IMPACT OF TILLAGE SYSTEMS ON THE MICROBIOTA OF CAMBIC CHERNOZEM SOILS IN THE MOLDAVIAN PLATEAU

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Keywords: microbiota, tillage, soil, fertilized, unfertilized

Abstract: The objectives of this study were to perform the quantitative assessment of the microbiota in soils of cambic chernozem type under the influence of different tillage systems, isolate the microorganisms and prepare pure cultures, and compile the collection of microorganisms. Our results showed that the soil microbiota exhibits quantitative variations both from one sampling point to another and between the depths examined. The comparative analysis of the samples of fertilized and unfertilized soil showed that in the fertilized soils the most effective systems consisted in fertilization at 7-10 cm and soil working using a Paraplow plough, or ploughing to 30 cm.

INTRODUCTION

From the ecological point of view, the soil can be considered the quarters of a collection of nutrients and energy of several organisms and microorganisms, characteristics and processes determined by both its own composition and construction, and the totality of pedogenetic factors, which continually influence it, especially the climatic and very often hydrological ones (Akkermans, A.D.L.,Van Elsas, J.D. 1995). To examine the participation of the microorganisms in the complex processes in the soil requires primarily knowledge of the soil biology both as it is at the time and as it has evolved. This allows the comparison of different types of soil, finding potential disturbances within the soil, as well as establishing the relation between the microbiota and the state of fertility (Angle, S., *et al.*, 1995). The purpose of soil preparation is to change its physical state so that the humidity, aeration, temperature and loosening conditions meet the optimal requirements plants need for growth, and promote microbiota activity in the soil (Giri, B., *et al.,* 2005). A first result of soil working is the enhancement of its porosity. This influences access of gases, and implicitly the access of oxygen, which regulates the aerobic or anaerobic living conditions in the soil micromedia (Bowen, G.D., Rovira, A.D., 1976). The size of the soil aggregates is another important element among the physical factors with an impact on the activity of the microorganisms. The aggregates of soil particles act upon the activity of the microorganisms by two factors, namely their specific surface area and size (Brimecombe, M.J., *et al.*, 2001). The surface area of aggregates is directly related to the quantity of gases that permeate the soil, being therefore a criterion for aeration (Limm, D.L., 1998). Scientists have proved that the activity of the bacteria involved in the mineralization of organic matter is inversely proportional to the size of the soil aggregate. A tight relationship has been established among aeration, the size of aggregates, and intensity of the nitrification process (Norrell, S.A., Messley, K.E., 1997). For instance, nitrification has been shown to be more intense within small aggregates, of approximately 0.25 mm, in aerated and highly aerated soils (Madigan, M.T., *et al.*, 2000). With compaction, big aggregates provide superior aeration and the nitrification process takes place at a higher level, the number of heterotrophic organisms depending on the size of the aggregates.

The objectives of this study were to perform the quantitative assessment of the microbiota in soils of cambic chernozem type under the influence of different tillage systems, isolate the microorganisms and prepare pure cultures, and compile the collection of microorganisms.

MATERIALS AND METHODS

The soil is populated by a varied microbiota, its microbiological examination requiring a wide range of culture media appropriate for each type of microorganism.

One of the routine methods used to determine **the number of microorganisms involves plate cultures.** This technique consists in counting the colonies grown as a result of the inoculation of agar media in Petri dishes with dilutions of the sample to be examined. The method relies on the fact that each viable cell produces a colony, and therefore the number of colonies on a Petri dish indicates the number of microorganisms contained by the sample capable of growing in the conditions provided by the culture medium used.

The *working procedure* includes the following steps: *dilution preparation* – the sample to be examined is diluted to obtain 50 to 300 colonies on a Petri dish; to this purpose, tenfold serial dilutions are prepared; *inoculation* – the Petri dishes are inoculated with the prepared dilutions by adding 1 ml of each dilution on to the dish containing the culture medium previously melted and cooled to 45^0 C; the dishes are rotated slowly to ensure uniform spreading of the inoculum onto the medium; a number of 2 - 3 plates should be inoculated with each dilution, calculating then the average number of colonies grown corresponding to each dilution; *incubation* – the inoculated dishes are placed in a thermostat chamber at an appropriate temperature, i.e. 37^0 C for bacteria and 28^0 C for fungi and actinomycetes; *colony count* – generally, the colonies of bacteria are counted after 24-48 hours, those of fungi after 5-7 days, while those of

actinomycetes are counted after 7-14 days of incubation in the thermostat chamber; the colonies are counted by the naked eye or using a magnifying glass (Duncа Simona *et al.*, 2004).

