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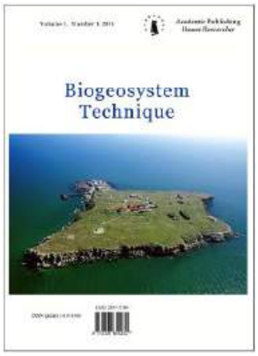


"MALDI-FLIP-on-a-chip" and "MALDI-FRAP-on-a-flap": Novel Techniques for Soil Microbiology and Environmental Biogeochemistry. II – Polymer Chip Prototyping (Invited Paper)

Arthur G. Jablokow[a, *], Philipp A. Nasirov[a], Feodor K. Orekhov[b], Oleg V. Gradov[a, b]

[a] Institute for Energy Problems of Chemical Physics, Russian Academy of Sciences, Russian Federation
Laboratory of Biological Effects of Nanostructures
[b] N.N. Semenov Institute of Chemical Physics, Russian Academy of Sciences, Russian Federation
Department of Dynamics of Chemical and Biological Processes

Abstract

In the second part of the article cycle «"MALDI-FLIP-on-a-chip" and "MALDI-FRAP-on-a-flap": Novel Techniques for Soil Microbiology and Environmental Biogeochemistry» the polymer chip prototyping techniques are described. In particular, the compatibility problems are discussed. The problem of the biocompatibility of polymer chip surface is eliminated using GIM-surfaced chip exposition in the high-humidity experimental media, which may be interpreted as a model of soils. The problem of compatibilities of fluorescence techniques and polymer chips is resolved (as a part of the general chip optics problem) using microscopic investigations of polymer chip transparency in some different textural variances and microfluorimetric measurements of fluorescent dyes in the chip geometry. The problem of the soil chip prototyping is solved using 3D-printing based on some biocompatible and, so possible, biodegradable polymers. The basic complexity of experimental data is provided in the tables placed in the general article text. Is it possible to create multiparametric analytical technique for synchronous biocompatible soil microbiome analysis and monitoring? It is a general question for the real time environmental control. We can say "Yes", but only if we have a minimal prerequisite case, which we have a good polymer, real "real time" analyzer, biocompatible and biodegradable coatings etc. In other cases the general problem of soil chip design is not a problem of engineering, but it is a problem of soil-chip interface chemical physics and physical chemistry. Such problem may be interpreted only as a principal physical, but not as a technical problem. This part of our article cycle is very simple and sensible, because such problems are not very strong and complexible, consequently, we can take only illustration of principle, but not a full verification and validation of fine and thin mechanisms of soil microbiome interactions with adhesive chip etc. Cureent opinions in soil biology are good compartible with our results, therefor we must not take full theoretical considerations in frames of the current concept paradigm in soil biology. De facto, it is a brief methodical and technical note before the closing of our projects in Russian Federation. It is not a normal research article, because all normal articles may be writed only in normal material and technical conditions.

* Corresponding author
E-mail addresses: retromicroscopy@gmail.com (A.G. Jablokow)

Keywords: FRAP (Fluorescence Recovery After Photobleaching), FLIP (Fluorescence Loss in Photobleaching), MALDI (Matrix-Assisted Laser Desorption/Ionization), “BIOTYPER”, CLSM (Confocal Laser Scanning Microscopy), LoC (Lab-on-a-Chip).

1. Введение

Проблемный подход

В предшествующей статье данного цикла были рассмотрены возможности создания аналитического чипа для почвенно-биологических задач как концепта фингерпринтируемого устройства с т.н. «несуррогатным ключом», то есть локально-телеметрируемого программно-аппаратного комплекса почвенного заложения, идентификация в котором производится на основе собственных физико-химических параметров идентифицируемых объектов. На данный момент препятствиями к массовому внедрению данного подхода являются материаловедческие проблемы – проблемы биосовместимости, биокоррозии и биоразложения тех элементов конструкции, которые при массовой закладке способны не только исказить своим вводом результаты эксперимента на локальных диффузионных промежутках, но и сместить дескрипторы биогеохимического и экологического равновесия. Поэтому вторая часть статьи посвящается, в существенной части, именно проблемам выбора материалов для изготовления чипа и их практической апробации. Кроме того, в силу телеметрического характера сбора данных и вполне формально математизируемого способа идентификации на «фенотипических» и биогеохимических «обменно-метаболических» чипах данного типа, необходимо, в первую очередь, проиллюстрировать главное преимущество чипов разрабатываемого нами типа – возможность синхронного анализа в реальном времени, недоступную другим чипам с возможностью обработки последовательностей и идентификации организмов, в том числе – с матрично-опосредованной десорбцией-ионизацией при масс-спектрометрическом секвенировании, а именно – генетическим чипам для анализа почвенной микробиоты. Если наш чип не обладает преимуществами данного типа, а современные активно распространяемые и используемые почвенные генетические чипы типа «GeoChip» способны делать тоже самое, то смысла в разработке, очевидно, нет. Если наши чипы могут выдавать лучший метрологический результат, но токсичны для среды – это тоже не выход. Если применяемые полимеры и другие среды нейтральны для почвы и микробиоты, но не выдают флуоресцентного и матрично-опосредованного масс-спектрометрического сигнала – аналогично, прикладной и отраслевой смысл в работе отсутствуют, ибо она не выйдет за пределы лаборатории.

