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Quantitative Assessment of Some Soil Health Parameters

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Abstract

The definitions of such concepts as soil, soil ecosystem, soil health, microbial community, etc. are done. A fundamental substantiation and practical protocols of proven methods for analyzing the number and activity of soil microorganisms in natural ecosystems are presented. The necessity of an analytical definition of innovative parameters of the soil ecosystem - soil health is substantiated. The protocols for the quantitative determination of the heterotrophic soil health parameter and the estimation of the parameter of soil health as "self-supply" with biophilic elements (N, P) are presented. Possibilities of using quantitative values of soil health parameters for choosing directions and specific methods of healing and treatment of a diseased soil ecosystem are considered.

Keywords: Ecosystem; Soil; Microbial Community; Microbial Activity; Soil Health; Parameters; Biophilic Elements; Diagnostics

Abbreviations: CFU: Colony-Forming Units; SS: Soil Suspensions; FITC: Fluorescein Isothioceanate; HPSH: Heterotrophic Parameter of Soil Health; PSBE: Parameter of its Self-Sufficiency in Biophilic Elements; SIR: Substrate Induced Respiration

Introduction

The soil is an object of study and research of geologists and soil scientists, agrochemists and agronomists, biologists, ecologists and soil scientists. Soils have many characteristics. They are evaluated and classified by type, properties and modes of use. Soils are diversified, often with the same or similar methods, pursuing different goals and objectives. A variety of biological and physicochemical processes, cycles of microorganisms and biophilic elements occur in soil. A unique natural object, traditionally referred to as soil, has different definitions. Due to combination of biological and geochemical processes, the soil has (up to a certain limit!) buffer capacity in relation to various disturbing influences. Most often, when studying and researching soils, the peculiarities of their coming to existence, origin and evolution, applying, as a rule, available physicochemical concepts and characteristics, are taken into account. Without diminishing the role of knowledge accumulated on the soil as a physicochemical substance, it is important to use such its definition that would more fully take into account modern knowledge. This is, first of all, the idea of the soil as a multi-phase global ecological system, without which neither earthly biodiversity, nor the special unique world of soil biota is possible.

Enough knowledge has already been accumulated in order to reasonably accept and use the concept of a soil ecosystem instead of the traditional, concise name - just soil. The concept of "soil ecosystem" most fully reflects the content of the unique natural formation, represented by modern soil. The soil ecosystem makes it possible to more objectively characterize its biotic component from a scientific, practical and consumer point of view. The concepts of "soil ecosystem" directly makes aware both practitioners and academicians of the need of distinguishing several of its categories, primarily natural and exploited by man. Modern social systems in a technologically transformed agroecosystem use intensive technologies (convention agriculture), organic (organic agriculture) and / or transitional, mixed technologies (low input). Depending on the system of operation (degree of man-made intervention) different quantities and quality of bioproduction as well as the resulting risks and problems should be expected from the soil ecosystem. Depending on the purpose of use of the soil ecosystem, a user must build a strategy for its maintenance and development, as well as protection from a variety of natural, man-made and social impacts. We emphasize that only for the purpose of brevity, in the present Guide, the terms "soil" and "soil

ecosystem" are used interchangeably with the dominant term - a soil ecosystem.

The soil ecosystem (soil) is the product of a long-term mutual assimilation-dissimilation activity of microorganisms and plants in the dominant mineral-organic matter. Modern soil (soil ecosystem) is an organic-mineral natural product created and maintained in accordance with the local climate regime by continuous micro-plant interaction in the initially quantitatively dominant inorganic substance. The product includes biota (organisms), its mortmass, residues and metabolites, biophilic, macro and mycoelements. Biological and physicochemical processes occur continuously in this product — biogeochemical cycles of elements and cycles of microorganisms [Kupriianov et al., 2014]. The soil ecosystem has significant buffering properties against a variety of stressors, provides plant nutrients and soil biota, and serves as a source and stock of biodiversity. It is the biological component of the soil ecosystem that creates and maintains the ecotopic soil environment, which, in turn, ensures the production, preservation, reproduction and functioning of the soil biota. That is why biologically ecological characteristics, such as soil health and / or its pathology, are valid and applicable to a sustainably functioning open soil ecosystem. Among the many characteristics and properties of soil biota, it is important to emphasize its self-reproducibility, self-sufficiency and dynamism.

Traditionally common categories characterizing soils are its quality and fertility. The quality of the soil is mainly expressed in physicochemical concepts and characteristics. Soil fertility - "birth of a fruit" is a consumer, anthropogenic characteristic of the soils of agrocenoses Semenov, Sokolov [1], Semenov [2], Semenov [3]. In the late 90s of the twentieth century, a new category appeared in demand, which more fully characterizes the biotic component of the soil ecosystem. This modern category, which takes into account the characteristics and relationships of soil biota with the abiotic component, is soil health. The health of a soil ecosystem is a biological category reflecting the state of the dynamics of the activity of the biotic component in the organo-mineral complex of the soil; this category is characterized, in accordance with the natural climatic zone, adequate activity of biotic processes (synthesis and hydrolysis), their resistance to disturbing influences (biotic and abiotic stressors), "closed" cycles of biophilic elements and microorganisms. The soil health of agroecosystems is also characterized by the compliance of its material and biotic composition with the normative indicators and adequate fertility. Such a definition of health of a soil ecosystem, applicable to any soil (excluding anomalous), does not contradict the substantive essence of traditional characteristics, since it integrates their content. Indicators of dynamics of the biotic component activity are interrelated with the quality of the soil, and with its actual fertility Semenov, Sokolov [1].

Let us consider the key concepts used in this manual. They are soil ecosystem, soil health and, of course, microbial community. With reference to any ecosystem, and especially to soil, microbial diversity is considered not as a set of homogeneous, isolated populations ("pure cultures"), but in the form of microbial communities. A microbial community is "a certain aggregate of taxonomically different but functionally interacting populations of microorganisms that exist for some time in a particular place." The components of the microbial community can be both closely interrelated (including physically), and the microbial community can be (with MC being) highly specialized, and weakly related, and the microbial community can be low specialized Semenov [4] Microbiology Manual, 2016. The more objective the concept of the structure of the microbial community is, the higher its informative and prognostic properties are, and the more likely is possibility of making the right decision in environmental and industrial biotechnology Semenov, Đukić [5]. The concept of the microbial community structure is supplemented by the following concepts: • r -K - continuum, reflecting the continuity and discreteness of the properties and distribution of microorganisms; • undulating development of microbial populations and microbial community; • regulation of the activity of natural microbial community, in accordance with this concept MC activity changes mainly by changing its structure - that is, through succession; • oligotrophy, according to which ecosystem oligotrophication is one of the mechanisms for maintaining healthy soil.

This Guide is intended to teach the user practical work. In general environmental terms, it allows to determine and quantitatively characterize biological activity of the soil, more specifically, to determine the number and activity of soil microorganisms to characterize its health parameters. The proposed approaches are quite acceptable for determining the "biological activity" of aquatic ecosystems. The purpose of the scientific - methodological guide is to propose protocols for the determination of colony-forming units of bacteria (CFU) in soil samples and / or soil microorganism activity (in the form of CO_2 emissions), allowing user to calculate health parameters of the soil ecosystem and diagnose its condition. The manual was created on the basis of the original long-term methodological development (research) of the authors, patented, tested and described in numerous publications Semenov [6], Semenov [7], Semenov, Sokolov [1].

Determining of the Number and Activity of Microorganisms in the Soil Ecosystem, for Characterizing of Soil Health Parameter

From the point of view of a microbiologist, the soil ecosystem is a variety of microorganisms living in the mineral-organic substrate. Although the concept of microorganisms includes everything that is not distinguishable with the naked eye, in the everyday scientific presentation they usually include bacteria (prokaryotes) and fungi (eukaryotes) - mycelial (micromycetes) and unicellular (yeast). Microscopic protozoa, algae and other microbiota, due to biological traditions and its ecological functions,

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although can be taken into account, but only when solving specific tasks. Why do we need information on the number and activity of microorganisms living in the soil? The laconic answer is to understand their ecological role and use the knowledge gained for practical purposes.