The optimal dilution is the one that results in a number of 50-100 colonies. If the number of colonies grown following the inoculation with a dilution is lower than 10 or higher than 300, the results are disregarded.

The ***quantitative assessment of the soil microbiota*** involved the counting of the colonies grown on Petri dishes inoculated with the dilutions (10^{-1} - 10^{-5}) of the soil samples. The results were expressed as colony forming units (CFU) per g of soil.

The number of colony-forming units (CFU) per g of sample is calculated by the formula:

CFU/g of soil = a x 10^n/V,

where: **n** = number of colonies;

10^n = dilution at which the colony count was carried out;

V = volume of the inoculum.

RESULTS AND DISCUSSIONS

For the **quantitative analysis of the microbiota in fertilized** (N80P80) **and unfertilized soils,** 26 soil samples were collected (10 samples of fertilized soils, 10 samples of unfertilized soils, 6 samples of soil to which different tillage systems were applied) from two different sampling depths (i.e. 7-15 cm, and 15-20 cm) during May 2008. Several soil working techniques were selected (e.g. disk harrow, Paraplow plough, Chisel plough + rotary harrow, ploughing to 20 cm, ploughing to 30 cm, Chisel plough and direct seeding) to make quantitative comparisons of the microbiota and select the locations for the subsequent investigations.

For the conventional notation of the soil samples, the following initials were used:

DF1- Disk harrow, **F**ertilized, 1 – depth: 7-10 cm;
DF2- Disk harrow, **F**ertilized, 2 – depth: 15-25 cm;
PPF1 - Paraplow plough, **F**ertilized, 1 – depth: 7-10 cm;
PPF2 - Paraplow plough, **F**ertilized, 2 – depth: 15-25 cm;
CF1 – **C**hisel plough + rotary harrow, **F**ertilized, 1 – depth: 7-10 cm;
CF2 – **C**hisel plough + rotary harrow, **F**ertilized, 2 – depth: 15-25 cm;
AF1 – Ploughing to 20 cm, **F**ertilized, 1 – depth: 7-10 cm;
AF2 – Ploughing to 20 cm, **F**ertilized, 2 – depth: 15-25 cm;
AF3 – Ploughing to 30 cm, **F**ertilized, 1 – depth: 7-10 cm;
AF4 – Ploughing to 30 cm, **F**ertilized, 2 – depth: 15-25 cm;
DN1- Disk harrow, **U**nfertilized, 1 – depth: 7-10 cm;
DN2- Disk harrow, **U**nfertilized, 2 – depth: 15-25 cm;
PPN1 - Paraplow plough, **U**nfertilized, 1 – depth: 7-10 cm;
PPN2 - Paraplow plough, **U**nfertilized, 2 – depth: 15-25 cm;
CN1 – **C**hisel plough + rotary harrow, **U**nfertilized, 1 – depth: 7-10 cm;
CN2 – **C**hisel plough + rotary harrow, **U**nfertilized, 2 – depth: 15-25 cm;
AN1 – Ploughing to 20 cm, **U**nfertilized, 1 – depth: 7-10 cm;
AN2 – Ploughing to 20 cm, **U**nfertilized, 2 – depth: 15-25 cm;
AN3 – Ploughing to 30 cm, **U**nfertilized, 1 – depth: 7-10 cm;
AN4 – Ploughing to 30 cm, **U**nfertilized, 2 – depth: 15-25 cm;
A1 - Ploughing to 20 cm, 1 – depth: 7-10 cm;
A2 - Ploughing to 20 cm, 1 – depth: 15-25 cm;
B1 - **C**hisel plough, 1 – depth: 7-10 cm;
B2 - **C**hisel plough, 2 – depth: 15-25 cm;
C1 - Direct seeding, 1 – depth: 7-10 cm;
C2 - Direct seeding, 2 – depth: 15-25 cm.

The research studies carried out in May 2008 showed quantitative variations in the soil microbiota both among the sampling points and between the depths considered for examination (*Table I*).

