



REPORT II.
ON TESTING OF
MicroSoil® SOIL INOCULANT
(in 2000-2001 years)

A summary of the trials made in Hungary under
coordination of KIRKUK Trade Ltd.

Performed at the Saint Stephan University, Hungary
Budapest, 2002 April

INTRODUCTION

In the year 1999 a strategical conception was made for the study of bacterium fertilizers, first for studying the effectivity and behaviour of the **MicroSoil** bacterium fertilizer. The trials were started in the same year and they are running also now. In this Report you will find a short summary concerning the results of the 2001-2002 year's tests. It is well known that the use of bacterium fertilizers reveal new fields of applications in the sustainable and environmental friendly farming. The soils with low nutrient content can be promoted and the farming on these fields can be economical as well.

The experiences with bacterium fertilizers look back for a short time. The summarizing of results could not be done until now since we did not have enough and reliable data. It is a principal statement that only one year test or series of tests can not present authentic results. For this reason we have to continue these tests in approximately similar conditions for a longer period. We have to remember that the tests with chemical fertilizers lasted more than 100 years. The trials of the 2001-2002 years followed our conceptual program.

The RD program for 2001-2002 contained the followings: test of effectivity of **MicroSoil** bacterium fertilizer on the nutrient supply of soil, test on decomposition and exploration of organic matters effect on the microbial life of the soil effect on the production mass, constituents and quality of corn and sugar beet

According to the conception the effectivity of **MicroSoil** was compared with the effect of organic manure and with the different amount of chemical fertilizers. The decomposition rate and effect of wheat-straw was also tested in respect of organic nutrient content of the soil. The trials were carried out, as in the last periods, in pots and in small parcels. The aim of these trials were to get reliable, controllable and reproducible results to compare them with each other. Further task is to make some basic tests to get methodology and connections for the following years. This aim will be fulfilled on the most reliable way if besides the number of species the number of treatments will be pointed out.

I. RESULTS of POT TRIALS

1. Purposes of the trials

The trials of the last year have been continued and the effect of the **MicroSoil** was further tested in respect of :

- the change of the organic and nutrient content of the soil
- the decomposition and revealing of organic materials
- the microbial life of the soil
- the production of the crops, the composition of the seeds and of the inner quality

The effect of **MicroSoil** was tested alone and compared with different amount and composition of inorganic fertilizers.

The trials are running already in the third year. For this reason the tests were carried out again in pots and small parcels of 30 - 50m². The number of plant species were not increased, however the number of treatments were extended.

2. Methods and materials

2.1 Nutrient treatments:

- control without treatment, compared after harvest
- MS = 1 lit/ha MicroSoil
- N30 = 30 kg/ha nitrogen fertilizer, ammonium-nitrate 34%
- N60 = 60 kg/ha nitrogen fertilizer, ammonium-nitrate 34%
- N30PK = N30 P60, K60 kg/ha combined fertilizer
- MS PK = 1 lit/ha MicroSoil + P60 K60 kg/ha fertilizer
- Sza+MS = 1 lit/ha MicroSoil on 4 t/ha straw

2.2 Plant species:

- corn, *Zea Mays*
- sugar beet, *Beta vulgaris*

2.3 Types of soils: chernozem

depth cm	Humus %	pH	CaCO ₃ %	plasticity K _A	capil. lifting 5h-20h
0-16	3,63	7,90	11,3	38	250-430
16-30	3,45	8,0	12,2	39	300-420
30-50	2,98	8,10	12,5	40	320-450

Brown sandy forest soil (in small parcels)

depth cm	humus %	pH	CaCO ₃ %	Plasticity K _A	capil. lifting 5h-20h
0-25	3,02	5,9	0	42	240-300
25-35	2,52	6,0	0	44	320-360
35-50	1,68	6,1	0	45	380-410

2.4 Method of trials

- Pot test: 63,5 diameter and 60 cm deep pots, with open bottom and set in the soil
- small parcels of 30 sqm (sugar beet) -50 sqm (corn)
- the tests are repeated 4-times in accidental blocks

2.5 Trials:

- germination intensity
- growing and development
- amount of production
- quality of production
- change in nutrient content of the soil
- microbiological tests

3. Agrotechnical data:

3.1 *seeding time*: corn: in pots May 14, 2001.

small parcels May 20, 2001.

sugar beet: in pots May 25, 2001.

small parcels April 05, 2001.

3.2 *harvest*

corn: November 08, 2001.

sugar beet November 09, 2001.

4. Results of the trials **Corn**

4.1 *Growing of corn during the season*

There were no differences in germination between the treatments.

The starting grow of corn was promoted best by the „conventional” inorganic fertilizers. Later the effect of **MicroSoil** was better than of any other comparing products.

At the second controll (07.20) the **MicroSoil** provided its effect though the biggest growing was not total reliable. Comparing the NPK treatment with **MicroSoil**+PK it was to see that the effect of nitrogen was quicker but of the **MicroSoil** was durable. At the 3rd time (08.20) the difference did not grown between the height of the treated and non-treated corn. The reason was supposingly because of the bigger amount of rainfall in this period which was over the average quantity. In this time the reliable growing was observed only at greater dosages of nitrogen (N60), independent from the treatment with **MicroSoil**. The effect of treatments on the height of corn is shown in Table 1.

In the last period, before the harvest, the height of crops reached the final stadium (09.20). The effect of treatments have been remarkable. The heighest were the corns after treating with **MicroSoil** but this is not significant, untill now.

4.2 Effect of treatments on the production of corn

4.21 Seed production

The last year of 2001 was favourable for the corn. The production of controll was also good, in spite of the late seeding. The N30 dosage did not yield reliable effect on the production. The greater dosage of nitrogen, N60, promoted primarily the vegetative mass. The amount of production was higher than of the controll but with use of **MicroSoil** it was remarkable higher. This production is depending on the humidity.

The smaller nitrogen dosage (N30) could be replaced by **MicroSoil** and so the increase of production was 5%.

The bigger amount of organic matter, straw turned into the soil did not yield relaiable increasing in production. The 3,3% increasing was due to the weather conditions. The **MicroSoil** reduced the „pentosan” effect and promoted the decomposition of straw, so the effect of straw nutrification could be remarkable.

4.22 Leaf and stem

The vegetativ mass of corn is important not only for production of silo-corn but by greater assimilation surface for the energy binding and incorporation. The development of vegetative parts show the effectivity of treatments. Testing the parts above the ground we can state that the most of treatments increased the vegetative mass but the effectivity of treatments were not comparable with the observations at the seed production.

The effect of treatments related to the controll was smaller than the differences between the seed production. The effect of **MicroSoil** compared with N30 was the same but did not reached the effect of N60. In combination of nutrients (NPK) the N30 part-dosage could be replaced by **MicroSoil** This means that 1 lit/ha **MicroSoil** treatment promoted better the vegetative increasing during the season than the N30. It was necessary the supplement of PK content as well because there was no difference between the sole treatment with N30 and **MicroSoil**

The decomposition of straw, treated with **MicroSoil** yielded 3% increasing in production, more than the straw alone (Sza). The results can be seen in Table 2.