There are several widely used methods for determining the number of microorganisms. Each of them has its advantages and limitations. The direct counting (counting) of bacteria using a light microscope (with its modifications and the corresponding dyes, traditionally referred to as the method of S.N. Vinogradsky, allows determining the total number of prokaryotic cells in the soil (mainly represented by bacteria). The advantages of this method are that it allows you to take into account almost all microorganisms. This is necessary to solve cognitive tasks, for example, to know the number of bacterial cells in one gram of soil, to determine the microbial biomass in the soil, etc. It is important for sanitary practice to identify pathogenic microorganisms, their cycles and possibilities of transfer from one habitat to others Kupriianov [8]. Disadvantages of the method are its complexity, high cost of equipment, the need for highly qualified personnel. A detailed description of the method and modern dyes is available in the manual Semenov, Shatalov [9], Semenov [10].

The determination of the number of active, viable, growing on different media microorganisms is carried out by their cultivation on nutrient media. These methods are based on the assumption that all cells in the sample under study show growth in the medium. Ideally, for this you need to have a universal environment that provides conditions for the growth of the whole variety of microorganisms, which is unattainable, first of all, precisely because of the diversity of their properties. Note that at the present time, microbiologists have succeeded in creating elective microbial media and conditions, that is, in differentiation of nutrient substrates, but not in their integration Methods of General Bacteriology [11], Methodological Guide [12], Practical Training in Microbiology [13], Practical Training in Microbiology [14].

At present, either solid media (mainly agarized) or, more rarely, liquid nutrient media are used for mass analysis. Grown colonies on solid media are counted, assuming that each colony is the result of reproduction of a single cell! Since this is an assumption, the number of colony forming units (CFU) is referred to rather than the number of cells determined by the seeding method. The method of counting CFU is quite simple and very well worked out, therefore, it has received universal recognition. The method of determining the number of soil microorganisms in liquid media is used less frequently. It is also known as the "limiting dilution method" (MPR) (most probable number method). The method is based on the same assumption and the assumption that each cell multiplies and the growth (clouding of the medium) found in the last test tube of dilution series is the result of the reproduction of that only cell that was found in one milliliter selected from the penultimate dilution. To calculate the number of microorganisms in the sample to be analyzed, a McCredy table is required. It was created based on the processing of numerous experimental results by the method of variation statistics Practical Training in Microbiology [13]; Practical Training in Microbiology [14].

The method of limiting dilutions, apparently, is not applicable to characterize the health of the soil. It provides for the activation of microbial populations in a soil sample by introducing a substrate (glucose in our method). The preparation of dilutions of microorganisms in a fresh (!) media makes it difficult or even impossible to reveal the wave-like development in the form of growth cycles and the death of microbial populations and communities (which is methodologically very difficult!). In addition, there is also a "double" stimulation of the growth of microorganisms by the substrate Methods of General Bacteriology [11]; Methodological Guide [12]; Practical Training in Microbiology [13]; Practical Training in Microbiology [14].

Monitoring and quantitative determination of the activity of microorganisms living in the soil, carried out by different methods. There are no substances on Earth that are not exposed to microbial enzymes. The depth and speed of manifestation of this effect is very different. The variety and significance of the transforming activity of microorganisms is essentially limitless. The soil itself is a product of the active interaction of microorganisms and plants. Since, according to the ecological rule: "the microbial process is only then significant and noticeable, if there are many microorganisms carrying out this process and they are active" Semenov Shatalov [9]; Semenov [10], we will limit ourselves, firstly, to assessing the activity of only soil aerobic heterotrophic microbial communities, and secondly, to defining only activities that can be expressly recorded and quantified (see below, Chapter 2).

Selection of the soil samples and their preparation for microbiological analysis

According to the traditions of soil science, an average soil sample is prepared by mixing individual samples taken from 3-5 points with 100 m^2 of cultivated soil or from 15 points per 1 hectare. Samples are collected by a soil auger, deepening it to 20 cm. Before each operation, the surfaces of the auger are sterilized with ethanol. When sampling from fallow lands, one should first remove the soddy layer (≈ 2 cm), only after that soil samples should be taken. The samples extracted from the drill are placed in fresh plastic bags, closed and signed with marker (indicating the date, time, site address, etc.) Zvyagintsev [15]; Methodological Guide [12].

When preparing the sample for analysis, the soil is mixed, removing pebbles, roots and biota (macroscopic dimensions), then the soil is sieved through a sieve with a cell size of 2 mm. Before each operation, the surface of the sieve is sterilized with ethanol. To determine the soil moisture by a gravimetric method, a sample of the soil (1 ÷ 5 g) in heat-resistant containers is taken from each sample, and the samples are dried in a drying cabinet at

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100 ÷ 105°C, during the day. Equipment and tools. 1. A soil auger with an inner diameter of 2 cm. 2. Fresh plastic bags. 3. Ethyl alcohol. 4. Sterile wipes for sterilizing auger. 5. Marker. 6. Sieve (cell size 2 mm). 7. Metal spatulas to remove soil from the drill and its mixing.

Determination of the CFU number of bacteria in a soil sample by plating the soil suspension on agar media for soil health parameters

The determination of the number of native microorganisms in the soil, in particular, bacteria, is carried out by growing them on nutrient media. Soil microorganisms differ in their requirements for the biological and physicochemical characteristics of the nutrient medium. The physicochemical characteristics include temperature, pH, rH, Eh, content of biophilic elements and compounds. The biological characteristics of microorganisms include, first of all, peculiarities of their metabolism: energy based (lithotrophs, phototrophs, organotrophs) and carbon based (autotrophs, heterotrophs). For the convenience of operating with a variety of microorganisms, they are divided in accordance with metabolic features and capabilities into "specialists" and "generalists"; by response to the content of organic carbon in the medium - to "copiotrophs" and "oligotrophs"; by response to enrichment / depletion of the habitat with an organic substrate and growth rates - to r-L-K- "strategists" Semenov [16]; Semenov [4]; Semenov, Đukić [5]. There is also a division of microorganisms into groups according to functional and physiological features.

From the above it is obvious that there cannot exist universal nutrient media and conditions that allow to grow all the microbial diversity of the soil. Therefore, use of such a conditional subdivision of microorganisms, which is objectively applicable to different metabolic groups, such as "majority" and "minority", is justified, although this division has recently been revised. However, by the end of the 20-th century, using cultured methods of moderately "poor" heterotrophic media, while optimizing physicochemical conditions of cultivation in soil suspension, it was possible to count the number of bacterial cells, quite comparable to their number, counted by direct counting of bacteria in the soil suspension by fluorescent microscope. Therefore, despite dominance of molecular-biological methods in recent years for estimating the number of microorganisms, it is quite reasonable and justified to use for practical purposes well-known optimal nutrient media and cultivation conditions by counting CFU for determination the number of microorganisms (bacteria, micromycetes) Semenov [16]; Semenov, Shatalov [9]; Semenov [17]; Semenov [18]; Van Bruggen, Semenov [19].

Composition and preparation of the medium to obtain CFU of bacteria: To determine the number of native microorganisms in the soil, nutrient media and regulated cultivation conditions are necessary. Liquid and solid (overwhelmingly) agarized media are used to account cultivated saprotrophic microorganisms, bacteria and micromycetes. Many recipes of such media are known, collections of their formulations and cultivation conditions for different physiological groups of bacteria have been published Methods of General Bacteriology [11]; Microbiology Manual [20].