In the ***fertilized soils,*** the number of microorganisms was higher at 7-10 cm than at 15-25 cm depth, except for the soil ploughed to 20 cm, in which the number of microorganisms was larger at the higher depth. The lowest value found in the samples of fertilized soils ***collected at the 7-10 cm depth*** was 99 x 10^5 CFU/g of soil (tillage system: disk harrow – sample DF1), while the highest one was 241 x 10^5 CFU/g of soil (tillage system: Paraplow plough – sample PPF1) – *Fig.1*.

The lowest value found in the samples of ***fertilized soil, collected at the 15-25 cm depth*** was 37 x 10^5 CFU/g of soil (for the same tillage system: disk harrow – sample DF2), while the highest was 195 x 10^5 CFU/g of soil (soil ploughed to 20 cm – sample AF2) – *Fig. 1*.

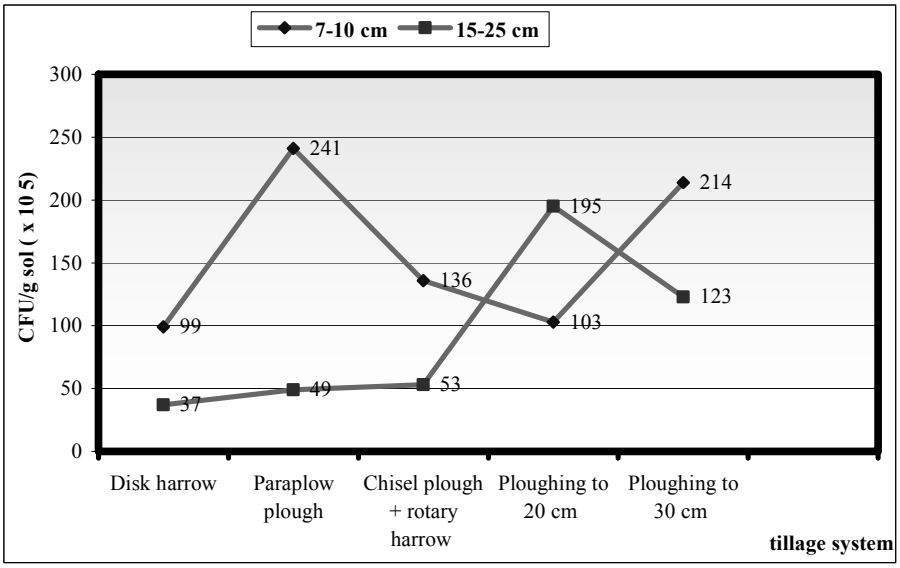

Fig. 1. Dynamics of the microbiota (CFU/g of soil) in the fertilized soils

Table I. The CFU/g of soil values in the soil samples collected in May 2008

Sample no.	Soil type	Tillage system	Sampling depth (cm)	Conventional notation	CFU / g of soil
1	Fertilized (N80P80)	Disk harrow	7-10	***DF1***	99 x 10^5
2		Disk harrow	15-25	***DF2***	37 x 10^5
3		Paraplow plough	7-10	***PPF1***	241 x 10^5
4		Paraplow plough	15-25	***PPF2***	49 x 10^5
				CF1	136x 10^5
5		Chisel plough +	7-10		

Sample no.	Soil type	Tillage system	Sampling depth (cm)	Conventional notation	CFU / g of soil
		rotary harrow			
6		Chisel plough + rotary harrow	15-25	***CF2***	53 x 10^5
7		Ploughing to 20 cm	7-10	***AF1***	103 x 10^5
8		Ploughing to 20 cm	15-25	***AF2***	195 x 10^5
9		Ploughing to 30 cm	7-10	***AF3***	214 x 10^5
10		Ploughing to 30 cm	15-25	***AF4***	123 x 10^5
11	Unfertilized	Disk harrow	7-10	***DN1***	67 x 10^5
12		Disk harrow	15-25	***DN2***	92 x 10^5
13		Paraplow plough	7-10	***PPN1***	115 x 10^5
14		Paraplow plough	15-25	***PPN2***	275 x 10^5
15		Chisel plough + rotary harrow	7-10	***CN1***	429 x 10^5
16		Chisel plough + rotary harrow	15-25	***CN2***	93 x 10^5
17		Ploughing to 20 cm	7-10	***AN1***	165 x 10^5
18		Ploughing to 20 cm	15-25	***AN2***	210 x 10^5
19		Ploughing to 30 cm	7-10	***AN3***	306 x 10^5
20		Ploughing to 30 cm	15-25	***AN4***	101 x 10^5
21		A - Ploughing to 20 cm	7-10	***A1***	153 x 10^5
22		A - Ploughing to 20 cm	15-25	***A2***	24 x 10^5
23		B – Chisel plough	7-10	***B1***	26 x 10^5
24		B - Chisel plough	15-25	***B2***	78 x 10^5
25		C – Direct seeding	7-10	***C1***	108 x 10^5
26		C - Direct seeding	15-25	***C2***	203 x 10^5