Sugar beet

4.3 Growing of sugar beet during the season

The results are shown in the Table 3 and Table 4. The mass of beets was measured in every 30 days. At the first measurement (07.20.) there were reliable differences between the treatments. The growing of sugar beet was promoted primarily by the nitrogen doses either in form of organic or inorganic compounds. The data of the table confirm that the balanced nutrient supply is beneficial. We can draw the consequence from this result that to get the effect of **MicroSoil** needs more time.

The nitrification with straw confirmed this statement.

These could be the result also with the treatment of straw and **MicroSoil**, since there are no differences between them. It is a result if the pentosan effect does not appear and does not decrease the starting development of the sugar beet. The decomposition of straw requests nitrogen. It is obvious that the inorganic nitrogen compounds promote better the starting effect than the **MicroSoil**.

In the second evaluation time several tendencies have been observed. The sugar beet reacts well on the higher nitrogen doses and also on the NPK nitrification, however the N30PK treatment did not provide better results than the MSPK treatment. In the time of third evaluation the availability of nutrients are in good condition and the decomposition of organic matters are also advanced, the beneficial effect of the **MicroSoil** is to see: it reached about 4,3%.

5. Effect of treatments on the nutrient content of soils and on the microbiological life

The examinations were carried out in the lab of the Institute for Agrochemie and Soil Science Researches of the Academy of Sciences with the following topics: (Table 5.)

Dehydrogenase activity: this is a microbiological parameter which shows the metabolic activity of the active material exchange of microflora, indirectly the size of the biochemical reaction, (Figure 1/1)

Phosphatase-activity: The activity of the abiotic accumulated phosphor-monoesterase, a biochemical parameter which is playing an important role in mobilization of organic bound phosphorus. It is generally indirectly related with the available phosphor-content, (Figure 1/2).

CO₂ production: This parameter is characteristic on the decomposing respective on general intensity of the metabolic activity or potential (Figure 1/3);

Microbial biomass: The effectively existing mass of microflora in the soil, giving realistic results related to the stereotyped-accounted, also indirect methods which was performed with the chloroform-fumigation method which is a more realistic one (Figure 1/4).

The results of the tests can be seen in the Table 5. We can see that the different treatments show greater effect on the soil-life than on the generative or vegetative production. The effect of these will be realized supposedly in the next years in the development and growing of post-plants. The activity of soil bacteria can be well measured with the activity of microflora. The **MicroSoil** alone produced about threetimes effects of the activity. The effect of MSPK is also confirmed in relation with NPK. In other treatments there are no essential differences.

Examining the *phosphatase activity* on the availability of phosphorus is as follows: MSPK, NPK.

From the decomposing ability of organic matters we can conclude from the developing of CO₂. The treatments with **MicroSoil** seems beneficial. These phenomena must be examined also in the next years in several combinations.

The healthy of a soil-life, the mass of microflora presents the production of biomass. The data of the Table 5. confirm these observations. The effect of **MicroSoil** was prominent in itself and also in combinations.

The effects of treatments on the total and available nutrients can be studied in the Table 6. The data of the table should be evaluated with great care. It can be stated in general the absolute nutrient capital, the starting amount of nutrients have been decreased the production by means of some treatments. Decreased the available both forms of nitrogen content also in control and by the treatment with **MicroSoil**. Increased both form of nitrogen fertilizers however, more in combination with **MicroSoil!** Since the productivity was beneficial influenced also alone with **MicroSoil** but the nitrogen content of the soil did not increased, we can conclude that as effect of treatment will produced more nitrogen however, this will received by the crops and does not remain surplus in the soil.

The role of the available phosphorus is similar to nitrogen in the soil. The P-content decreased in greatest rate in starting status in the control and practically remains stable in the MS and nitrogen treatments. The other treatments increased the P-content. Prominent was the effect in treatment of straw with **MicroSoil**. (Figure 2.1 and 2.2)

The K content of the soil in control parcels did not change related to the starting status.

Since for the increase of corn production it is necessary to add some potassium, it is obvious that this was explored from the soil without K treatment too. This is valid for the parcels treated with nitrogen fertilizer as well. The K content of the soil was increased also by the treatments with K containing fertilizers as well. The K content of the soil was decreased in case when the treatment was only with **MicroSoil**. That means that the **MicroSoil** has not enough capability for explore the K-content. If this will be controlled and confirmed than it have to be suggested in some cases to supplement the treatment with K. May be that the straw will be able to supplement a part of K demand.

II. RESULTS OF SMALL PARCELL TRIALS

Effect of MicroSoil on the production-yield and quality of corn (*Zea mays*)

1.1 Purpose of the trials

The trials in pots allowed the evaluation of important basic connections in absolutely exact circumstances. In the small parcell trials it was possible to test the connections between **MicroSoil**-Environment-Plant relations in field experimentation. In small parcell trials have been tested also the same treatment methods as in the pot-tests.

1.2 Methods and materials

1.2 Nutrient treatments

- Control, nutrient status after harvest
- MicroSoil 1 lit/ha
- N30 = 30 kg/ha nitrogen fertilizer, ammonium-nitrate 34%
- N60 = 60 kg/ha nitrogen fertilizer, ammonium-nitrate 34%
- N30PK = 30kg/ha N+60 kg/ha P+60 kg/ha K fertilizer
- MSPK = 1 lit/ha MicroSoil+60 kg/ha P+60 kg/ha K fertilizer
- Sza + MS = 4,0 t/ha straw+1 lit/ha MicroSoil

1.2.2 Soil properties

Type: Ramann-type brown sandy-forest soil

Humus %	pH	plasticity	CaCO ₃ %	NO ₃ +NO ₂ ppm	P ₂ O ₅ ppm	K ₂ O ppm
1,86	5,05	33	0,0	4,8	206	123

1.3 Circumstances of the trials

1.3 Timing:

- Stubble tilthing: 07.20.2000
- tlth
- dosage of MicroSoil: 05.10.2001.
- seeding: 05.11.2001.
- stubble treatment: 08.15.2000.
- dosage of straw and manure: 02.03.2001.
- seeding-bed making: 05.10.2001.
- weed control: with Primextra Gold 3,0 lit/ha

1.3.2 Method: parcels 50 m²
repetition: 4

Observations: homogeneity in germination
starting grow
quantity of the production kg/ha
constituents: protein %, starch %, oil %, humidity %

1.4 Results of the trial:

1.4.1 Homogeneity in germination:

It was observed that in the phase of germination there was no difference in general only in that parcels where straw was turned in. This heterogeneity disappeared until 06.20 because of the rainfall.

1.4.2. Quantity of the production

The quantity is shown in the Figure 4. below in t/ha and also the relative changes related to the treatments. The + mark means that with the treatments the production yield was statistically more related to the control. The SD5 % marked (proven) that between the two treatments were reliable differences, which difference was greater than the value of SD5 %. The smaller dosage of nitrogen did not promote the decomposition of the straw. It seems that the straw was not decomposed without treatment in greater part during the corn vegetation, so as nutrient did not stand at disposal for the corn.