For the purposes mentioned in this Guide - accounting soil suspension of mesophilic, saprotrophic, copiotrophic bacteria (i.e., cultured, active set) - the following medium composition is recommended (g. / l. distilled water): $MgSO_4 \bullet 7H_2O$ - 0.5 ; KNO_3 - 0.5; $K_2HPO_4 \bullet 3H_2O$ - 1.3; Ca (NO_3) 2 $\bullet 4H_2O$ - 0.06; glucose - 2.5; enzymatic casein hydrolyzate - 0.2; bacteriological agar - 15.0; pH = 7. Sterilization is carried out twice. The first time the composition is sterilized without glucose and casein at 1 atm. Then again, after inserting (administering) glucose and casein it is sterilized at 0.5 atm. When large number of micromycetes is expected to be found in the analyzed soil, it is recommended to add 100 ppm of cycloheximide to a liter of sterile medium. The total amount of carbon available for bacterial growth is estimated at 1000 mg / l of medium. Repeated experimental verification has shown that the composition of such a medium allows obtaining the maximum number of CFU from both rhizospheric and non-rhizospheric soil Semenov [18]; Van Bruggen, Semenov [19]; Zelenev [21].

The amount of medium is prepared on the basis of the number of soil samples analyzed and the number of staff. The duration of analyzing of each sample is (4 - 5 days, one analysis / day). The day before their use, the medium, is "melted", cooled down to ~ 50 °C to prevent the formation of abundant condensate on the surface of frozen agar and at the flame of a gas burner, poured into prepared sterile cups, taking into account: the number of analyzed soil samples, the number of staff, duration of analyzing of each sample (4-5 days). Depending on the used cups - glass or plastic - 13 to 17 ml. of agar medium are poured into each cup. After solidification of the agar medium, the cups are turned upside down and left at a room temperature for at least the next day to dry the surface of the medium. On the outer surface of the bottom of the cups put the necessary information (date, etc.) with a marker. Necessary equipment, tools and reagents. 1. Scales (analytical). 2. Marker. 3. Dishes and reagents for the preparation of a nutrient medium. 4. Autoclave. 5. Sterile Petri dishes. 6. Gas burner (alcohol lamp). 7. Laminar box.

The procedure of plating soil suspension on agar medium: One gram of soil (prepared as described above, section 1.1) is weighed, the sample are transferred to test tubes with 9 ml. of sterile distilled water. Spatulas for the selection of soil weights are sterilized with ethanol before each operation. Soil suspensions (SS), obtained in the first test tubes, for greater desorption of microorganisms from soil particles are subjected to ultrasonic treatment. The treatment is carried out according to the instructions for the ultrasonic device or according to the recommendations of D.G. Zvyagintsev Zvyagintsev [15]: frequency 15 kHz, current power 0.4 A, exposure - 3 min; heating of the soil suspension during this operation should be prevented.

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After ultrasonic treatment immediately proceed to a tenfold dilution of suspensions. To do this, after shaking it on the "shaker" (for example, Vortex company, for at least 5 seconds, prevent cap of the shacked tube from soaking!) With a sterile tip or a sterile pipette, take 1 ml. of the soil suspension. An aliquot is transferred to another tube with 9 ml. of sterile (distilled) water. This procedure is performed to obtain a dilution of suspension 10-5 and / or 10-6, depending on the expected abundance of bacteria in the samples. Shaking the tube with the soil suspension on the Vortex (for at least 5 seconds) is done every time before taking an aliquot of the suspension; the procedure of dilution of soil suspension is always performed only with sterile tips (pipettes).

An aliquot of 50 µl is taken from the last dilution and the Petri dishes are placed on the surface of the nutrient agar (in the middle of the cup). Aseptically, at the flame of a gas burner under the lid of the cup, suspension is distributed (rubbed) with circular motions with a sterile (preferably glass) spatula over the surface of the agar medium; each Petri dish is signed with a marker on the outer side of the bottom of the dish (indicating the degree of "dilution", the date of sowing and other necessary information). The cups are thermostated (at 28 °C) by incubating them upside down. Required equipment, tools: 1. Test tubes with 9 ml. of sterile distilled water, closed with air-permeable stoppers, pH neutral, 5-6 test tubes for each analyzed soil sample and taking into account the number of staff. 2. Spatulas for soil selection. 3. Ultrasonic device. 4. Ethanol. 5. Spatulas, preferably glass, sterile, for the "distribution" of the soil suspension over the surface of the agar medium in a Petri dish. 6. Vortex type shaker. 7. Automatic pipettes and sterile tips with a volume of 0.1 and 1.0 ml or traditional sterile pipettes (with a cotton swab!). 8. Marker. 9. Thermostat at 28o. 10. "Contact" colony counter.

CFU counting: After the time of incubation of crops (≈ 60 hours), the grown colonies are counted, followed by calculating the number of CFU per 1 g of absolutely dry soil. For convenience, each recorded colony on the outside of the bottom of the cup is marked with a marker. An indicator of the optimality of the used cultivation of soil suspension is growing on a cup from 50 to 150 colonies. With a large number of colonies on the cups and / or with a large number of cups, it is desirable to use a contact counter of colonies to account CFU.

Digital results of counting colonies are entered in an Excel file, in tables. All other results are entered in the tables of the Excel file, for example, concerning the determination of the moisture content of soil samples, etc. The following procedure is proposed for labeling and filling of the tables in the Excel file, which is approved for operations with a large data array. Excel tables have columns labeled by letters and the lines labeled by figures. It is suggested that the first column in the table be named (labeled) in the row cell as [No.] and subsequently placed vertically with sample numbers, dates of crops, etc. It is strongly recommended that all so-called "intermediate" data be entered into the tables; further calculations of the main indicators will be made. Further, several columns (up to five) will be occupied by the data necessary for calculating soil moisture. The next at least three columns will be occupied by the CFU data for each cup and the column for calculating the average value for three cups.

In the next column (No. 11) enter the data on the number of CFU calculated in as per the formula: CFU = (a • b • c / d). Legend: a - the number of CFU, the average value of the three cups; b - is the numerical coefficient for bringing the applied volume of the soil suspension into a Petri dish to 1 ml; c - is a numerical factor equal to the order of dilution of the suspension, for example, 10-5 or 10-6; d - mass of the soil sample after drying 1 g of the original native soil. The results are calculated for at least three replicates of samples, which makes it possible to calculate the average value of the number of CFUs and the standard deviation Semenov [3]; Zelenev [21].

Determination of the bacteria cells number in the soil suspension by total microscopy counting

The data necessary for determining the parameter of soil health can be obtained by accounting of microorganisms under a microscope. The complications and limitations of this method were mentioned above. In addition to the complexity of the method, when using the simplest approach to the registration of bacteria cells in the soil suspension, all cells are taken into account: live, active; alive, but not active (viable); dead but still intact cells. To determine health of the soil, of course, only living and active organisms are significant. If you use differentiating dyes, for example, "live - dead kit", then all the same, live, but inactive, surviving (time and place!) cells will get into the recorded organisms, which can lead to significant errors.

In the framework of author's experiments was carried out comparison of the counting of bacteria under a fluorescent microscope using fluorescent dyes Fluorescein isothioceanate, FITC (detecting all intact prokaryotic cells) Fluorescein diacetate, FDA or 5-Sulfofluorescein diacetate, SFDA (detecting cells of physiologically active prokaryotes). It was found that only ~ 10% is detected by FDA or SFDA staining compared with the amount detected by FITC. In other words, determination of the number of bacteria cells in the soil by the method of microscopic examination the dilutions of the soil suspension is not recommended for use for the method of determining the parameter of soil health. A detailed description of equipment and reagents, preparation of dyes, staining procedure, counting and calculation of the number of cells in a soil sample is given in the methodological manual Semenov, Shatalov [9].