Итого, ключевыми проблемами для решения задачи комплексирования MALDI (что уже аннотировалось выше – матрично-опосредованная десорбция и ионизация при разных видах масс-спектрометрии, начиная от времяпролетной, заканчивая орбитальными ловушками» и ловушками с динамической гармонизацией, масс-спектрометрами ионного циклотронного резонанса и т.д.), FRAP (метод исследования кинетики флуоресценции после фотообесцвечивания / фотовыжигания – см. предыдущую статью) и FLIP (установление уровня затухания и потерь флуоресценции в процессе фотообесцечивания / фотовыжигания) на чипе, погружаемом для экспонирования в почвенную среду, являются в комплексе:

1) Наличие/отсутствие специализированных чипов для почвенно-биологических задач (фенотипического и биогеохимического плана) реального времени.

2) Если нет – возможность быстрого 3D-прототипирования чипов данного назначения на тех материалах, которые оптимальны для данных задач (см. п. 3, п. 4 ниже).

3) Совместимость полимерных подложек или покрытий чипов с почвенной биотой.

4) Наличие полимерных подложек, красителей *in vivo* и матриц, совместимых с MALDI.

5) Возможность исследования чипов с данными текстурами флуоресцентным методом.

Рассмотрим эти проблемы несколько подробнее ниже.

Специализированные чипы для почвенно-биологических задач

Наиболее распространенным типом чипов для геобиологических и биолого-почвенных задач является т.н. GeoChip – разработка Университета Оклахомы (University of Oklahoma) в США. GeoChip представляет собой «геночип», т.е. ДНК-биочип, содержащий олигонуклеотидные зонды для детектирования генов, участвующих в биогеохимических циклах (углерода, азота, фосфора, серы и различных металлов), определяющих устойчивость к антибиотикам и бактериальную деградацию органических загрязнителей, вирусологические дескрипторы, реакции стресса, мембранно-биоэнергетические процессы, а также филогенетические дескрипторы (маркеры). GeoChip способен специфически детектировать сотни тысяч функциональных генов/групп микробов, важных для биогеохимических, экологических и экологических процессов. GeoChip является универсальным инструментом в аспекте способности анализировать микробные образцы из любого источника окружающей среды. GeoChip может анализировать микробные образцы без какого-либо культивирования и без предварительного знания микробной композиции образца (это важно, так как более 90 процентов микроорганизмов почвы не культивируемы как чистая культура на искусственных средах). GeoChip является беспрецедентным инструментом в его способности быстро и всесторонне идентифицировать функциональную структуру, активность и динамику микробного сообщества, используя образцы ДНК и РНК сообщества. GeoChip обеспечивает компаративный анализ данных микрочипов в различных условиях (экспериментах, лабораториях, временных периодах) с использованием универсальных стандартов. GeoChips имплементируется на стеклянном слайде. В данном разделе приводится перевод элементарных сведений о геочипе, что позволяет не отсылать читателя к молекулярно-биологической и биотехнологической литературе для длительного определения собственного мнения о предмете, так как геочип, по сути, представляет собой обычный генетический чип, позволяющий определять гены, общие для разных микроорганизмов, обеспечивающие различные аспекты их активности в естественной почвенной / геохимической / гидрохимической среде (устойчивость к химическому стрессу, возможность экспрессии хемотрофных свойств в данном сообществе и т.д.). GeoChip – это функциональный генный микрочип, предназначенный для обнаружения тысяч функциональных генов-микробов за один раз. Он производится собственными силами. Олигонуклеотидные зонды (содержащиеся в 384-луночных планшетах справа) печатаются на специально покрытых слайдах, которые позволяют химическим связыванию зондов со слайдом. Печатные штифты (в золотой печатной головке справа) окунаются в лунки, содержащие раствор зонда, и затем попадают в массив (Рис. 1).

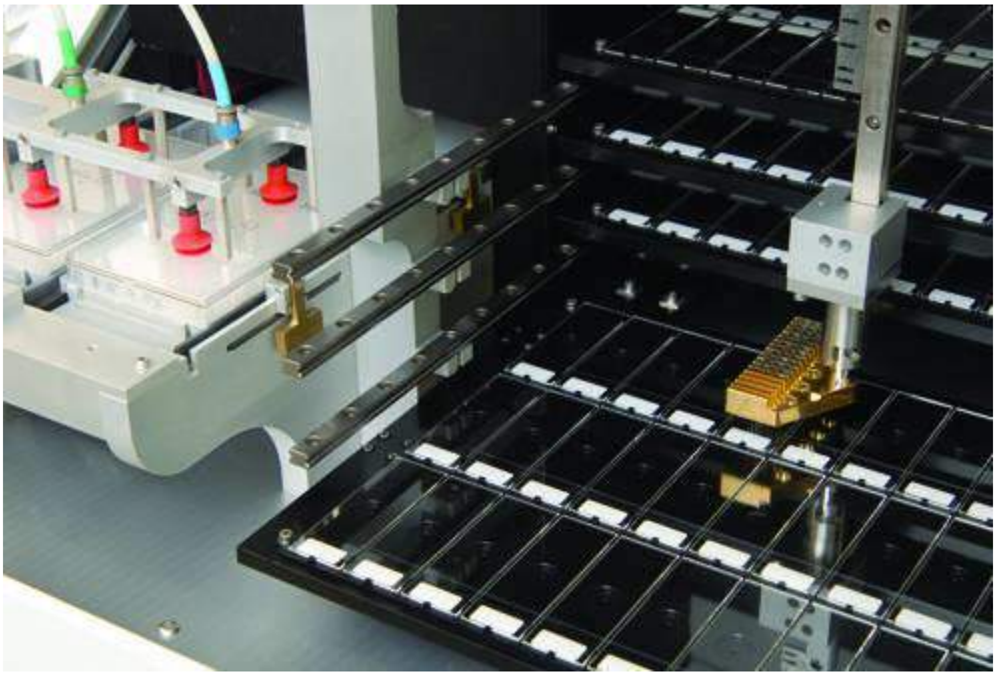


Рис. 1. GeoChip (фото Роберта Тейлора с сайта Офиса вице-президента по исследованиям Оклахомского Университета в Норман-Кампусе; https://vpr-norman.ou.edu/geochip-printing)

