Proceedings of the third international conference on the Global Impacts of Applied Microbiology (GIAM III), Bombay, 1969 (under press).

RHIZOSPHERICAL AND SPERMATOSPHERICAL SULFATE REDUCTION AND RHIZOSPHERICAL NITROGEN FIXATION IN SALINE SOILS

DOMMERGUES (Y.)[1], JACQ (V.)[2], BALANDREAU (J.)[1], COMBREMONT (R.)[3]

In large parts of North Africa, and especially in Tunisia, the use of brackish water in irrigation raises many problems (VAN HOORN, 1966), the study of which has been undertaken by the Tunisian Government with the assistance of the United Nations' Special Fund and U.N.E.S.C.O. (C.R.U.E.S.I.). Among the problems which have been dealt with, some are related to an abnormal activity of the rhizosphere microflora (dying out or slackening of the growth of certain plants) or of the spermatosphere microflora (dying out of germinating seeds).

A - IN SITU OBSERVATIONS

Three types of deleterious phenomena have been observed in situ in NAKTA - C.R.U.E.S.I. experimental station (central eastern Tunisia).

1. Dying out of plants correlated to light intensity modifications

This phenomenon occurs only in waterlogged areas and only at the beginning of bright periods following cloudy ones. The plants - lucern, broad bean, maize, cotton for instance - wilt very quickly, outer leaves being first affected ; then blackening, but not drying, takes place.

(1) Centre de Pédologie biologique du C.N.R.S., Vandoeuvre-les-Nancy (France)

(2) Office de la Recherche Scientifique et Technique Outre-Mer, Dakar (Sénégal),

(3) U.N.E.S.C.O., C.R.U.E.S.I., Tunis (Tunisie).

At the same time, roots appear to be covered by a black sheath of iron sulfide. Within 7 to 10 days after the manifestation of the first symptoms, the plants die out.

2. Slackening of the plant growth after cutting

When plants grown for forage, such as sorghum, are cut and when this cutting happens to be followed by soil waterlogging, the growth of the shoots is slackened, forage yield being then reduced to circ. 50 %. Meanwhile roots appear to be covered by a sheath of iron sulfide as in the previous case.

3. Dying out of germinating seeds

When soil is waterlogged after sowing, germinating seeds - especially those which are too deeply sown - die out after sulfide has accumulated in the surrounding soil.

B - EXPERIMENTAL STUDY

1. Materials and methods

The soil was taken from the surface (0-10 cm) horizon of the Nakta saline soil (table 1) irrigated with brackish water (table 2). The experiment was conducted in large test tubes or flat columns described in previous reports (DOMMERGUES et al., 1969 ; HAUKE-PACEWICZOWA et al.,1969). The experimental designs of factorial type included 4 treatments :

Treatment 11 : soil without maize, maintained at field capacity (non rhizospherical, non waterlogged, non compacted soil) ;

Treatment 12 : soil without maize waterlogged and compacted (non rhizospherical, waterlogged and compacted soil) ;

Treatment 21 : soil with maize, maintained at field capacity (rhizospherical, non waterlogged, non compacted soil) ;

Treatment 22 : soil with maize, waterlogged and compacted (rhizospherical, waterlogged and compacted soil).

In the case of treatments 12 and 22, waterlogging and compacting were completed only after the plants were circ. 10 cm high. Rhizosphere soil was obtained by vigorous shaking of the maize roots ; non rhizosphere soil was taken out of the tubes or columns without maize growing. Analysis were carried out 7 or 14 days after waterlogging and compaction were completed. Methods used were as follows :

- sulfide content according to CHAUDHRY and CORNFIELD (1966) ;
- sulfate reducers enumeration according to STARKEY (PICHINOTY, 1966) ;
- nitrogen fixation by the acetylene method as described by STEWART et al., (1967-1968) and modified by HAUKE-PACEWICZOWA et al. (1969).
- *Clostridium* sp. enumeration according to POCHON and TARDIEUX (1962).

The maize hydroponic axenic culture was obtained on Börner and Rodemacher's mineral medium (CHALVIGNAC, 1958).

2. Results of the experimental study

a. Rhizospherical sulfate reduction

We consider that sulfate reduction occurs when sulfides accumulate in the soil and when the sulfate reducing population increases simultaneously.

(1) Soil conditions

The first necessary condition is, of course, the presence of sulfate in the soil itself or in the irrigation water : preliminary experiments have shown that irrigation with fresh water never induces sulfide accumulation, whereas irrigation with Nakta water, or water containing sulfate at the same concentration, may induce sulfide accumulation.