The Activity of the Soil Microorganisms (Microbial Community) Assay in The Soil Samples

The observation and quantification of the activity of microorganisms living in a soil ecosystem can be carried out using several methods. These methods also have their limitations due to specificity and limited sensitivity. General methods as well as specific group methods are known. In particular, there are known methods of determining microbial activity while decomposition

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in the soil (by microbial community) of various polymers. The types of relevant activities are called: cellulolytic, proteolytic, nuclease, chitinase, etc. There are known methods of determining methanogenic and methanotrophic activities, sulfate-reducing activity, etc. However, to determine the most common activity of heterotrophic mesophilic aerobic prokaryotic and eukaryotic microorganisms, the method of measuring respiratory (oxidative) activity is most often used. Since some substrate is often introduced into the habitat of microorganisms to stimulate respiratory activity, which increases the "threshold" of the sensitivity of the method, this method was labeled with the abbreviation (or was named after the abbreviation) SIR - substrate induced respiration.

Methodically, the respiratory activity is determined and calculated by the amount of absorbed oxygen or the amount of emitted carbon dioxide (CO_2). Stoichiometric ratios between the amount of absorbed O2 or released CO_2 have already been calculated for most of the known organic substrates. Due to the development of chromatographic technology, its miniaturization and high sensitivity, this method of quantitative determination of CO_2 emitted by soil samples has become widespread Semenov [22]; Semenov [2]; Semenov, Sokolov [1], (http://bankpatentov.ru/node/62779). In this Guide, we will limit ourselves to consideration of assessment of the activity of the soil microbial community by the method of quantitative determination of CO_2 emitted by soil samples after initiation of respiration by a disturbing effect - by introducing glucose solution into soil samples Semenov, Bubnov [3].

The substrate induced respiration (SIR) of soil microorganisms (microbial community) by gas chromatographic method for characterizing their activity at soil health parameter

The activity of microorganisms is a dynamic process, manifested in space and time. Therefore, the calculation of the activity of microorganisms includes such dimensions as time and volume (mass). In determining any type of activity of soil microorganisms, it is important to use the most recent soil samples. Long-stored dry samples are not suitable for determining the health of the soil. Since the initially selected samples may have different humidity, affecting the physiological state of soil microbial communities, it is recommended to dry the samples in air (but not overdry!). To do this, scatter the soil on a clean, dry surface with a very thin layer, dry in the absence of direct sunlight, without forced ventilation, and cover with large, clean paper napkins. At a temperature of "comfort", this procedure will take several hours (~ 2 - 5 hours). The drying procedure must also be carried out when transporting samples to the place of analysis. Sample preparation is carried out as described in section 1.1. Before carrying out the analysis for the activation of microorganisms, soil samples should be moistened.

Numerous experiments have shown that moistening should be carried out not only with sterile distilled water, but with a low concentration of glucose solution - 0.5 mg / g of soil. The volume of such a glucose solution for moistening a soil sample is selected so that the final value of the weight moisture of soil samples for determining the SIR does not exceed 18-20%. For example, if the moisture content of soil samples after drying was 5%, then to obtain the final value of its mass moisture content (for example, 20%), 0.15 ml of fluid per gram of soil must be injected. At the same time in the introduced volume - 0.15 ml / g, such amount of glucose should be contained so that its final concentration in 1 g of soil is 0.5 mg / g. Therefore, to moisten 100 g of the sample, it is necessary to prepare an appropriate volume of glucose solution. Moist and activated soils are immediately covered with plastic wrap (food grade, "saran wrap") to prevent the sample from drying out. The soil is packed in clean, dry (penicillin) bottles of such a volume that after adding the soil the volume of air space above it is at least three volumes occupied by the soil. For example, if you take 5g of soil into a vial, the total volume of the vial should be at least 15 ml. Pre-bottles labeled with the designation of options to be applied to the soil. Weighing procedures and packing of soil should be carried out as quickly as possible, after adding the soil to the vial, it is immediately closed with plastic wrap (food, "saran wrap") to prevent drying. Flasks with soil are placed for 2-4 hours in a thermostat with a constant temperature (for example, 22 ± 1 ºC) to "equalize" the temperature in the soil of the vials. The exposure temperature is determined experimentally.

A gas analyzer (gas chromatograph) is prepared for operation to determine the concentration of CO_2 in the sample. Flow-through gas analyzers are not recommended for determining CO_2 emissions due to the constant additional external influence on the soil microbial community (its aeration!). After removing the bottles from the thermostat, the polyethylene film from the bottles is removed and left for ~ 15 min to ventilate the CO_2 accumulated under the film. After airing the vials are tightly closed with standard rubber stoppers. To reduce errors in measuring CO_2 concentrations, it is highly desirable to keep vials, both open and closed with stoppers for a certain time, for example, 1 hour. With this purpose it is recommended to place the bottles closed with stoppers in the thermostat alternately, at the required equal intervals, using the numbering of the bottles to remove them for CO_2 measurements, observing the standard incubation time. A 1 ml gas sample is collected from the closed vials with a syringe (1 or 2 cm_3 in volume). To do this, insert the needle of the syringe into the gas space under the cap of the bottle, holding the plunger of the syringe in the extreme, zero position and holding it with your fingers. It is recommended that the end of the syringe needle be introduced into the gas space under the lid of the vial ~ 1-1.5 cm from the inner surface of the lid and about the same from the soil surface. Gas phase is collected by smoothly raising the piston of the syringe up to the end of the graduations on the syringe.

Without removing the syringe needle from the vial, smoothly pushing the syringe plunger down until it stops, squeeze the gas phase into the vial. For mixing the gas phase in the vial, this procedure is repeated 2-3 more times. After completion of the gas phase mixing in the vial (above the soil), a sample is taken for analysis. After collecting a gas sample into the syringe, gently

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withdraw the needle from the bottle cap, holding the syringe piston with your fingers and immediately turn the syringe needle up to reduce the possible gas leakage. The syringe piston is installed as precisely as possible at the 1 ml mark, the syringe needle is inserted into the gas analyzer and the gas sample is squeezed out vigorously but energetically into the analyzer until the piston stops. Vigorously withdraw the needle of the syringe from the analyzer, and, following on the monitor or on the recorder tape, complete the analysis of the introduced sample, repeat the procedure with the same soil sample at least 2 times. Thus, the analysis of each soil sample should be at least threefold. (Three bottles are prepared for each soil sample in advance). On the recorder tape make the necessary marks (time, sample number, etc.).

Note that the above sequence of procedures (paragraphs 1-11), refers to the very first stage or the zero procedure of analyzing each soil sample. The subsequent analysis procedure consists of two phases. The first phase (I) is determination of the concentration (emission rate) of CO_2 from soil samples in vials, closed with caps until they are incubated (for 1 hour!) In a thermostat. The second (II) is the determination of the concentration (emission rate) of CO_2 in all vials by samples after 1 hour incubation in a thermostat with closed lids. After completing the determination of CO_2 concentration in all the vials, the rubber caps are removed from the vials, the vials are aerated for 15 minutes, then carefully covered with food film and placed in a thermostat until the next measurement of CO_2, that is, for 1 day. To calculate the parameter of soil health, determining the amount of CO_2 emitted (for 1 hour of incubation with soil samples in vials closed with caps) is performed daily at the same time for 4 to 5 days.

Calculation of the concentration of CO_2 (ng C-CO_2 / h • g dry soil) is carried out for samples collected before their 1 hour incubation with the cap closed on all vials and calculation of the concentration of CO_2 is carried out after their 1 hour incubation with caps closed on all vials. If the determination of CO_2 is carried out on traditional chromatographs with tape recorders, measure the height of the peaks of CO_2 on the recorder tape with a ruler and enter numerical values into the Excel file in the tables. All other results are included in the tables of the Excel file, for example, concerning the determination of the moisture content of soil samples, quantitative results of calibration of the instrument with different concentrations of CO_2, etc. The following procedure of creating and filling in tables in an Excel file, which was experimentally tested and was helpful to the author in operating with a large data volume, is proposed.