The effect was better when the straw was handled with **MicroSoil**. The yield increased with 860 kg/ha (17,9%) and it was reliable. This quantity was higher than with N30 or with N60. The reason is that the 1 lit/ha **MicroSoil** supplies more nitrogen than the N60 fertilizer or the P content of the original soil would be explored as well. This variation is also possible since the total fertilizer supply (NPK60) gave reliable more yield, (+900 kg/ha) as the sole treatment with **MicroSoil**. The effect of the P and K seems to be in the increased yield of NPK related to N30 which was 820 kg/ha.

The 1 litre/ha diluted **MicroSoil** could replace the nitrogen demand in the MSPK treatment. The reason was that the **MicroSoil** could supply more nitrogen and also the phosphorus exploration was better.

1.4.4 Quality tests of corn

Protein content, %

The nitrogen fertilizers do not increase automatically and reliably the protein content of the plants. The synthesis of the proteins is a very complicated process, which is not only by the nitrogen supply influenced.

This situation could be seen from the trials. The greater doses of nitrogen, N60, did not increase alone the protein content. Only the complex nutrient supply can do this process. The dosage of NPK confirmed the importance of the harmonic nutrient supply. In the complex nutrient supply the **MicroSoil** could replace the nitrogen fertilizer because the protein content of the two treatments resulted the same quantity.

The **MicroSoil** showed a duration-effect and is able to supply more phosphorus for the next season, however this effect shall be confirmed. (Figure 4.)

Humidity, %

In the respect of economical evaluation there should be important to test the humidity of the product resulted by the different kind of treatments. This influences the costs of transport. Those treatments were favourable which does not increase the humidity related to the control. The data show that only the higher amount of nitrogen doses increased significantly the humidity of the seeds.

The **MicroSoil** increased the amount of the production without increasing the humidity but related to the N60 decreased it. This means the advantage of the continuous treatment with **MicroSoil** and the problems of nitrogen fertilizing.

Comparing the effects on straw the treatment with **MicroSoil** decreased the humidity and N30 increased the humidity in small scale decreasing the pentosan effect but this shall be confirmed.

Oil, %

The oil content was not changed by any treatment in significant scale related to the control. That means that the oil formation process is in the nutrient supply not unambiguous.

There are verifiable differences between the treatments. The bigger doses of nitrogen, N60 and the effect of straw was significant smaller than of the NPK, MSPK treatments. These effects shall not be overvaluated however we have to examine further.

2. Effect of MicroSoil on production and constituents of sugar beet

2.1 Purposes of the trials

The purposes of the trials were the same as at the corn. It is important that as at the pot-tests the single effect on some plants was tested, than at the small parcell tests the „stand” and the „environmental” effects have been tested which is better evaluable. The connection between the treatment and the average production is more reliable and corresponds better to the results of field tests. It can be stated that the pot tests confirm the potential effects and the small parcell tests present the effective attainable results.

2.2 Methods and materials

2.2.1 Nutrient treatments:

- control
- MicroSoil 1 lit/ha, 1:100 diluted
- N30, = 30 kg/ha nitrogen fertilizer, ammonium-nitrate 34%
- N60, = 60 kg/ha nitrogen fertilizer, ammonium-nitrate, 34%
- N30PK, = 30kg/ha nitrogen + 60 kg/ha P + 60 kg/ha K fertilizer
- MSPK, = 1 litre/ha MicroSoil + 60 kg/ha P + 60 kg/ha K

2.2.2 Soil types:

Type: Ramann-type brown forest soil

Humus %	pH (KC1)	Plasticity K _A	CaCO ₃ %	NO ₃ +NO ₂ ppm	P ppm	K ppm
1,75	5,70	30	1,6	3,1	136	110

Notice: The soil was poorer than at corn that means a greater effect in treatment.

2.3 Conditions of the trials

- deep loosen 2000. 09.25.
- tillage 2000. 10.05.
- treatment 2001.04.05.
- tillage 2001 04.05.
- seed bed 2001.05.05.
- seeding 2001.05.10.
- weed controll Safari 30 g/ha + Betanal Pr. 1 lit/ha
- harvest 2001. 11.05. by hand
- measure of parcels 30 m2
- repetition 4
- observations
 - homogeneity in germination
 - balance in starting development
 - mass of production
 - constituents, digestion, sugar content, %

2.4 Results

The germination and homogeneity was uniform, there were no differences between the treatments.

The mass of production are presented in table 6. The production is given in t/ha but the relative % to controll is also shown. The + sign means the statistically reliability, SzD5%. The average production was low because of tzhe late seeding. The production of controll was specially low wich can be declair with the low nutrient content of the controll soil. In this area there was no nutrient supply in the last 3 years. This is similar to the reality.

Concerning the the effect of treatments we can state that the effect of the treatments could be well succeeded. The exception was the smaller amount of nitrogen, N30 that it was no increasing in production.

The production was increased by the N60 with 12,1%. The **MicroSoil** alone increased the production with 35,4%. The treatment with N30PK60 we could reach a production of 45%. In combination MSPK the MS could replace the effect of nitrogen and the production was increased with 8,3%.

The constituents (digestion) can be improved by total nutirent supply. Because the receipt of the product is depending on the sugar content (digestion), it is necessary to evaluate these data as well The reacheable mass of sugar and the root production was different related to the treatments. The difference between the treatmernts are diverse. The difference between the NPK and MS is decreasing from 9,6% to 8,7% and increased for the MSPK related to the NPK form 8,3 upto 15,4%.

SUMMARY

Evaluating the results of the trials of the 2001-2002 years we can state the efficiency of the bacterium fertilizers are influenced by several factors therefore the test shall be continued also in form of pots, small parcels and in fields. The differences in scale show the variances in details and promotes the elaboration of suitable production technologies.

In this year only two species of plant have been tested with corn and sugar beet which are the most important fodder and industrial plants. The variations of the treatments have been extended. The data of control means in this respect the conditions after harvest and this was related to the effectivity of treatments, the absolute control meant the basic nutrient content of the soil at the very beginning of the tests.

Testing the development of corn in pot-tests it was found that the most reliable starting increasing was observed using the „conventional” treatment with organic manure and inorganic fertilizer. At the second month, in the following period the effect of MicroSoil bacterium fertilizer was remarkable and overtook the conventional treatments. A consequence could be stated that the effect of inorganic fertilizers are rapid however the effect of MicroSoil is more continuous and durable. The effect of greater amount of nitrogen fertilizer due to the greater amount of beneficial rainfall and independent from the treatment of the MicroSoil, produced a significant development. In the last period the effect of bacterium fertilizer presented a prominent durable and reliable development.

The increase in production due to the treatment with MicroSoil was alone 18,6% and 30,1% when supplemented with 60 kg/ha P and K inorganic fertilizers related to the control.

At the tests with sugar beet in pots we got also reliable results. The balanced, harmonic nutrient supply especially the effect of organic manure have been prominent. The effect of inorganic fertilizers in the later 2.-3. month period of the cropping season was not as perceptible against the effect of MicroSoil. The root-mass increased on the treatment with MicroSoil increased with 7,3% and with the supplemented treatment with 60 kg/ha P and K treatment about 10,4% against the control. This increased also the sugar content. The result having got in small parcels are confirmed.