The second necessary edaphic condition is anaerobiosis ; the results of the experiment gathered in fig. 1 reveal that sulfide accumulation and sulfate reducers proliferation occur only in the rhizospherical waterlogged and compacted soil.

It has been shown that sulfide accumulation is discrete as long as the soil bulk density is less than 1.60 ; but when it is higher than this threshold, the intensity of the phenomenon increases quickly and proportionnally to the bulk density (fig. 2).

(2) Plant growth conditions

Figure 1 implies that the accumulation of sulfides and the proliferation of sulfate reducers are strictly localized to the rhizosphere ; moreover it has been demonstrated that the rhizosphere is the only microhabitat affected.

It is known that hight intensity influences the qualitative and quantitative nature of root exsudates (ROVIRA, 1965).

This influence was checked by a preliminary pot experiment using broad bean ; this plant appeared to be more affected by the deleterious consequences of sulfide accumulation when exposed to direct sunlight than when kept under shade.

Cutting of the shoot of hydroponic maize promoted proliferation of sulfate reducers in the nutrient medium where roots were growing (table 3). When the plant was intact, the sulfate reducers micropopulation increased from 0.1 to 1 thousand per ml ; when the stem was cut off, the micropopulation increased from 0.1 to 13,400 thousands per ml.

b. Spermatospherical sulfate reduction

The requisite soil conditions are the same as in the case of rhizospherical sulfate reduction ; but strict anaerobiosis is established more quickly and more intensely in the seed than in the root neighbourhood, likely because the exsudate production of seeds is more important than the exsudate production of roots. As in the case of rhizospherical sulfate reduction, the increase of bulk density enhances the phenomenon, but the accumulation of sulfide in the spermatosphere is much greater than in the rhizosphere (fig. 2), conditions being the same in other respects.

c. Rhizospherical nitrogen fixation

Nitrogen fixation appears to be strictly localized to the rhizosphere placed in anaerobiosis. Proliferation of Clostridium sp. is governed by the same environmental factors (fig. 3).

Sulfide accumulation does not seem to impede nitrogen fixation for no correlation could be evidenced between sulfide content of the rhizosphere soil and nitrogen fixation intensity ; yet when sulfide concentration was more than 20×10^{-6}, nitrogen fixation appreciably decreased.

An experiment conducted with a soil of different pedological type, established the stimulating effect of light on rhizospherical nitrogen fixation ; this stimulation would likely occur in the case of Nakta soil.

C - DISCUSSION AND CONCLUSION

The results presented here emphasize the significance of the rhizosphere and spermatosphere effects which are generally overlooked by agronomists.

These effects are of a real practical interest every time sulfate reduction may appear, that is in soils characterized (1) by the presence of sulfate (soil sulfate or irrigation water sulfate) (2) by a compact structure inducing an abnormally high bulk density (table 1). These two characteristics are commonly found in North Africa and Middle East soils ; so one may think that the phenomena observed for the first time in Nakta soil (rhizospherical and spermatospherical sulfate reduction) are likely to occur in many other places.

Though the influence of light has not been thoroughly studied, preliminary results suggest that the rhizosphere effect is much more important in sunny climates - such as mediterranean climates - than in cloudy temperate ones.

As to nitrogen fixation, we have not yet tried to evaluate its effective importance. But the indications so far obtained, show that the benefit that crops could get through this process, is largely obliterated by the damage induced by sulfate reduction, for the environment conditions governing both nitrogen fixation and sulfate reduction are identical in the Nakta soil type.

The cause of the death of plants or seeds, or of the slackening of growth has not been cleared up. Toxicity of free hydrogen sulfide or mercaptans, which has been stressed by VAMOS (1959) and Japanese searchers (MITSUI et al., 1954.; YAMADA and OTA, 1958 ; TAKAI and KAMURA, 1966) in the case of sulfate reduction in whole profiles, may be held responsible for the damages observed in the case of rhizospherical or spermatospherical sulfate reduction. But one may also suppose that the iron sulfide sheath prevents water or nutrients from being absorbed by the plants.

At last, we have to notice that, by itself, the evaluation of the number of microorganisms at a given time is not sufficient to give an idea of the effective activity of those microorganisms in the soil ; for instance, fig. 1 and 3 show that, in the non rhizosphere soil, sulfate reduction and nitrogen fixation are non-existent whereas the number of sulfate reducers and Clostridium is higher than 10^4 per g of soil. On the other hand an increase of the number of microorganisms during an interval of time suggests an effective activity during this interval.