После получения образцов ДНК или РНК многочисленные образцы из одной или нескольких конкретных сред могут быть проанализированы столько, сколько нужно. При использовании GeoChip-конвейера все результаты могут быть обработаны сразу после гибридизации. Такое быстрое обнаружение позволяет отслеживать функциональные процессы за короткий промежуток времени (хотя и не в реальном времени), чего раньше не было возможно. Альтернативой схеме GeoChip являются трудоемкие техники, такие, как методы клонирования на основе генов на основе 16S рРНК, денатурирующий электрофорез в градиентном геле (DGGE), аналитика по полиморфизму длины концевого рестрикционного фрагмента (T-RFLP), количественная ПЦР и гибридизация in situ. Методы с использованием GeoChip-а чрезвычайно просты и адекватны использовавшимся ранее для аффиметрических измерений. Комплексный микрочип, называемый GeoChip, содержит 24 243 олигонуклеотидного зонда и покрывает > 10 000 генов в > 150 функциональных группах, участвующих в биогеохимических циклах (см. выше). Это особенно полезно для исследования прямых связей микробных генов / популяций с экосистемными процессами и функциями. По термодинамике аффинные зонды весьма специфично отличаются: 20 оснований – свободная энергия 35 ккал / моль; 35 оснований – 60 ккал / моль и т.д. Разработанные зонды валидированы ProbeChecker и синтезированы MWG Biotech Inc. (High Point, NC, USA). Концентрация всех олигонуклеотидов доходит до 100 пмолей / мл. Все олигонуклеотидные зонды и контрольные образцы размещаются на слайдах Corning UltraGAPS (Corning, NY, USA) с использованием Microgrid II Arrayer (Genomic Solutions, Ann Arbor, MI, USA). Процесс нанесения показан на Рис. 1. Олигонуклеотиды, которые комплементарны зондам, обнаруженным на матрице, синтезируются и метятся на 5'-конце с помощью красителя во время синтеза. Целевые фрагменты гена амплифицируют с помощью ПЦР. Обычно 50 пг каждого синтезированного олигонуклеотида или ПЦР-ампликона используются отдельно (одноцелевые эксперименты) или в смеси (многоцелевые эксперименты) нескольких мишеней. ДНК сообщества (для задач метагеномики) извлекают из образцов, как описано в работе (Zhou et al., 1996). Маркировку, гибридизацию и сканирование массивов проводят, как описано в работе (He et al., 2005). Отсканированные изображения количественно определяют с помощью программного обеспечения типа ImaGene 6.0 (Biodiscovery Inc., El Segundo, CA, USA) с использованием адаптивного Perl-сценария (скрипта). Как можно видеть, несмотря на позиционирование как метода мониторинга (Van Nostrand et al., 2011), геочипинг не может быть рассмотрен как true real time метод. Разработчики чипа (Рис. 2) и не рассматривают его как метод контроля *in situ*, если не считать масштабов геологического времени и эволюции микробных популяций.

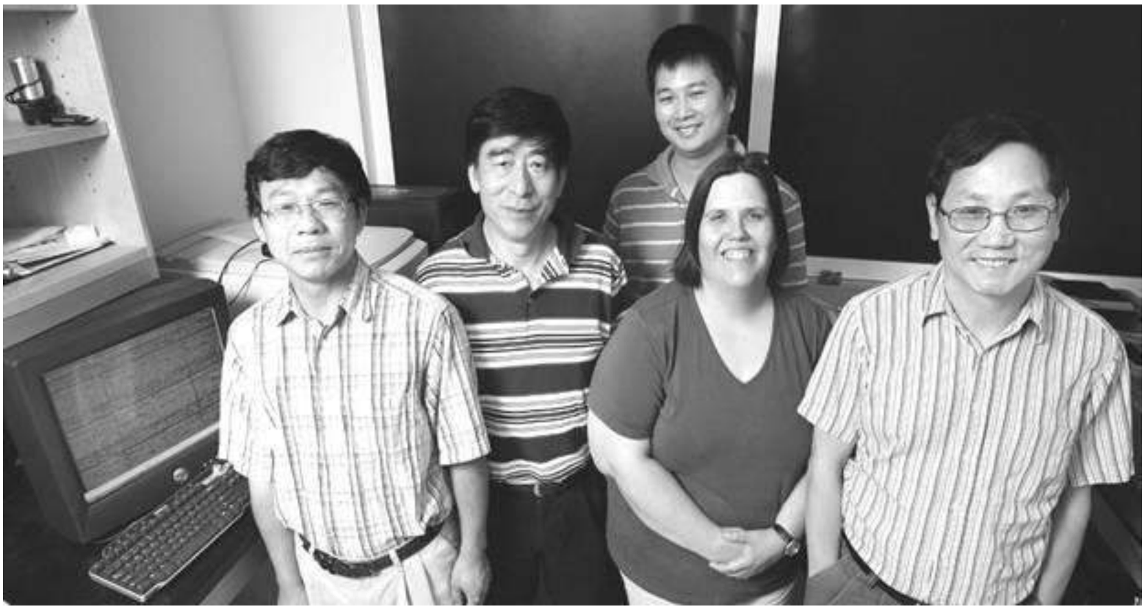


Рис. 2. Команда разработчиков «GeoChip»: Zhili He, Liyou Wu, Ye Deng, Joy Van Nostrand, Jizhong Zhou (Photo by Jaconna Aguirre, THE OKLAHOMAN)