Excel file columns for CO_2 sample data taken before 1 hour incubation with a closed cap of all the bottles and the same order for columns with results after 1 hour of incubations with a closed cap of all vials, are marked in the following way: (1) the sample number; (2) M - "multiplier" (numerical factor), reflecting the sensitivity of the device; (3) L is the peak height of CO_2 on the tape, mm; (4) V is the volume of the gas phase in the vial above the soil, cm_3; (5) t is the incubation time, hour; (6) m- dry soil mass, g; (7) K is a numerical coefficient for converting the peak height of CO_2 (mm) on a tape to CO_2 concentration in%, according to the calibration of the instrument; (8) CO_2,% concentration, volume; (9) V- CO_2 emission, mkg C-CO_2 / hour • g of dry soil.

Calculation of the CO_2 flow released from the soil samples in 1 hour of incubation is carried out by subtracting from the results obtained after 1 hour of incubating bottles with closed caps the data obtained before incubating the bottles with closed caps. To calculate the mass of CO_2 released in one hour by a sample of soil of known dry mass, i.e., ng C - CO_2 / h * g dry soil (using a chromatographically measured volume of 1 ml of gas) it is proposed to have the following variables: (C-CO_2 / h * g sp.) Ng = [(Y2 - Y1) * 12 * 104] / 22.4 * W, where: Y2 - Y1 are CO_2 concentrations (percent of volume), measured at intervals of 1 hour; 12 - molar mass of CO_2 (g / mol); 104 - the numerical coefficient obtained after the transfer: ml to liters,% in ml, grams to nanograms; 22.4 - the volume of 1 mol of gas (l); W is the mass of dry soil (g).

Sources of errors that occur when determining the concentration of CO_2 in soil samples: the volume and mass of the soil used, soil moisture, the incubation conditions of the samples (temperature), the incubation time, the volume of gas sample taken, the quality of analyzer, individual features of the operator. Necessary reagents and equipment. 1. Distilled sterile water. 2. Glucose solution. 3. Gas chromatograph, or other analyzer, for example, an infrared analyzer with sensitivity to CO_2 not lower than the concentration of CO_2 in the ambient atmospheric air (~ 300 ppm). 4. Set of calibration gases (CO_2) for analyzers. 5. Bottles with a capacity of at least 10 - 15 ml (penicillin), closed with rubber (not permeable to CO_2) stoppers, but easily punctured by syringe needles. 6. Syringes for 1-2 ml. 7. Polyethylene (food grade, "saran wrap") film. 8. Technical scale. 9. Marker. 10. Computer with appropriate software.

The Soil Health Parameter Determination According to the Criteria of the Number of Cfus and/or SIR

A health characteristic is a dynamic biological category that applies only to a living object. In medicine, the category of health is characterized by a list of observationally experimentally identified and consistently adopted qualitative-quantitative indicators and standards. For the microbial community of the soil, such a category as health is also legitimate. The need for its introduction in relation to the soil ecosystem arose relatively recently. Therefore, the developed qualitative and quantitative indicators, characteristics, standards and parameters of soil health need to be constantly improved. A model of this development (see below, Chapters 3.2 and 3.3) is a method for determining the health parameter of soil samples, composts, and other solid substrates Semenov [2]; Semenov [4]; Semenov, Sokolov [1].

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Specific requirements for the state of soil samples and the analysis procedure in determining the parameter of soil health

In determining the parameter of soil health, an indispensable condition is compliance with the requirements for the state of soil samples and following the analysis procedure. The key provisions for its successful implementation is the obligatory compliance with the principles and requirements as follows. 1. Comparison of the studied soil with the selected healthy (conditional reference) soil of the same genesis and territorial landscape is the principle of comparability. 2. Use of only freshly selected soil samples for determining the parameters of soil health is the principle of nativeness. 3. The impact on the comparable soil adequate, the same disturbing effect - the principle of initiation. 4. Conducting dynamic observations and definitions - the principle of dynamism Semenov [4], Semenov A.M., Sokolov [1].

It was established experimentally that any stress effect on soil samples, composts and other solid organic substrates with subsequent tracking the number of CFU or respiratory activity at a frequency of determination of not less than daily, at the same time, within 3 - 5 days, manifests itself in the form of a wave-like dynamics of the number of CFU and / or respiratory activity - CO_2 emissions. Stress effects on samples of soil, composts and other substrates can be performed in the form of physical effects (for example, drying - moisturizing, freezing - thawing, etc.), or biochemical - making easily accessible nutrient substrate, such as glucose in our method. With a daily tracking the number of CFUs or measurements of respiratory activity (CO_2), that is, these dynamic indicators over time, the type of curve obtained can be either unimodal or polymodal (have one peak or several).

The parameter of soil health determination based on results of the number of CFU bacteria

It is recommended that two performers should participate in plating suspensions of soil samples. Since plating has be carried out every day for at least 4 days, this work should be carried out and the results should be counted in turn (to exclude subjectivity). The composition of the medium and its preparation for the cultivation of bacteria with obtaining CFU are described in section 1.2.1. The amount of medium is prepared on the basis of the number of analyzed soil samples, the duration of analysis of each sample (within 4-5 days, times / day) and the number of analyzes repeated by the executor. The procedure for planting soil suspension on agar medium is carried out as described in section: 1.2.2, CFU counting - as described above (section 1.2.3).

If the number of CFU is tracked in different soils, and suspensions are prepared from them at least once a day, then dynamic results will be obtained. Such data obtained in tracking the number of CFU from different, at least two soils, for example, reference and analyzed, for, for example, 4-5 days and calculated as the number of CFU / g of dry soil, are copied in a separate column opposite the column with time data for tracking the number of CFU / g of dry soil with a time dimension (days). Based on these results, it is necessary to create graphs of the dependence of the number of CFU / g of dry soil on time. Such graphs allow us to calculate the parameter of soil health (Figure 1).

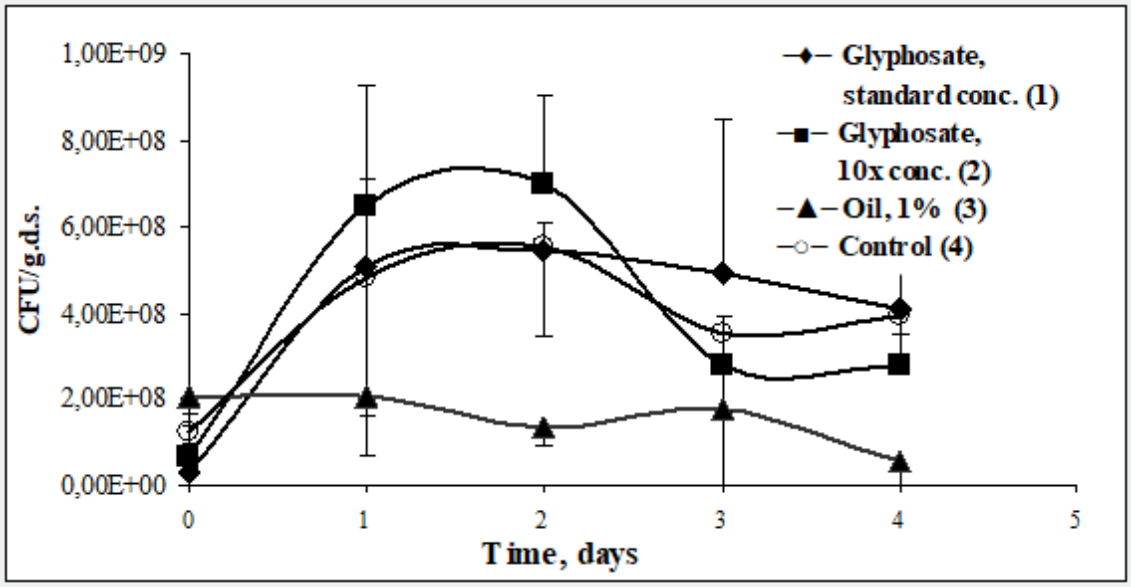

Figure 1: Dynamics of CFU of bacteria in polluted soils after the disturbing action (DA) in the form of 0.5 mg of glucose /g.d.s. for calculation of the heterotrophic parameter of soil health.