The soil life was also prominently increased. The enzyme activity increased with 275% after treatment with MicroSoil, with the PK fertilizer the combined effect varied with 168% related to the control. The phosphatase activity increased with 172%, the carbon-dioxide production with 330% and the biomass with 180% against the control. The effect of MicroSoil was prominent. In order to confirm this the durability of the post-effect shall be tested in the future.

The small parcel trials were made with treatment on corn and sugar beet. The results were in these tests similar to field tests and also the relation between bacterium fertilizer in treatment-environment-plant could be tested.

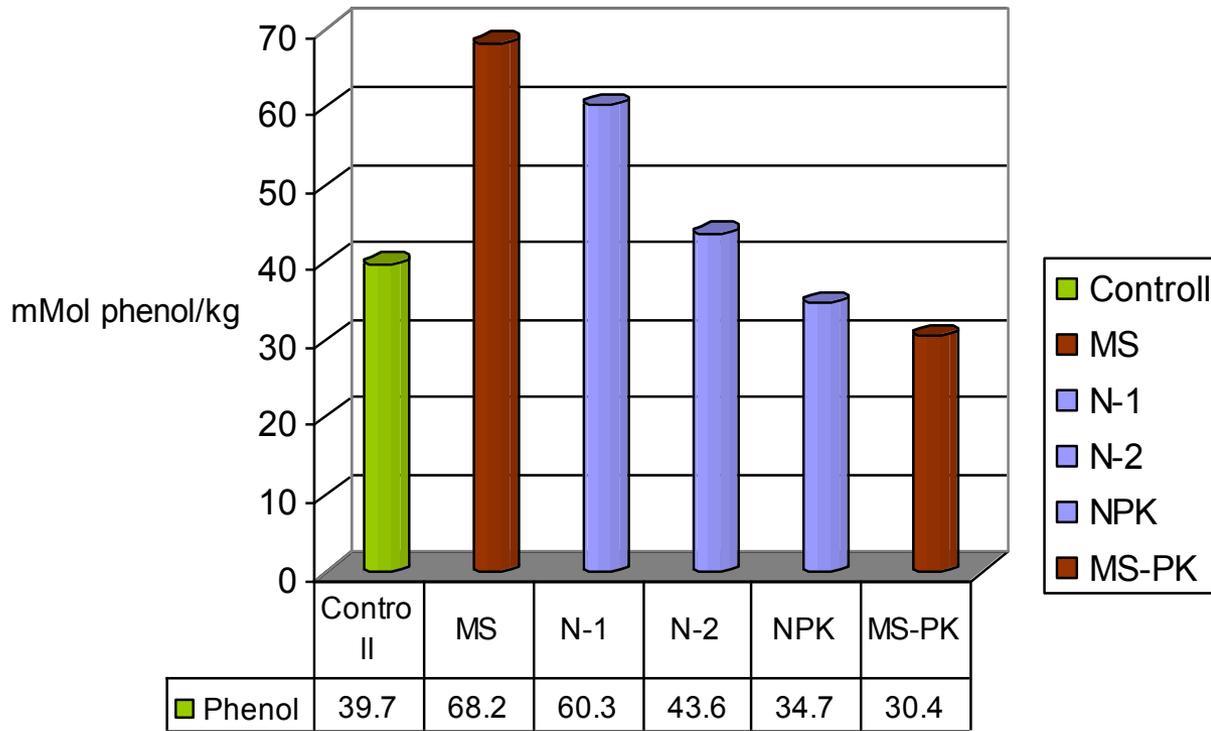
It can be seen in small parcel tests that as an effect of bacterium fertilizer the increase in production was 18% and in combination with inorganic fertilizers 20,2% related to the control. They can reach or overtake the conventional treatments with inorganic fertilizers and the MicroSoil can supplement totally the nitrogen fertilizers and also the exploitation of phosphate resources can be also promoted.

The tests with sugar beet in small parcels the effect of stock effect and the environmental effect prevails successfully. We got reliable production and the quality was also in correlation with the real results.

The mass production of sugar beet after the treatment with MicroSoil increased with 35,4%, the sugar content of the beet increased from 14,5% up to 15,2% and the relative sugar mass increased with 49,2% related to the control. Combined with inorganic fertilizers, 60 kg/ha PK, the production increased with 53,3%, the sugar content of the beet from 14,5% up to 16,5% and the relative mass of sugar increased with 73,3%.

As a consequence we can state that the trials in this year were successful. The tendencies have been confirmed our aims and for the further proofs and in order to the elaboration of reliable technology the durability and the season-effect should be tested in the next year as per program.