Существенным преимуществом системы «GenoChip», с точки зрения биогеосистемотехники (Kalinichenko, 2014, 2015; Kalinichenko and Starcev, 2015; Kalinitchenko, 2016), является возможность параллельного анализа биогеохимических и экологических процессов в контексте структурно-функциональных дескрипторов активности микробного сообщества (He et al., 2007, 2010; Van Nostrand et al., 2010; Wawrik et al., 2010). Биогеохимические возможности чипа определяются анализом генетической информации, отображающей связи между метаболизмом микробных клеток и геохимической средой или, что точнее, определяющей потоки элементов геохимической среды в микробном сообществе как часть метаболизма этого сообщества (Wang et al., 2009; Xie et al., 2011; Bai et al., 2013). С помощью геочипинга исследованы следующие биогеохимические процессы: реоксидация биовосстанавливаемых уран-содержащих водоносных горизонтов (аквиферов) – осадочных пород с одним или несколькими переслаивающимися подземными слоями горных пород с различной степенью водопроницаемости (Van Nostrand et al., 2009); системы подповерхностного биовыщелачивания тяжелых металлов и биоремедиации после агрохимической нитратной контаминации (см., например, постер от Национальной лаборатории имени Лоуренса в Беркли – Lawrence Berkeley National Laboratory (Waldron et al., 2008)); параллельная денитрификация и десульфуризация, опосредованная микробным сообществом (Yu et al., 2014); десиликация как форма выветривания с образованием биогенных осадочных пород (Song et al., 2015); активность микробного сообщества в условиях оттока кислых вод из металлогенных рудников и угольных шахт, то есть в условиях необратимого дренажа кислотных руд / кислотного металлоносного дренажа [AMD] / дренажа кислых пород [ARD] (Xie et al., 2011; Tan et al., 2017); активность молекулярных систем микроорганизмов, отвечающих за эффективность цикла азота, в условиях изменения климата и агрокультурной модернизации (Xue et al., 2014); микробно-биогеохимические тренды при нитрификации и анаэробном окислении аммония (Zhao et al., 2014). Изучены различные микробные сообщества (Van Nostrand et al., 2007; Zhou, 2009; Zhou et al., 2010; Tu et al., 2014), что было отражено также в оперативной информации «Lawrence Berkeley National Laboratory» (GeoChip 3.0 as a high-thoughput tool for analyzing microbial community composition, structure [2010]). Помимо биогеохимических отличий и вариаций (вплоть до экстремальных, отраженных выше), геочипинг микробных сообществ может отображать на карту структурно-функциональных связей «фингерпринты» идентификации различных популяций, штаммов, географических таксономических вариаций микроорганизмов и биоматериала естественного происхождения вообще. На данный момент биогеографическое тестирование геочипов уже проведено в условиях биома влажных тропических дождевых лесов / гилеи (Cong et al., 2015) и мангровых лесов, с анализом почвенных микробных сообществ (Van Nostrand et al., 2012; Bai et al., 2013), биогенных рифообразующих структур в морских / океанических условиях солености / удельной электропроводности среды (Bayer et al., 2014), а также различных типов почв – от альпиского типа луговых систем (встречающихся как тип не только в Альпах, но и в иных, в частности, азиатских горных структурах, таких, как Тибет (Zhang et al., 2013)) до лавовых / лавоподных почв готьявальского леса в Джеджу (Kim et al., 2012), где преимущественной (зачастую – генотипически-эндемичной) микробиотой являются археи (Kim et al., 2014). Прикладные применения химически-реактивной метаболической идентификации микроорганизмов в биогеосистемотехнике на чипе могут быть проиллюстрированы внедрением в экологически-чистую биоэнергетику и биоремедиацию загрязненных сред. В качестве примеров первого направления целесообразно аннотировать, с 2008 года (Zhou, 2008) фиксируемые работы с использованием геочипов в области контроля или мониторинга процессов микробной ферментации отходов с получением водорода для целей водородной энергетики будущего в биоэлектрохимических микробных реакторах (Wang et al., 2008), вплоть до генетического анализа «анодофильных сообществ» в микробных ячейках для биоэлектролиза микробно-обогащенных сред (Liu et al., 2010). В качестве примеров второго направления целесообразно указать на работы по анализу с использованием геочипов функциональной структуры микробных сообществ и растительности загрязненных вод (Wang et al., 2014), почв и иных геосред, в том числе – в целях биоремедиации (Van Nostrand and Zhou, 2014). Агентом загрязнения могут быть как органические (например – фенантрен (Ding et al., 2012) или масляно-нефтяные загрязнения (Zhong et al., 2010; Liang et al., 2014)), так и неорганические (например – продукты выщелачивания, в том числе и в особенности – минерального (Lu et al., 2012)) загрязнения. В 2016 году проведены исследования по резистентности бактериальных сообществ к антибиотикам в рамках анализа т.н. «резистома» городских вод (Low et al., 2016)*.