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The parameter of soil health determination based on results of the substrate induced respiration dynamics soil samples

To prepare samples for analysis to determine the parameter of soil the health according to results of the counting for the flow of CO_2 is recommended to be carried out by two performers. Since the dynamics of CO_2 emissions should be measured for at least 4 days, then measurements should be taken and results recorded (to exclude subjectivity) in turn.

If the determination of CO_2 emissions is carried out simultaneously for different soils at least daily (see sections 1.1. and 2.1), then dynamic results will be obtained. Such data, obtained for at least two types of soil, for example, "reference" and "analyzed", within 4-5 days and calculated as the amount of μg C - CO_2 / h • g of dry soil, are copied in a separate column opposite to the column with data of the time to determine the number of CFU / g of dry soil with a daily dimension. Based on these results, it is necessary to construct graphs of the dependence of the amount of carbon dioxide emitted by the soil (μg C - CO2 / hour • g of dry soil) on time.

To calculate the heterotrophic parameter of soil health, a logical algorithm and a formula are proposed. The sample under study should be considered the more healthy the less it is numerically different from the zero value of the fraction modulus; in the numerator of such a fraction is the difference between the digital value of the reference sample, obtained by measuring the width of the maximum peak in amplitude at its half-height [Lc] and the same characteristics obtained for the sample under study [Le]. In the denominator of this fraction, the numerical value obtained for the control sample [Lc], that is, ZP = | (Lc - Le) / Lc | (Figure 2).

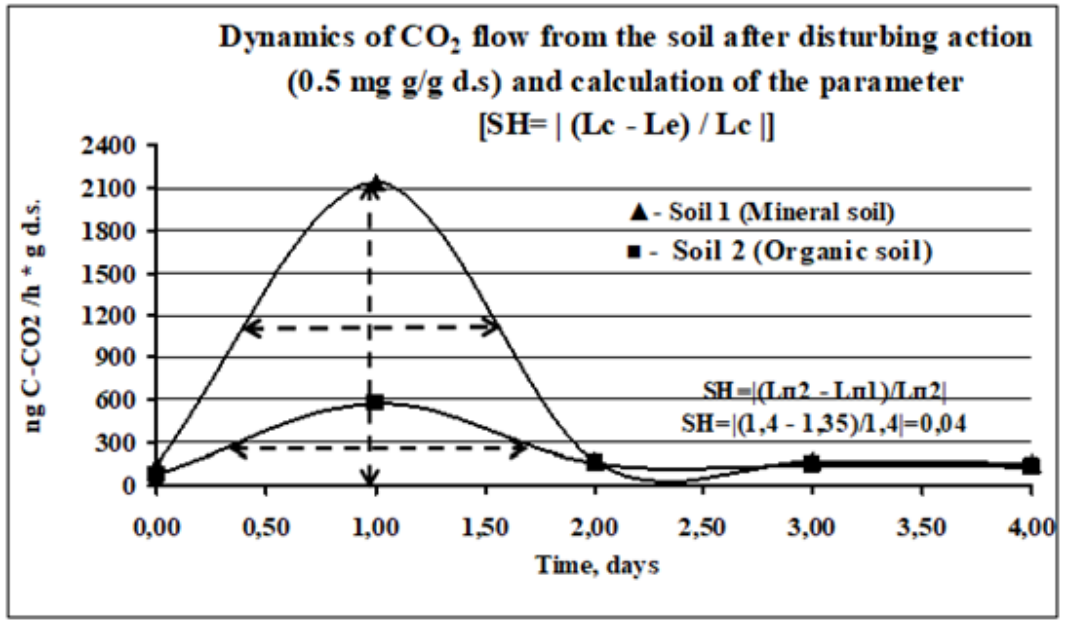

Figure 2: Dynamics of substrate induced respiration in soils after disturbing effects (0.5 mg of glucose /g.d.s.) for the calculation of the heterotrophic parameter of soil health.

A preliminary scale of the obtained values of soil health assessment

If the heterotrophic parameter obtained in the sample under investigation is in the range from 0 to 0.1, then the sample is considered healthy. If the parameter exceeds 0.1, but less than 0.2, then the soil is considered practically healthy. If the parameter is numerically higher than 0.2, but less than 0.4, then the soil is moderately not healthy. For large values of the heterotrophic parameter, the soil is considered not healthy. However, the proposed "scale" was obtained on experimental soil plots and should therefore be considered an "example." For soils of different origins, landscapes, and histories of use, databases with their own "scales" of quantitative indicators of health and ill-health should be created.

Determination of "self-sufficiency" parameter of the soil ecosystem by biophilic elements (N, P) for assessing health status of the soil

Assessment of soil health clearly indicates the importance of not only knowing and evaluating the activity of the most common biotic processes (which reflects the heterotrophic parameter), but also the state of "closure" of the cycles of the biophilic elements

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of the soil ecosystem. At the same time, "closure" should be understood in the sense of "self-sufficiency" of biophilic elements (primarily N and P) of the local soil ecosystem of a particular genesis and landscape under study. It is important to maintain a balance between the "loss" of these elements, primarily due to the removal with the crop and their return to the ecosystem with a diverse mortmasse. It is well known that the balance of the compounds N, P and some other elements is critical for the functioning of any natural ecosystem, but especially for the consumers of the agroecosystem.

To the assessment of the "closure" of the cycles of biophilic elements in the soil: Traditional thinking, when discussing the nitrogen cycle in soil, directs researchers to detect the indicators of nitrogen-fixing-ammonifying inflow and denitrifying-nitrifying loss of nitrogen. In theory, it looks convincing. However, our research has shown that such an approach is unacceptable in solving the practical task of assessing soil self-sufficiency with nitrogen.

So, in the fallow soil, which is generally assumed to be healthy, using the acetylene method in the daily detections for a month, we did not find signs, and even more so, significant amounts of not only relevant, but also potential nitrogen-fixing activity. At the same time, in intensively exploited field soil, receiving up to 180 kg / ha of nitrogen fertilizers per season, in the absence of actual nitrogen fixation, potential nitrogen-fixing activity is detected Emer [23]. Since this activity is manifested after glucose is added to the test samples of the soil, it is appropriate to consider this technique as a variant of the effect on the soil by a certain stimulant, "doping" or stressor on the soil biota. Therefore, the activity of nitrogen fixation was not always and not for all soil ecosystems quantitatively significant and easily detectable. In this regard, the nitrogen-fixing activity cannot be considered as a routine, significant indicator for estimating the influx of N into the soil ecosystem Semenov, Sokolov [1]. It is quite obvious that nitrogen fixation is an exclusive process, caused only by prokaryotes and dependent on specific physicochemical soil conditions. Therefore, due to limitations, it can not always and not for all soils is reliably identified and quantified.

Ammonification also cannot be recommended for use as a daily indicator of the dynamics of quantitative assessment of the influx of N into the soil, since the potential ammonifying activity was manifested in similar values for both soils, the field (studied!) one and fallow (reference) one. Natural biological processes associated with the removal of N from the soil are nitrification and denitrification. However, due to significant methodological and other restrictions, they also can not be used as reliable valid indicators for estimating the loss of N from the soil ecosystem.