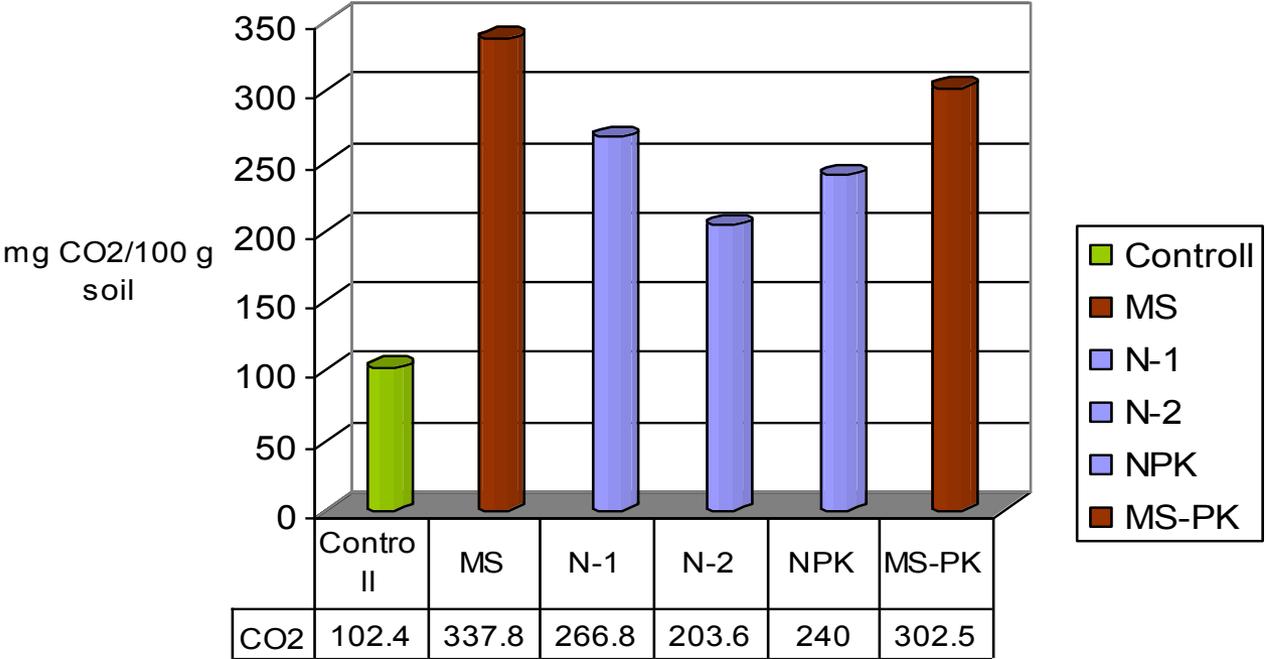
Phosphatase activity



Treatments

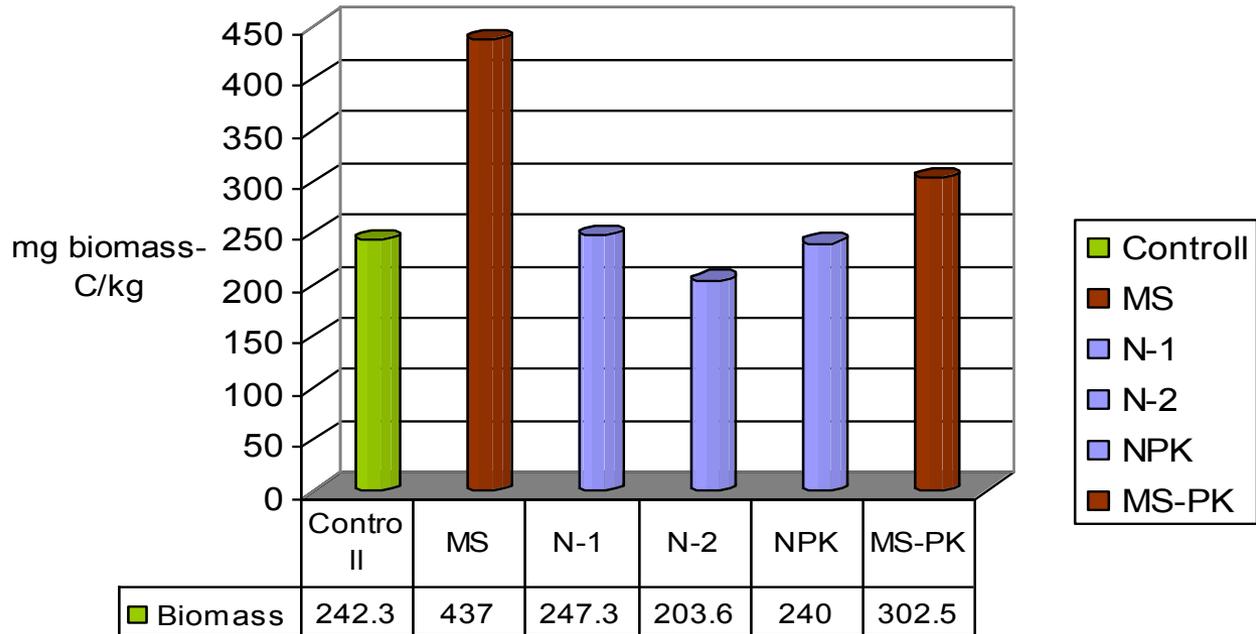
Figure 1/2

CO2 production



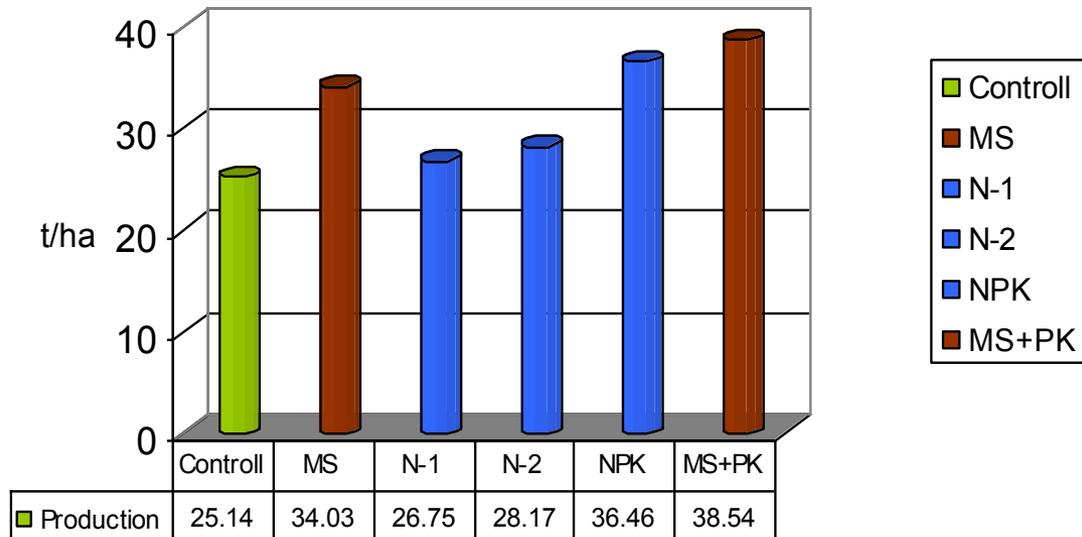
Treatments
Figure 1/3

Microbial biomass



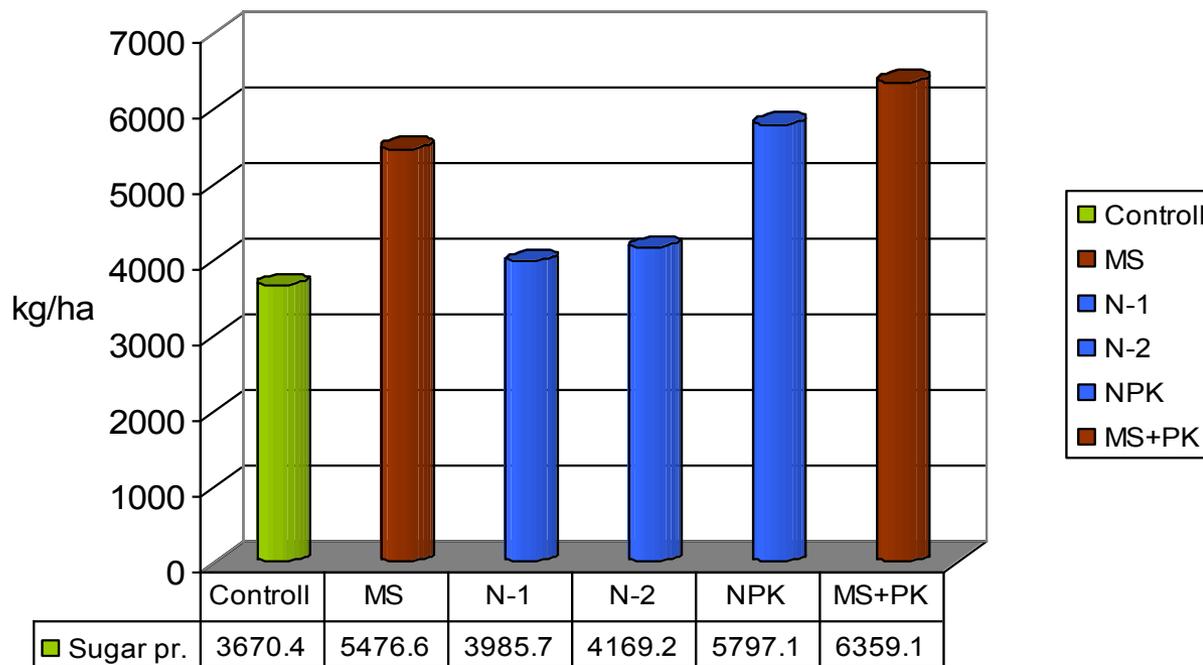
Treatments
Figure 1/4

Effect of treatments on sugar beet production.
(in small parcels)