In the ***unfertilized soils,*** from the five types of samples examined only two (namely sample CN1 – Chisel plough + rotary harrow and sample AN3 – soil ploughed to 30 cm) exhibited a larger number of microorganisms at the depth of 7-15 cm, i.e. 429 x 10^5 CFU/g of soil, respectively 306 x 10^5 CFU/g of soil. The values of the CFU/g of soil were between 67 x 10^5 CFU/g of soil (sample DN1 – disk harrow) and 429 x 10^5 CFU/g of soil (sample CN1 – Chisel + rotary harrow) in the ***samples collected from the 7-10 cm depth,*** and between 92 x 10^5 CFU/g of soil (sample DN2 – disk harrow) and 275 x 10^5 CFU/g of soil (sample PPN2 – Paraplow plough) in the ***samples collected from the 15-25 cm depth***(*Fig. 2)*.

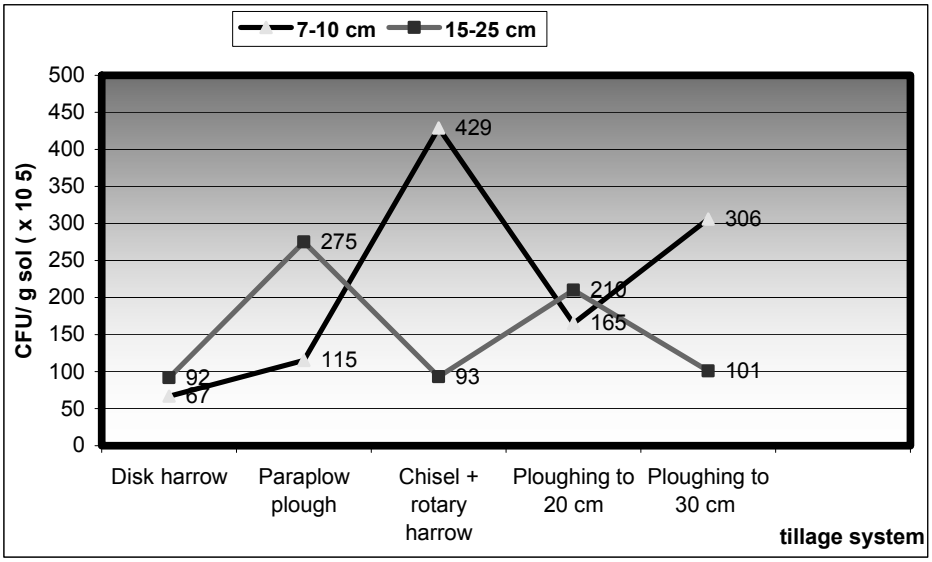

Fig. 2. Dynamics of the microbiota (CFU/g of soil) in the unfertilized soils

In what concerns the ***samples of soil to which different tillage systems were applied***, conventionally marked with A1, A2, B1, B2, C1 and C2, the lowest number of microorganisms was found in the soil worked using the chisel plough: 26 x 10^5 CFU/g of soil at 7-10 cm depth (sample B1) and 78 x 10^5 CFU/g of soil at 15-25 cm depth (sample B2). Also in the soil to which direct seeding was applied, the number of microorganisms was larger deeper in the soil, but higher than in the chisel ploughed one: 203 x 10^5 CFU/g of soil (sample C2). In the soil ploughed to 20 cm (sample A1) the number of microorganisms was higher at 7-10 cm depth (153 x 10^5 CFU/g of soil) as compared to the number at 15-25 cm depth (24 x 10^5 CFU/g of soil) – *Fig.3*.