Точность анализа приповерхностных и подповерхностных реактивных популяций бактерий и иных микроорганизмов может быть верифицирована и валидирована путем сопоставления данных геночипинга (с систем типа GeoChip) и филочипинга (с систем типа PhyloChip, используемых для филогенетического анализа и молекулярной систематики). Комплексность анализа достигается здесь путем метагеномного геочипинга (Van Nostrand et al., 2011, 2016; Xue et al., 2014; Gao et al., 2014; Tu et al., 2014; He et al., 2015). Надо сказать, что смычка принципов и методологии прикладного геочипинга и филочипинга является очевидным следствием единства объекта и техники исследования – генетического анализа, вплоть до пиросеквенирования и т.п. техник. Филочипы также используются для анализа микробных сообществ (Nelson et al., 2009; Hamady et al., 2010; Schatz et al., 2010; DeAngelis et al., 2011; Krebs et al., 2014) и бактериального разнообразия (Sagaram et al., 2009; Weinert et al., 2011), включая бактериальное разнообразие узких прослоек почвы ризосферы, содержащие симбиотические азотфиксирующие виды со стриголактонной чувствительностью (Weinert et al., 2010, 2011; Reich, 2009), и также могут характеризовать химическую реактивность микроорганизмов (Handley et al., 2012; Ding et al., 2012) и использоваться с хемометрическими методами (редоксметрия, респирометрия (Hery et al., 2010), нитрометрия для азотфиксаторов (Zhang, 2005; Zhang et al., 2005)). Также, как и геочипы (см. анализ микрофлоры водоносных горизонтов / аквиферов (Van Nostrand et al., 2009)), филочипы могут быть использованы для анализа микрофлоры почв субповерхностного слоя и подземных вод (Handley et al., 2012). Также, как и геочипы (ср. анализ губок и рифообразующих построек кишечнополостных на геочипах (Bayer et al., 2014) и филочипах (Kellog et al., 2012)), филочипы могут быть использованы для анализа биоминерализации и биорециклинга минерального вещества и солей, дающих селективный или супераддитивный вклад в удельную электропроводность среды и, как следствие этого, химизм биологических сред (особо учитывая каналомные эффекты и электрогенную природу активности биологических мембранно-отграниченных структур). В связи с этими и многими другими параллелями, на которых останавливаться не имеет смысла, в силу очевидности критериев идентификации и принципа идентичности для данного множества методов, можно резюмировать, что, не считая специфики множественных медицинских (Nelson et al., 2011; Kellog et al., 2013; Baudart et al., 2017) и социально-значимых фактов применимости в прикладных задачах (Lynch et al., 2007; Huang et al., 2009; Korves et al., 2013) данных филочипов и основанных на них технологий интерпретации данных (вплоть до сетевых баз данных с веб-интерфейсом – таких, как «Datensystem qChip-Entwicklung und Implementierung einer Phylochip Microarray Datenbasis und der zugehörigen Webapplikation» (Badnjevic, 2011)), уровни применимости (молекулярные, в общем случае, не считая отличия в акцентах на 16S РНК в филочипах (Brodie et al., 2007; Metfies and Medlin, 2008; Sagaram et al., 2009; Huang et al., 2009; Slusher, 2010)) у геочипов и филочипов идентичны, хотя и качество мультиплексирования и data mining-а / KDD у геочипов на порядки выше. Однако, при всём этом, последние также остаются чисто геномными, а не фенотипическими или прямыми хемометрическими системами. Чипов для гибридного фенотипического хемометрического (колокализационного) анализа в реальном времени не существует. Вид данных, получаемых с ГеоЧипа, представлен на Рис. 3.

* Опорными данными о норме могут являться динамические данные о микробных сообществах в естественных условиях (Van Nostrand et al., 2011; 谢建平, 2011); в интересном для нашего рассмотрения случае – почвы (Xue et al., 2014; Zhao et al., 2014; 김종식, 김대신, & 고석형, 2012).