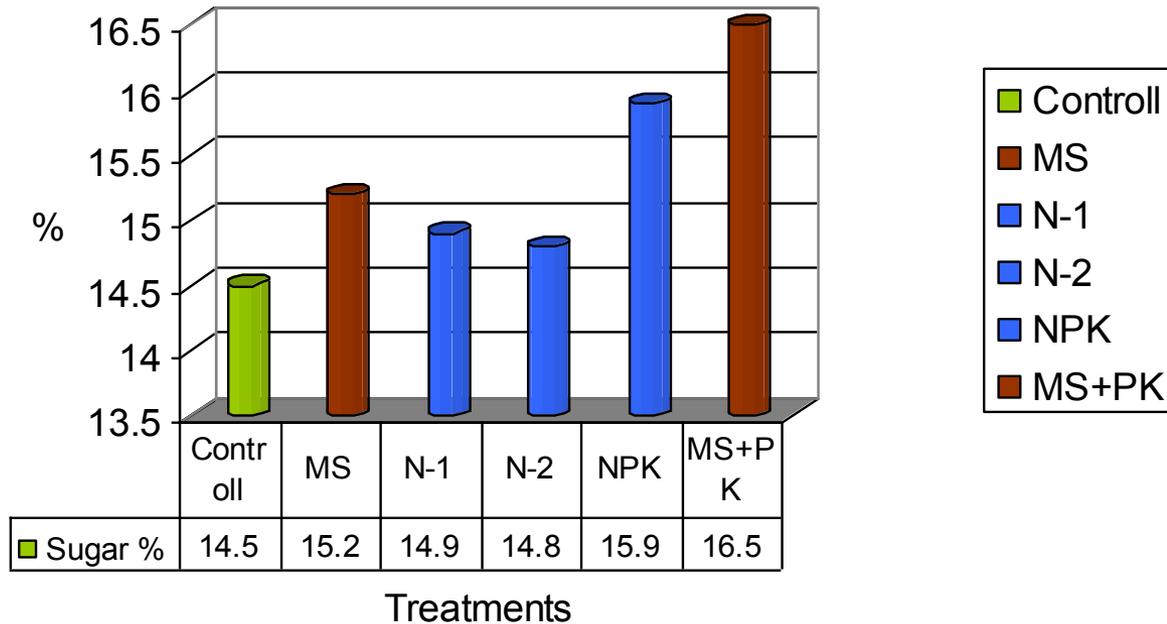
Treatments
Figure 6

Effect of treatments on sugar production of sugar beet.
(small parcels)



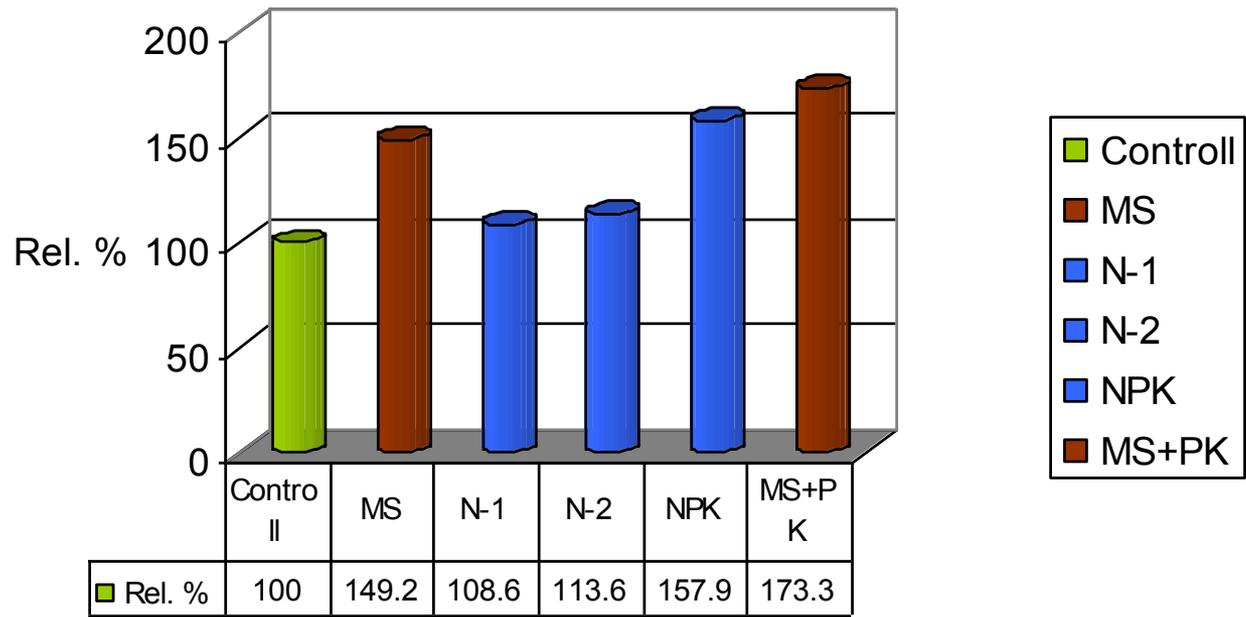
Treatments
Figure 7

Effect of treatments on sugar %.
(small parcels)



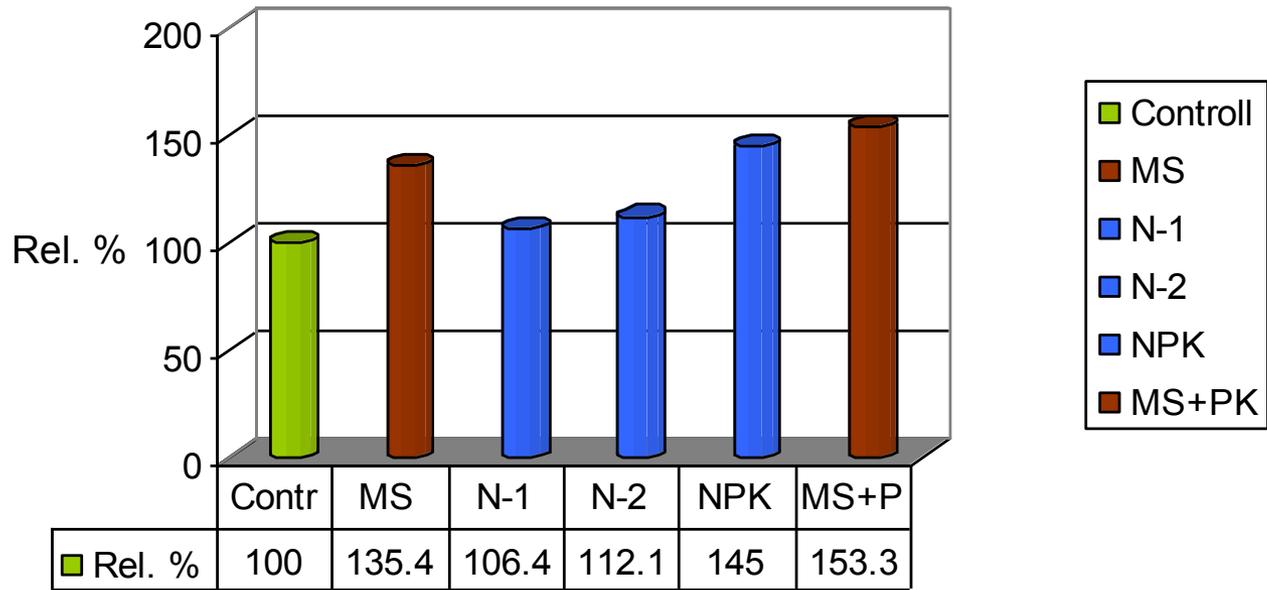
Treatments
Figure 8

Effect of treatments on sugar masse in rel. %.