The comparative analysis of the samples of fertilized and unfertilized soil collected at the 7-10 cm depth (*Fig. 4*) and 15-25 cm depth (*Fig. 5*) showed the effectiveness of the fertilization at 7-10 cm and soil tillage using a Paraplow plough, or of ploughing to 30 cm, when the number of microorganisms found was larger at the higher depth (15-25 cm).

In what concerns the unfertilized soils, the most effective systems were those consisting in soil working using a chisel plough (at 7-10 cm depth), and ploughing to 20 cm for the 15-25 cm depth. This can be explained by the fact that while turning over the beds, the lower beds with a better structure are brought up to the surface and therefore better humidity conditions are maintained in the soil from microbiological point of view, thus promoting biological processes within the tillable soil.

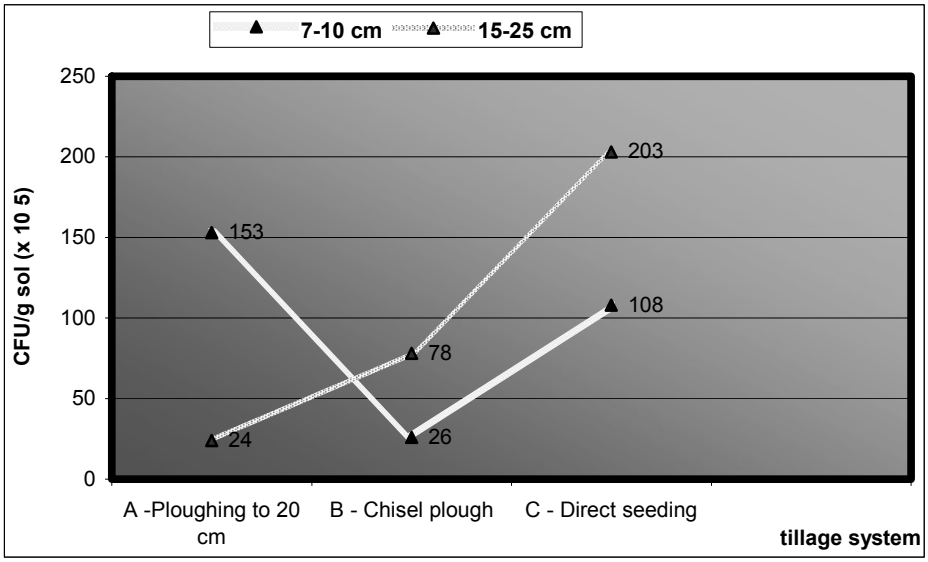

Fig. 3. Dynamics of the microbiota (CFU/g of soil) in soils with different tillage systems

The effectiveness of the tillage systems was assessed based on the number of microorganisms found in the soil samples investigated.

The results were correlated with enzymatic analyses which showed that there are correspondences between the microbial activity in the soil and the enzymatic equipment of the microorganisms.

The comparative analysis of our results to the results of the chemical tests showed that there is a relation between the highest number of microorganisms investigated and the pH, the optimal one for bacterial growth being close to neutrality (6.92-7.28), which was found in the samples subjected to investigation, as well as between the same number and increased content of Nt, P-Al, K-Al, organic C, Zn, and Cu, elements which are essential for the growth and multiplication of microorganisms.

The differences found demonstrate that there is a real microbial diversity. The large number of microorganisms in a place depends on the availability of the carbon and energy sources and the presence of other essential nutrients.

The results confirm the literature data according to which microorganisms are found in great number particularly in the upper layers of the soil. The number and specific composition of the microbiota may, however, vary according to the soil type and particularities.