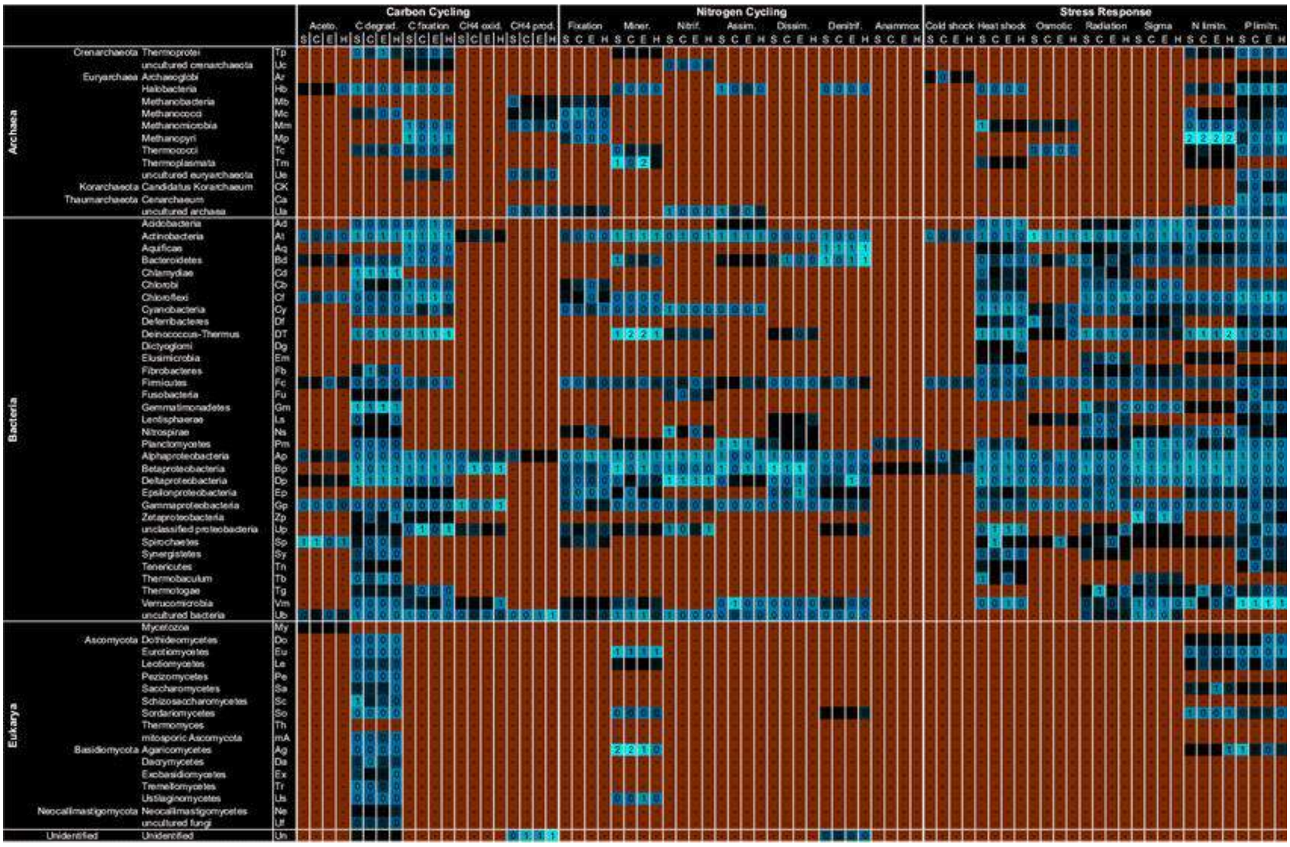


Рис. 3. Данные с ГеоЧипа

Строки представляют видовой состав таксономически сепарированной (бактерии, археи, эукариоты) почвенной микробиоты. По столбцам отложена биогеохимическая функция, соотнесенная с идентифицированными при чип-анализе генами: участие в циклах углерода и азота, стресс-реакции и т.д.

2. Методы

Быстрое прототипирование чипов и 3D-принтинг. Теория и практика

Достаточно очевидно, что для полноценного осуществления функций мониторинга (*in situ* / в реальном времени) система должна представлять собой не пассивный чип-матрицу, а систему, обеспечивающую сбор и передачу данных с непосредственного контакта со средой, обеспечиваемого капиллярными / интерфейсными процессами. То есть, иными словами, во-первых, система должна представлять собой активную «лабораторию на чипе» с наличием в ней флюидного микрорельефа (и микрорельефа поверхностно-активных сенсоров), обеспечивающего оптимальные биоадгезивные, сорбционные и биоинтерфейсные процессы, а, во-вторых, быть биосовместимой, что, в данном случае, является эквивалентным отсутствию в ней источников артефактов, поскольку любые не-биосовместимые материалы и технологии в случае биологического мониторинга в естественных условиях будут вполне очевидным образом влиять на источник аналитического сигнала, смещая его («негативная реактивность» вследствие действия ксенобиотиков и физических факторов, связанных с гидрофобностью / гидрофильностью, импедансом и т.п. характеристиками, которые должны подбираться для комплекса «сенсор-образец-среда-сенсор», исходя из предварительного анализа литературы и модельных мультифизических вычислений). Исходя из технологий, обеспечивающих как возможность модифицирования поверхности, так и быстрое прототипирование рельефных структур на ней, предположительно, не повреждающих клетки, было принято «тактическое решение» об использовании 3D-принтированных чипов на базе полимеров, являющихся, по данным литературы, биологически-совместимыми и нецитотоксичными.

Нами был использован 3D-принтинг на системе стандартного типа с подогреваемым столом (инженер по 3D-печати Ф.А. Насиров), показанной на Рис. 4.