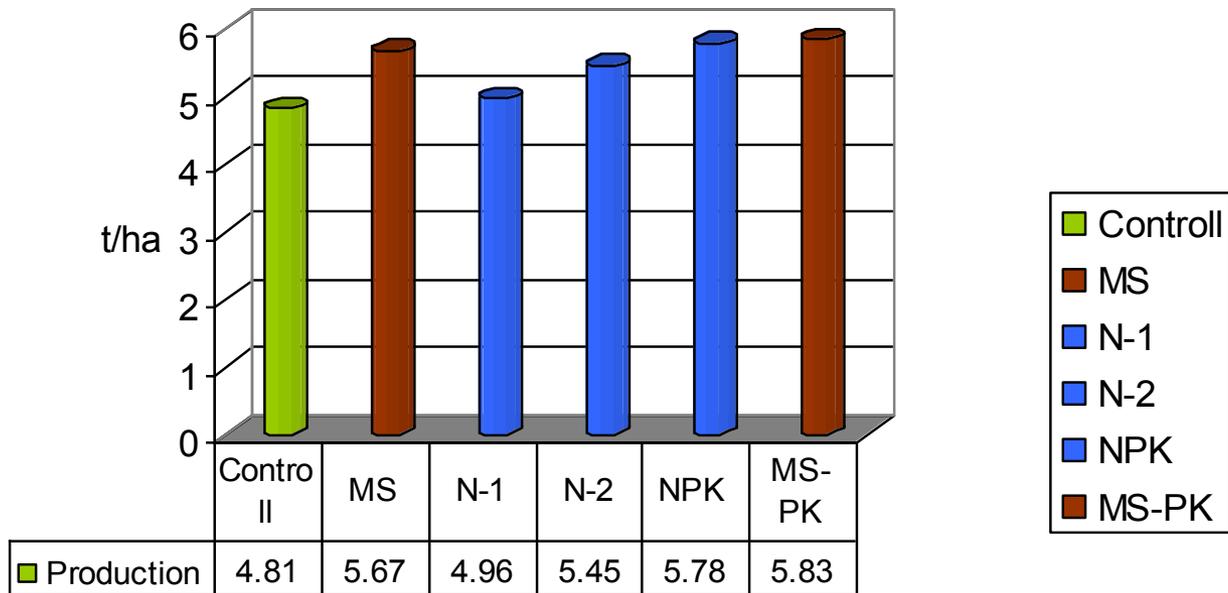
Treatments
Figure 9

Effect of treatment on sugar beet production in rel. %



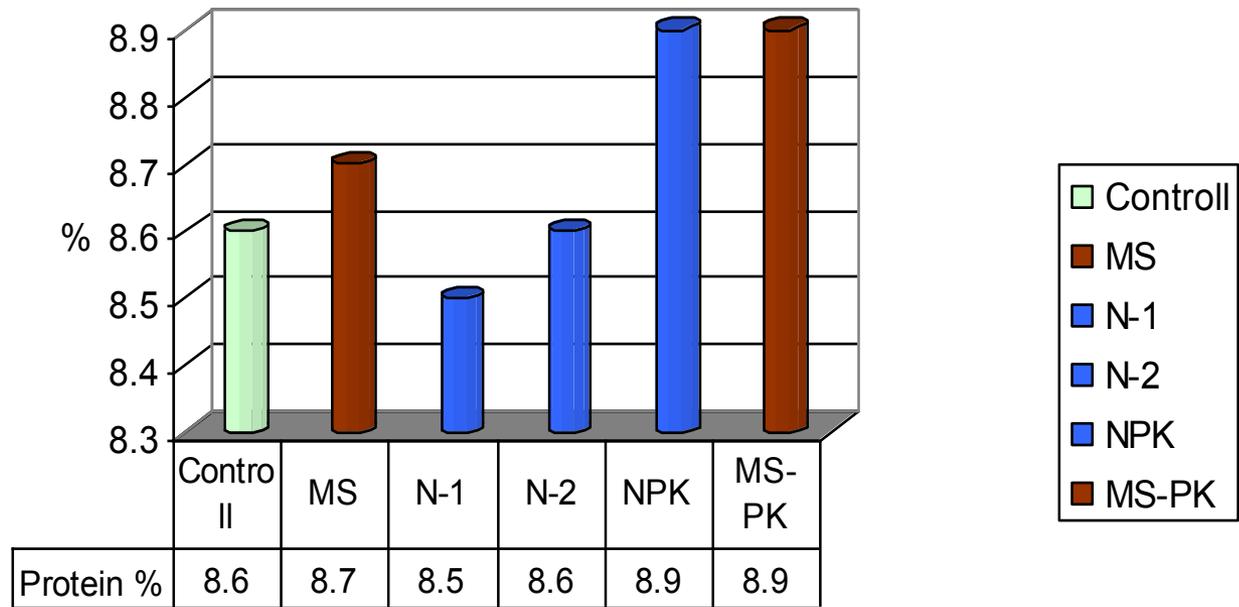
Treatments
Figure 10

Effect of treatments on production of corn (in small parcels)