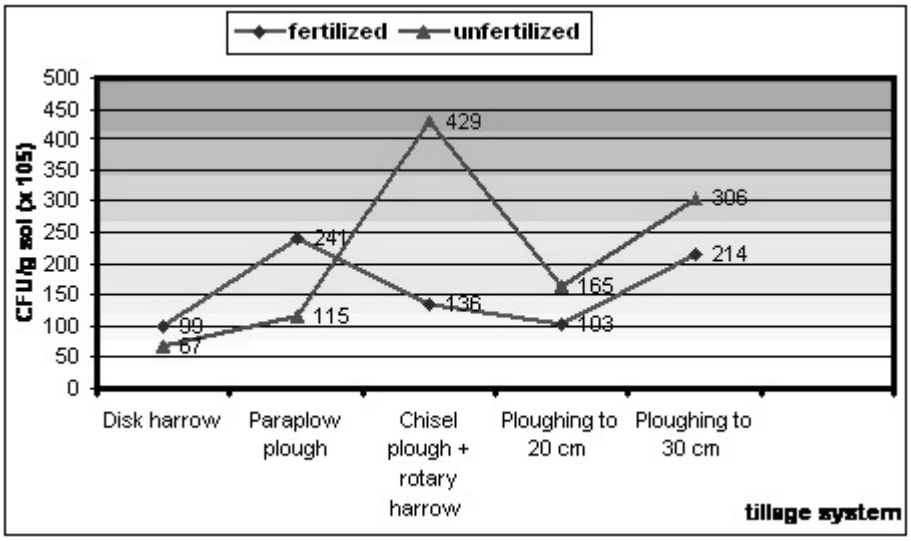

Fig. 4. Dynamics of the microbiota (CFU/g of soil) in the fertilized and unfertilized soils (7-10 cm)

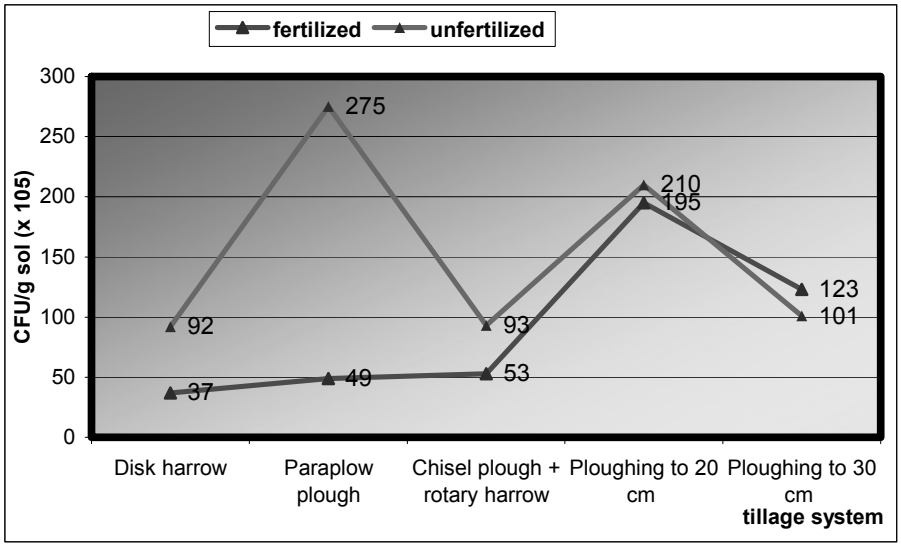

Fig. 5. Dynamics of the microbiota (CFU/g of soil) in the fertilized and unfertilized soils (15-25 cm)

The results confirm the literature data according to which microorganisms are found in great number particularly in the upper layers of the soil. The number and specific composition of the microbiota may, however, vary according to the soil type and particularities.

In terms of vertical spreading, in general, the higher the depth the smaller the number of microorganisms found; sometimes, there are sterile layers between layers populated with microorganisms. Thus, it can be assumed that the continual decrease of the microbiological activity in the lower horizons of the soil are the result either of the absence of air, or alkalinization of the soil solution, or lack of sufficient nourishing substances, or, more likely, of the complex and concurrent action of all these factors.

CONCLUSIONS

The soil microbiota exhibits quantitative variations both from one sampling point to another and between the depths examined.

In the fertilized soils**,** the number of microorganisms was higher at 7-10 cm than at 15-25 cm, except for the soil ploughed to 20 cm, in which the number of microorganisms was larger at the higher depth.

In the unfertilized soils**,** the number of microorganisms was high at 15-25 cm, except for the soil worked by chisel plough + rotary harrow and the soil ploughed to 30 cm.

The comparative analysis of the samples of fertilized and unfertilized soil showed that in the fertilized soils the most effective systems consisted in fertilization at 7-10 cm and soil working using a Paraplow plough, or ploughing to 30 cm - when the number of microorganisms found was larger at the higher depth (i.e. 15-25 cm) -, while in the unfertilized soils, the most effective systems consisted in soil working using the chisel plough (at 7-10 cm depth), or ploughing to 20 cm (for the 15-25 cm depth).

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