To the assessment of the state of self-sufficiency of the soil ecosystem with biophilic elements (N, P) as a parameter of its health: The state of the development direction and activity of the process of providing the soil with nitrogen is proposed to be judged by the activity of the microbial community after the disturbing effect in the form of its enrichment with mineral nitrogen compounds. The proposed approach, based on well-known and logically non-contradictory provisions, has been experimentally confirmed by our research [Semenov, Sokolov, 2016]. The intensity of circulation in the soil of biophilic elements, in particular N, depends on the activity of microorganisms. Plants and animals act mainly as consumers of soil nitrogen. Thus, they force microbial transformants of nitrogen compounds to function more intensively and more efficiently. To the optimum extent, their activity should manifest itself in a native, healthy soil ecosystem.

It is proposed to determine and compare the dynamic response of the microbial community of the studied and reference (healthy!) soil to the introduction of biophilic elements, in particular nitrogen and / or phosphorus. Episodic measurements of the concentration of nitrogen compounds in the soil (in the form of, for example, balance of total N or its compounds — ammonium, nitrate, ammonia, nitrous oxide) are not informative.

It is known that the dynamics of the main metabolic processes carried out by microorganisms and, as a result, their emission of carbon- and nitrogen-containing metabolites in the soil occur in waves and coincide in time. Therefore, an analogy is appropriate in determining the parameter of the biological activity of the soil on it, for example, "nitrogen enrichment" with the parameter determined when the soil is enriched with glucose (like the definition of a heterotrophic parameter Semenov, Sokolov [1]. Since there can be no stable, universal criterion or standard scale that characterizes natural and cultivated soils according to the availability of biophile's, only a comparative method is applicable as such as a criterion of soil health. It allows to reveal the difference in the reactions of the microbial community - dynamics of soil ecosystem activity - for short-term enrichment with the biophilic element of the studied and reference soils.

Guidelines for assessing the self-sufficiency of soil ecosystems in biophilic elements, nitrogen or phosphorus: To assess the self-sufficiency of soil ecosystems in biophilic elements, nitrogen or phosphorus, it is proposed to carry out the following procedures. To carry out simultaneous enrichment of soil samples (the way it is carried out for definition of a heterotrophic parameter) with carbon and nitrogen substrates, and if necessary, phosphorus. To measure the SIR in soil samples with reference to rate (V) of CO_2 emissions (induced by biophiles and carbon substrates) daily for at least five days at least once a day at the same time. To incubate samples of comparable soils (test and "reference") under optimal hydro and thermal conditions. To use as inductors of SIR simultaneously applied aqueous solutions of glucose (for glucose concentration, see paragraphs 3.3. and 3.4.), ammonium nitrate and / or disubstituted sodium phosphate.

The values SIR acts as a parameter of the soil health, which characterizes the intensity of its carbon, nitrogen, and, if necessary, phosphorus exchange. The parameter should be determined experimentally when a "wave-like" response is received in the form of a peak (burst) of the SIR value until it decreases (or at least

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until it starts decreasing). Graphically, this is expressed by single or double vertex curves. To determine graphically (similarly to the heterotrophic parameter), using the dependence of speed (V) on time (T) and calculate the parameter PSBE - the parameter of self-provision of the biophilic elements of the soil ecosystem. In this case, only one maximum peak should be analyzed. To calculate the induced parameter (PI), a formula similar to the one used for calculation of the heterotrophic parameter is used: PI = [(Lkp - Lip) / Lkp].

It is necessary to calculate PSBE also in absolute value. Using the modulus of the above fraction (and not just the modulus of the difference between the widths of the above peaks at their half-height) allows you to eliminate the dimensionality of the parameter of soil health and correctly compare the studied and control samples by this indicator. It is necessary to evaluate the obtained values of PSBE by means of the same approach as the heterotrophic parameter: the closer the PI to the zero value, the more healthy is the studied soil. If the calculated parameter turns out to be equal to zero, then the soil is quite healthy, that is, the activity of the microbial community (as a result, the enrichment of the soil with biophiles) of the studied and reference soils is similar. Experimental detection of the parameter of the activity of the microbial community of the soil in response to the introduction of biophilic elements (together with glucose!) showed acceptable correctness, sensitivity and reproducibility of the proposed method (Figure 3 & 4).

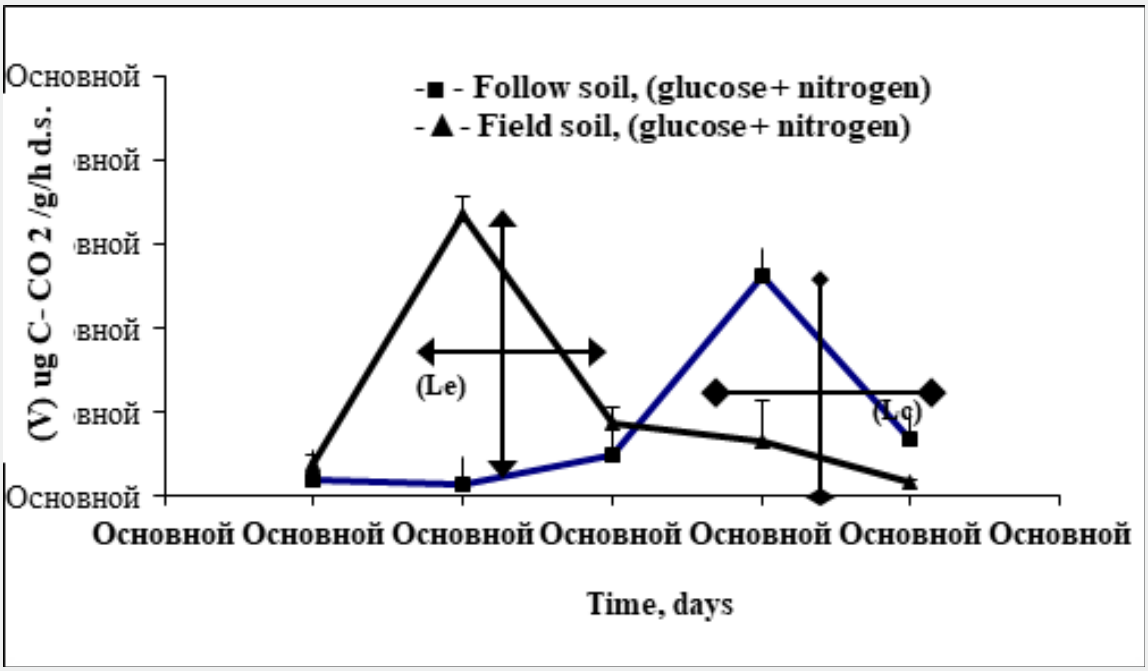

Figure 3: Example of determining the soil self-sufficiency parameter for biophilic elements based on the SIR dynamics after a disturbance (enrichment with glucose and nitrogen). Horizontal and vertical lines with arrows on the SIR graphs with the designations Lc and Le are supplemented as examples for measuring the "CO2 peak width at half-maximum" values in the compared soils.

Use of the heterotrophic health indicator and indicators of soil self-sufficiency in biophilic elements for soil health diagnostics and algorithms for its improvement and treatment

To solve the practical problems of soil processes management, approaches are proposed that allow to make decisions on the improvement or treatment of the soil ecosystem based on indicators of the heterotrophic parameter of its health and the parameter of its self-sufficiency in biophilic elements. So, the object of diagnosis and subsequent managerial impact is the soil ecosystem. This is a heterogeneous system with biodiversity. Its biotic component is reproduced, exists and is supported by microorganisms, the natural microbial cycle. The soil ecosystem is highly stable and has buffering ability, but at the same time, is characterized by high sensitivity to physicochemical changes and biotic disturbing influences.