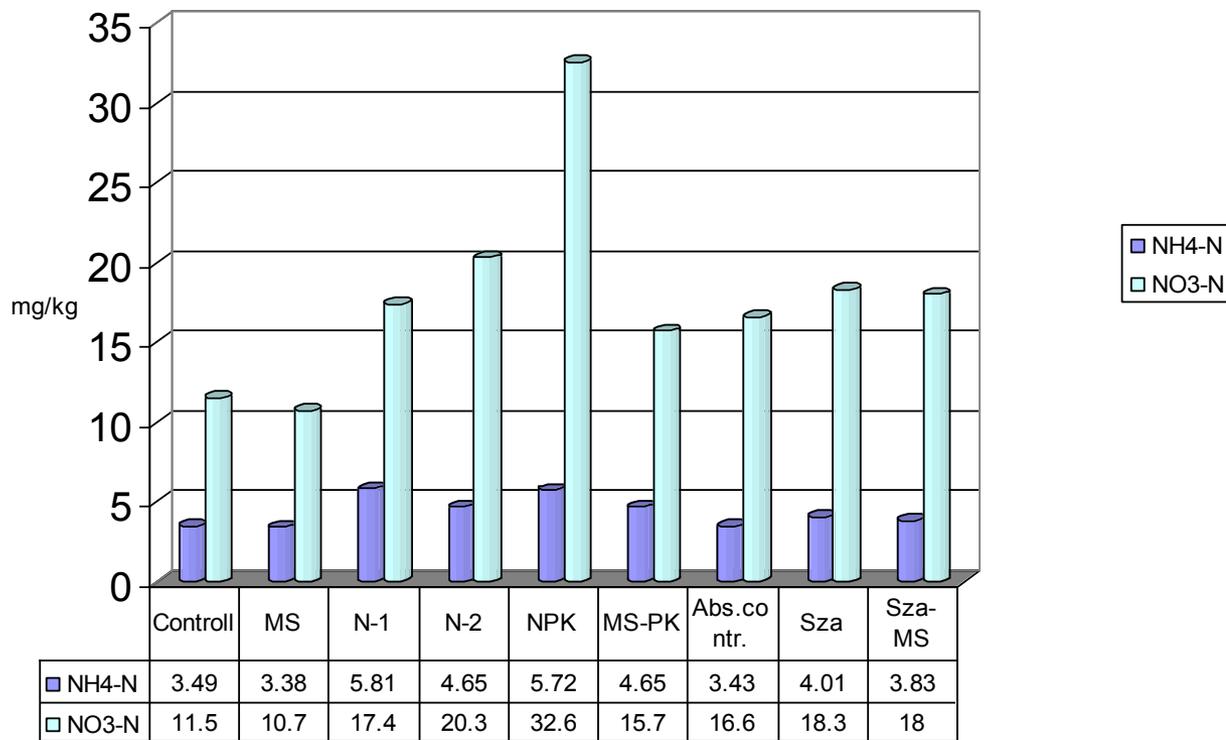
Treatments
Figure 4

Effect of treatments on protein content of corn.
(in small parcels)



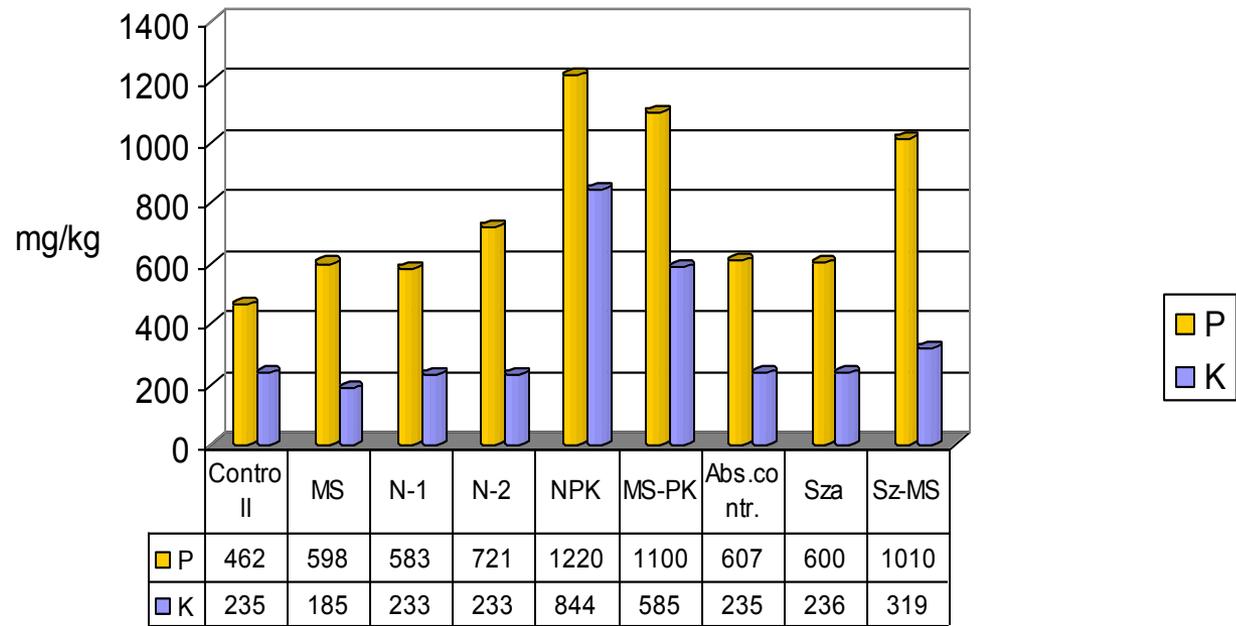
Treatments
Figure 5

Available nutrient content of soil in small parcels



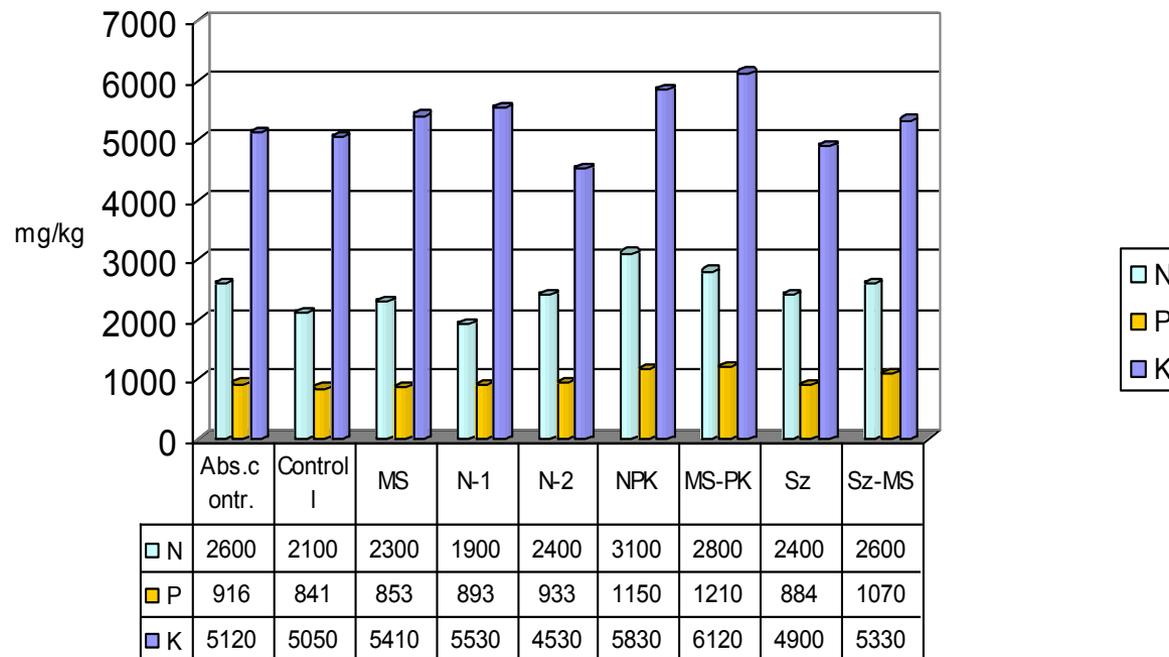
Treatments
Figure 2.1

Available nutrient of soil in small parcels



Treatments
Figure 2.2

Total nutrient content of soil in small parcels.



Treatments
Figure 3