REFERENCES

CHALVIGNAC (M.A.), 1958 - Effet rhizosphère comparé du lin en culture hydroponique et en terre. Ann. Inst. Pasteur, 95, 474-479.

CHAUDHRY (I.A.) and CORNFIELD (A.R.), 1966 - Determination of sulphide in waterlogged soils. Pl. Soil, 25, 474-478.

DOMMERGUES (Y.), COMBREMONT (R.), BECK (G.) and OLLAT (C.), 1969 - Note préliminaire concernant la sulfato-réduction rhizosphérique dans un sol salin tunisien. Rev. Ecol. Biol. Sol, 6, 115-129.

HAUCKE-PACEWICZOWA (T.), BALANDREAU (J.) and DOMMERGUES (Y.), 1969 - Fixation microbienne de l'azote dans un sol salin tunisien. Soil Biol. Biochem. (under press).

MITSUI (S.A.), KUMURAWA (K.) and ISHIWARA (T.), 1954 - The nutrient uptake of rice plants as influenced by hydrogen sulfide and butyric acid abundantly evolving under waterlogged soil condition. Trans. 5th Int. Congr. Soil Sci., 2, 364-368.

PICHINOTY (F.), 1966 - Mesure de l'activité de quelques réductases de microorganismes. Oxidative phosphorylation and terminal electron transport. Information exchange group n° 1 - Scientific memo n° 555, 1-13.

ROVIRA (A.D.), 1965 - Interactions between plant roots and soil microorganisms. Annual rev. Microbiol., 19, 241-266.

STEWART (W.D.P.), FITZGERALD (G.P) and BURRIS (R.H.), 1967 - In situ studies on N_2 fixation using the acetylene reduction technique. Proc. Nat. Acad. Sci., 58, 2071-2078.

STEWART (W.D.P.), FITZGERALD (G.P.) and BURRIS (R.H.), 1968 - Acetylene reduction by nitrogen-fixing blue-green algae. Archiv für Mikrobiologie, 62, 336-348.

TAKAI (Y.) and KAMURA (T.), 1966 - The mechanisms of reduction in waterlogged paddy soil. Folia Microbiol., 11, 304-313.

VAMOS (R.), 1964 - The release of hydrogen sulphide from mud. J. Soil Sci., 15, 103-109.

VAN HOORN (J.W.), 1966 - Recherches sur l'utilisation de l'eau salée en irrigation en Tunisie. Nature et ressources, 2, 3-7.

YAMADA (N.) and OTA (Y.), 1958 - Study on the respiration of crop plants. Effect of H_2S and lower fatty acids on root respiration of rice. Proc. Crop. Sci. Soc. Japan, 27, 155-160.

TABLE 1 - SOME CHEMICAL AND PHYSICAL PROPERTIES OF NAKTA SALINE SOIL (0-10 cm horizon)

SOIL	pH		7,8
	Carbon		5.0×10^{-3}
	Nitrogen		0.7×10^{-3}
	Clay (2 µm)		19.7×10^{-2}
	Silt (2-20 µm)		15.3×10^{-2}
	Silt (20-50 µm)		8.0×10^{-2}
	Fine sand (50-200 µm)		50.5×10^{-2}
	Coarse sand (200-2,000 µm)		4.5×10^{-2}
	Bulk density *in situ*		1.71
SATURATED EXTRACT	pH		7.9
	Conductivity 25°C ($mmho.cm^{-1}$)		12.9
	Anions ($me.l^{-1}$)	Cl^-	92.7
		$SO_4^{=}$	65.1
		CO_3H^-	2.5
	Cations ($me.l^{-1}$)	Ca^{++}	41.7
		Mg^{++}	23.6
		K^+	0.9
		Na^+	95.3

TABLE 2 - SOME CHEMICAL PROPERTIES OF NAKTA IRRIGATION WATER

Conductivity 25°C ($mmho.cm^{-1}$)		5.5
Dissolved solids ($g. l^{-1}$)		3.8
pH		7.6
Anions ($me.l^{-1}$)	Cl^-	36.7
	$SO_4^{=}$	20.8
	CO_3H^-	3.0
Cations ($me.l^{-1}$)	Ca^{++}	13.5
	Mg^{++}	7.5
	K^+	0.5
	Na^+	37.8
S.A.R. (*)		11.7

$$(*)\ S.A.R. = \frac{Na^+}{\sqrt{\frac{Ca^{++} + Mg^{++}}{2}}}$$

TABL. 3 - STIMULATION OF THE GROWTH OF A RHIZOSPHERE SULFATE-REDUCING MIXED MICROPOPULATION BY CUTTING THE PLANT STEM (hydroponic culture of maize)