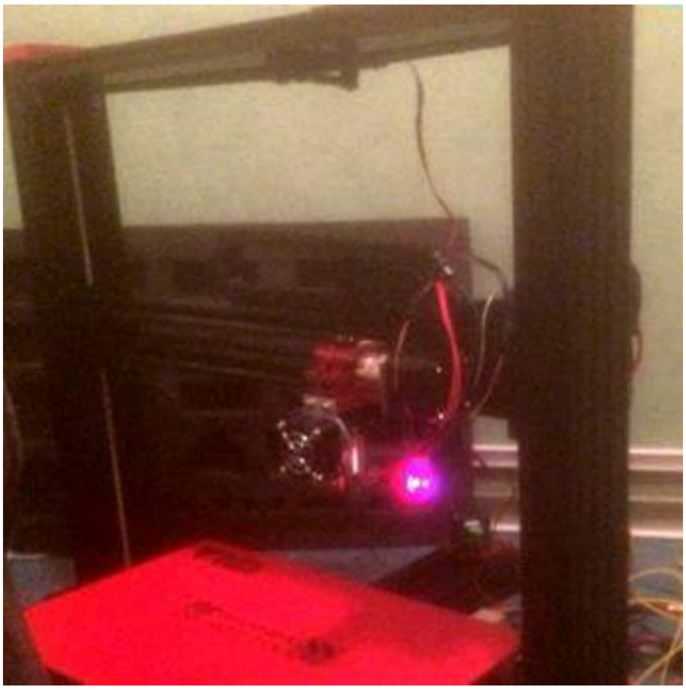


Рис. 4. Собранный 3D-принтер для печати прототипов чипов на больших поверхностях

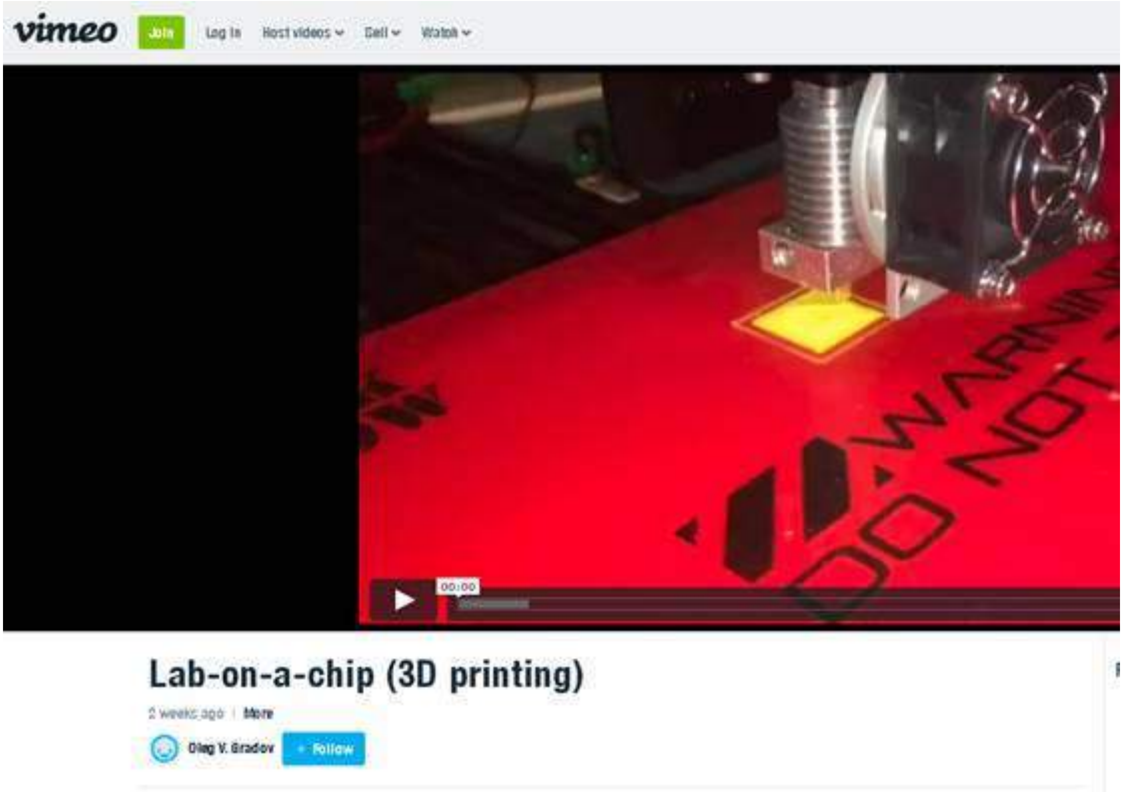
К сожалению, сопла стандартных промышленных 3D-принтеров выходили из строя при работе с функциональными композитами, которыми мы пытались для придания специализированных свойств поверхности печатать указанные структуры. Однако на стандартных пластиках (ABS, PL и др.) процесс шел достаточно эффективно. Видео печати структур-прототипов, на которых, с учетом критериев подобия, исследовались потоки, сорбционные и биоадгезивные свойства в топологии каналов, приведено на ресурсах:

- https://vimeo.com/273508210 (Рис. 5а);
- https://vimeo.com/273508513 (Рис. 5б);
- https://www.youtube.com/watch?v=tlb2jtr7Wqo (Рис. 5а);
- https://www.youtube.com/watch?v=HapB97OMiGc (Рис. 5б);

а также распространено путем микроблогинга на ресурсе лаборатории:

- https://twitter.com/Lab005/status/954308076315783168 (Рис. 5а);
- https://twitter.com/Lab005/status/979402936580296704 (Рис. 5б);