The following list of provisions and procedures for diagnosing soil health and making decisions about its treatment is proposed. In the social sphere, the concept of health involves such actions as its maintenance and treatment. Treatment is applicable only to an individual, but in medicine, any individual is a combination of biosystems. In medicine, treatment is undertaken after establishing the causes of the disease, that is, it must be preceded by a diagnosis. The biota of the soil ecosystem is basically a

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microbial community. When working with it, you first need to understand which ecosystem functions are impaired and what specifically needs treatment. Further, it is important to state whether treatment is possible based on the fundamental laws and functions of the microbial community of the soil, its specific physicochemical characteristics. It should be kept in mind that there really are the diseases which are difficult to diagnose, difficult to treat and which are even incurable. With regard to the soil for conducting its enhancement, rehabilitation, and even more so for its treatment, it is necessary to have a diagnosis, the objectivity of which is confirmed by the expert's experience, special database and other arguments. As for any living system, the objectivity and correctness of the diagnosis should be confirmed by instrumental quantitative indicators. These are the heterotrophic parameter of soil health (HPSH) and the parameter of its self-sufficiency in biophilic elements (PSBE).

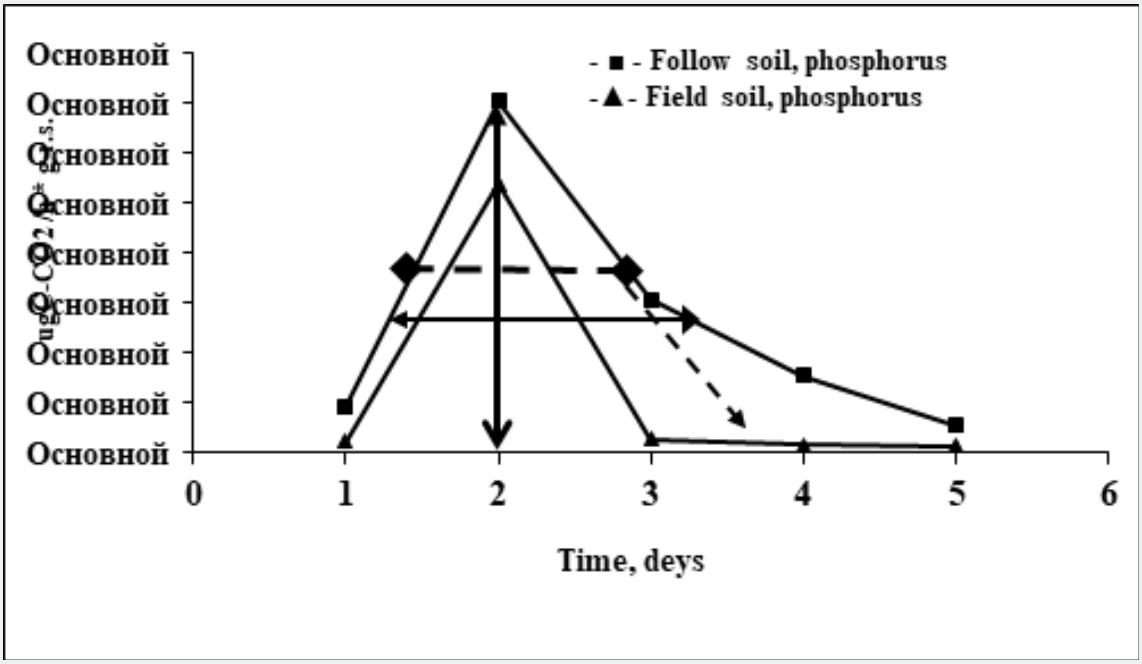

Figure 4: An example of detecting the parameter of soil self-sufficiency with biophilic elements according to the dynamics of SIR after a disturbing effect (enrichment with glucose and phosphorus). The horizontal and vertical lines with arrows on the SIR graphs are supplemented as examples for measuring the "CO2 peak width at half-maximum" values in the compared soils.

HPSH quantitatively characterizes at least two compared soils. They should be used to diagnose health of the test soil and, if necessary, to select methods of treatment. The obtained parameter of soil health must be interpreted not only as an indicator of "similarity" or "difference" between the reference and the studied soils (see chapter 3.4, a preliminary scale of the obtained values of soil health assessment."). The obtained quantitative indicator of the studied soil may quantitatively either coincide with the reference soil or be quantitatively lower or higher than the reference soil. The following questions are logical: a) how much is "below" or "above" the value of the parameter of the studied soil in comparison with the reference one? b) how significant are these differences? c) what should be the specific methods and means of treatment if the HPSH indicates that the soil is not healthy? The correctness of the answer to these questions depends on the expert's qualifications, as well as on the representativeness of the "data bank" information used by the expert.

Using the values of PSBE, it is possible to draw a conclusion about how the soil is depleted or "overfed" with specific biophilic elements. Based on this conclusion, a reasonable management decision is made. It is important to keep in mind that many traditional agricultural practices and means (plowing, fertilizers and pesticides introduced in excess), giving a short-term effect of increasing productivity, at the same time play the role of external disturbing effects of the soil ecosystem. This is reflected in the patterns of functioning of microbial populations and, as one of the consequences, there are problems with the health of the soil ecosystem, for example, in the form of excessive, uncontrolled development of some microbial populations of phytopathogens or other pathogens.

In particular, the excessive distribution of the most harmful root rot while growing the most important, staple food crops of Russia, the loss of suppressive functions by our soils, is directly related to the state of soil health. An objective diagnosis of such

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stressful effects is possible and promising by determining the parameters of soil health (HPSH, PSBE, etc.). For the treatment of such soils, traditional agrotechnical products (organic fertilizer, oligotrophy of soil ecosystem, etc.), as well as an arsenal of organic farming techniques, can prove to be very effective. It should also be borne in mind that there are very serious and even incurable soil diseases. Radical methods are used for their treatment - total fumigation, sorption detoxification, or even excavation of poisoned, degraded soil and its total exchange with a healthy one.

Conclusion

This publication, entitled "A convenient and informative method for quantitative assessment of soil health parameters", is essentially a guide for quantitative assessment of such an important characteristic as soil ecosystem health. In this case, it is proposed to detect the dynamic parameters - general (HPSH) and specific (PSBE). These parameters are detected ex situ, which makes the method mobile and quite operational. We emphasize that soil health is a comprehensive indicator of soil characterizing both its quality and fertility. The "Guide" allows the user to perform a scientific and practical task - to evaluate the activity of soil microorganisms (bacteria) and, based on the results obtained, to detect an important characteristic of the soil ecosystem - its health. A rather obvious, but essentially rarely used, concept is introduced and substantiated - soil ecosystem, instead of the term "soil" - traditional, but ambiguously interpreted. From the ecosystem position of the concept, the soil ecosystem allows a more meaningful assessment of the functions of the microbial community, the most important biotic component of the soil ecological system.

This "Guide" includes not only protocols, that is, a description of "what and how to do," but also contains introductory theoretical sections that introduce the user to the problem, thereby preparing him in more details to procedure of determining soil health parameters. Justification and description of those methods that are the basis of the determined parameters serves the same purpose. The work implements a clear two-pronged task - determining the number and / or activity of microorganisms - native, viable, active, able to form colonies on a nutrient medium and generate the most common biota metabolism product - CO_2. The proposed soil health parameters are determined on the basis of experimental dynamic indicators - CFU and CO_2 emission intensity.

The "Guide" discusses methods for determining the activity of the soil microbial community. Illustrative graphical examples of calculation and determination of these parameters are given. Their determination based on the assessment of soil microbiological processes, the preservation and integration of the results obtained will allow in the near future to create a "data bank", demanded by interested users. We hope that the "Guide" will help to train and educate not just the operator, but a creative specialist in the field of diagnosing the health of the most complex ecological system, traditionally called soil. It urges the user to understand the importance of diagnosing the health status of the soil ecosystem, since an objective diagnosis is the most important basis for its successful rehabilitation and treatment.

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