		Number of sulfate reducers/ml of hydroponic medium (x 10^3)	
		on the inoculation day	13 days later
Rhizosphere	No cutting (whole plant)	0.1	1
	Cutting (roots only)	0.1	13,400
Control (no plant)		0.1	0.1

fig 1

SULFATE REDUCTION IN THE RHIZOSPHERE OF A MAIZE GROWN IN A SALINE SOIL

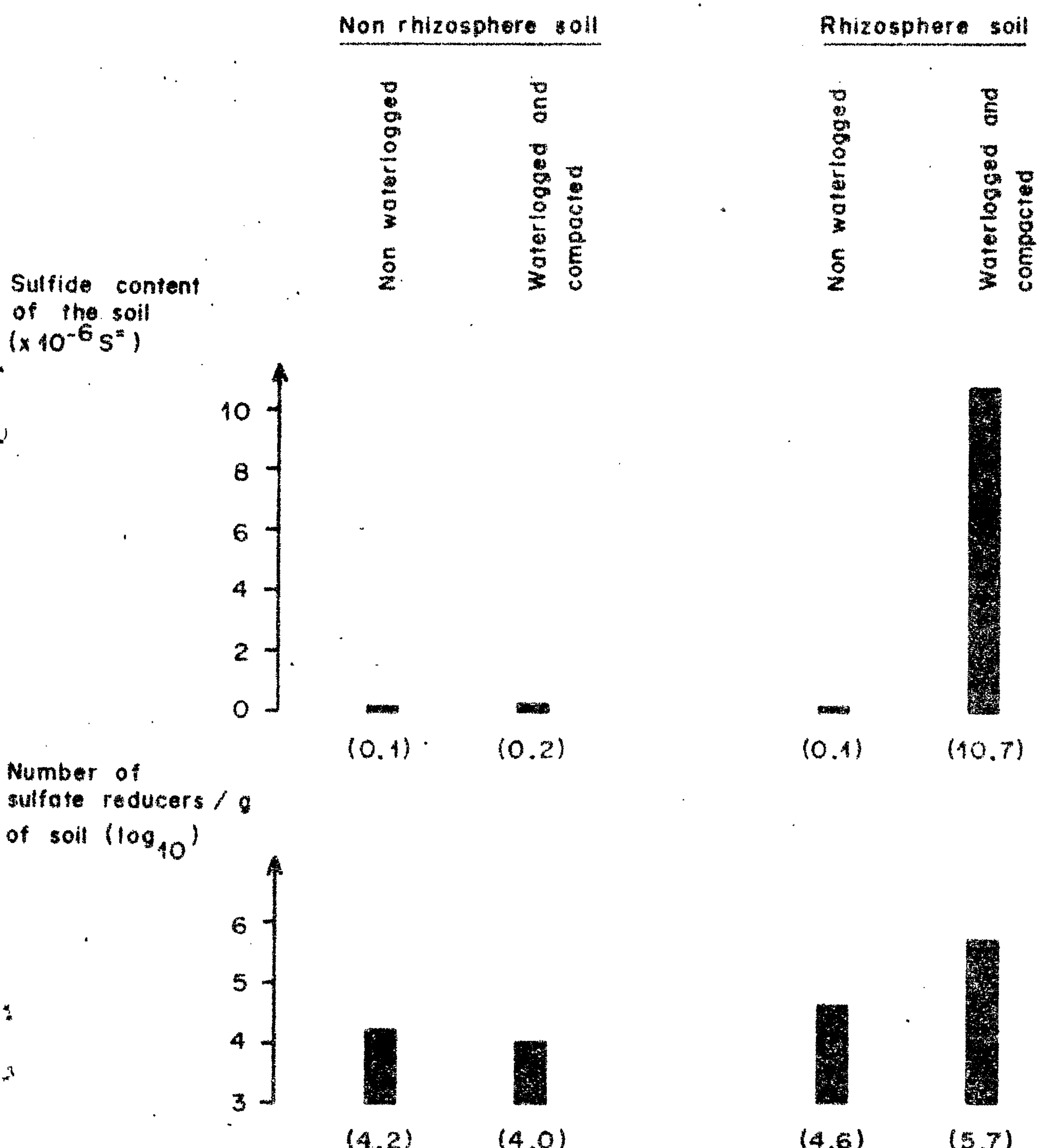
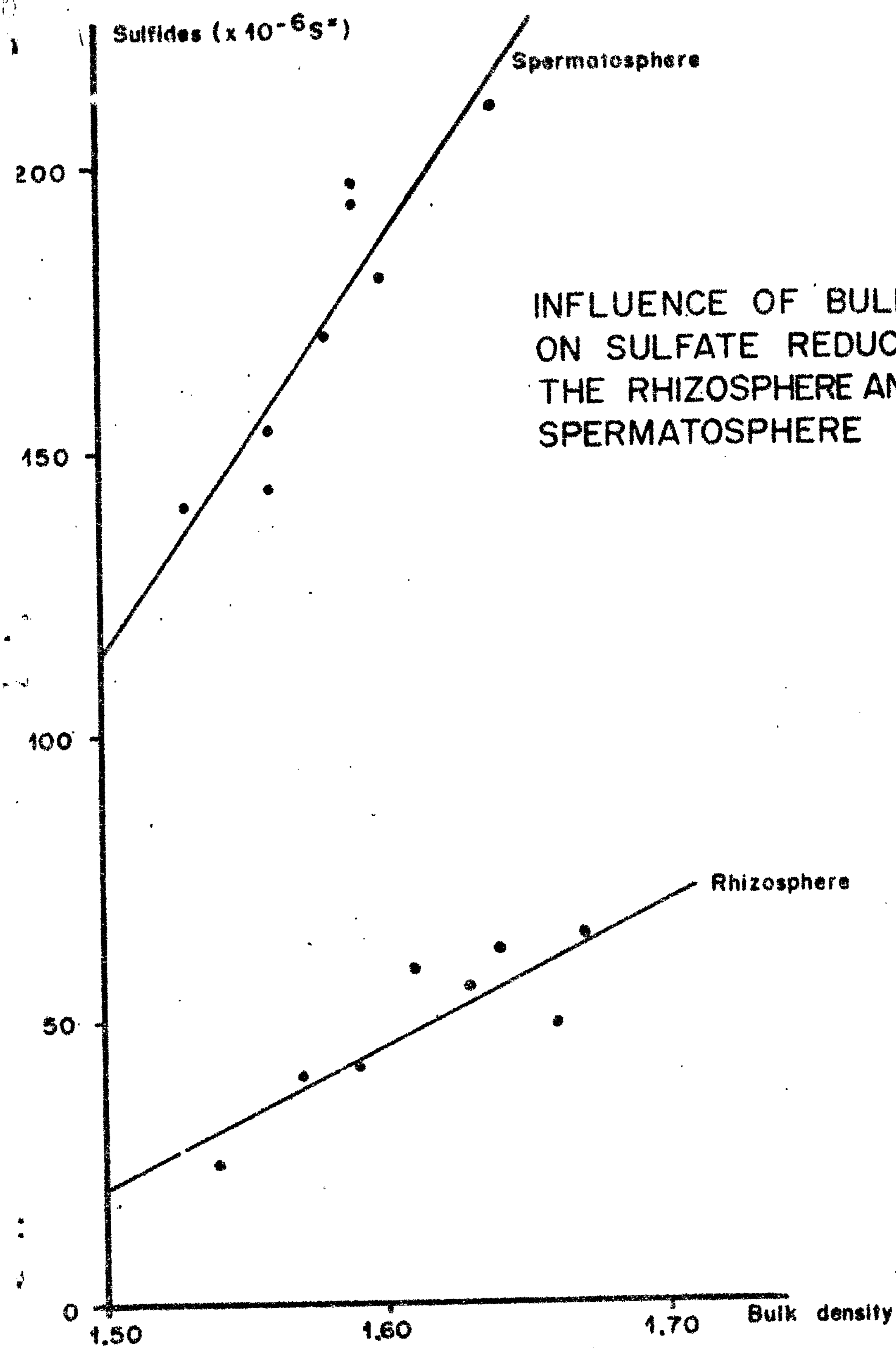


fig 2



INFLUENCE OF BULK-DENSITY ON SULFATE REDUCTION IN THE RHIZOSPHERE AND IN THE SPERMATOSPHERE fig. 3. NITROGEN FIXATION IN THE RHIZOSPHERE OF A MAIZE GROWN IN A SALINE SOIL