbial production of sulphate and sulphide in some Australian soils. *Soil Biol. Biochem.* **5**, 773 (1973).

SWABY R.J., VITOLINS M.I.: Sulphur oxidation in Australian soils. *Trans.Internat.Congr.Soil Sci., Adelaide* **4**, 673 (1968).

TOMÍČEK O.: *Principles of Quantitative Chemical Analysis.* (In Czech) Publ. House A. Krupička, Prague 1933.