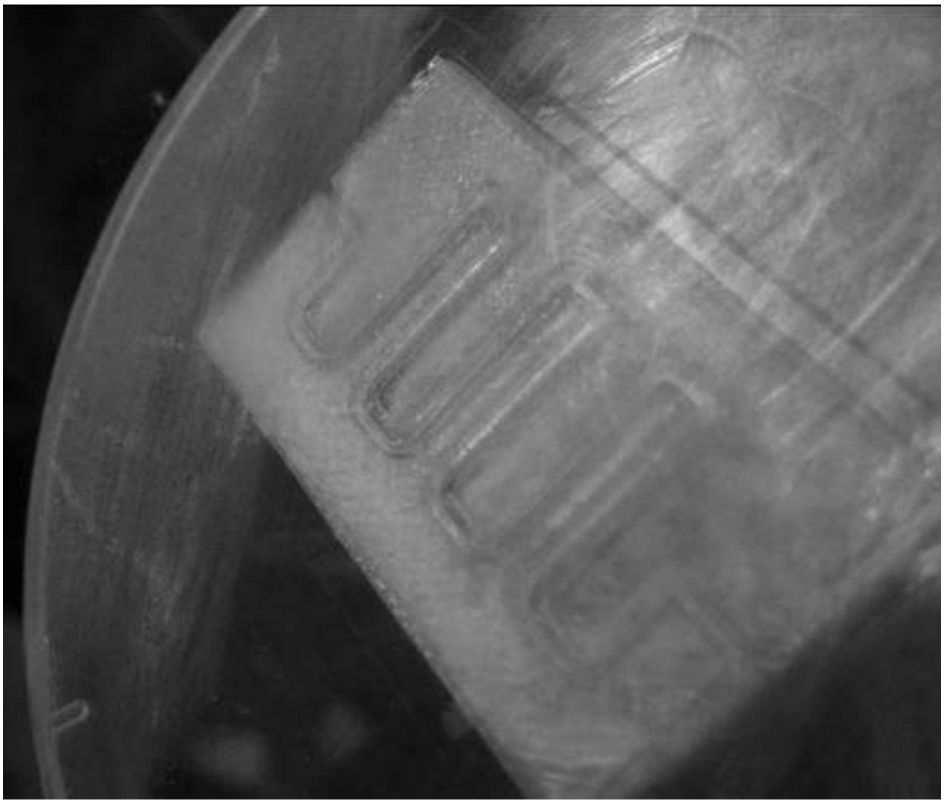
Фото установки для их печати приведено на Рис. 5б. Фото двух структур-прототипов lab-on-a-dish приведены на Рис. 6. Прототипы чипов для оптических испытаний, нанесенные на ленту из гнущегося и достаточно жесткого специально подобранного полимера приведены на Рис. 7. Использование прототипов чипов на высокополимерных лентах в прототипе конвейерной фотометрической или денситометрической калибровочной схемы считывания, показанной ниже на Рис. 8, позволило проанализировать границы применимости фотометрического подхода в массовой конвейерной аналитике на одноразовых пассивных чипах, предлагавшихся ранее как альтернатива многоразовой системе на чипе, а также установить источники ошибок.



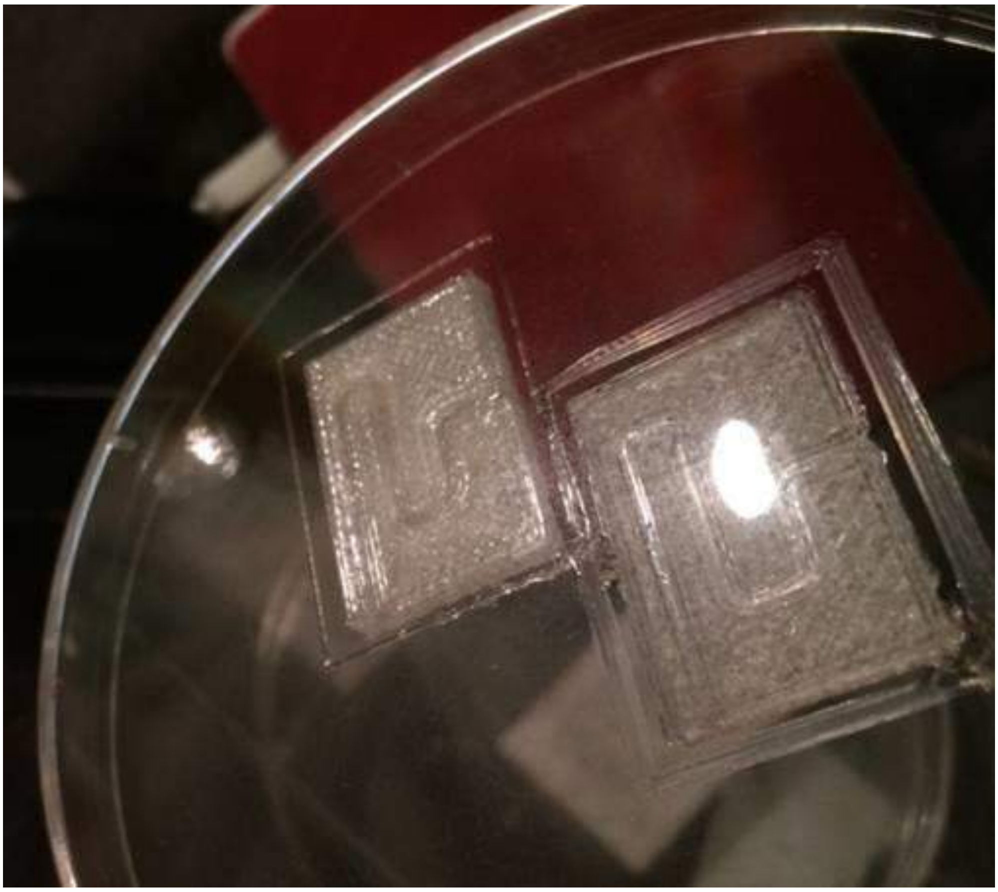
а

б

Рис. 5. Печать структуры-прототипа lab-on-a-chip (а) и lab-on-a-dish (б)



а



б

Рис. 6. Фото двух топологий структур-прототипов lab-on-a-dish

Использование доступных аддитивных технологий для формирования чипа возможно обосновать следующим образом. Аддитивные технологии формирования аналитических чипов (принтинг с использованием разогреваемых форсунок), пассивных лабораторий на чипе интенсивно используются в микрофлюидике и перспективной нанофлюидике (Comina et al., 2014; Wang et al., 2015; Walczak et al., 2016; Yazdi et al., 2016; Lee et al., 2016; van den Driesche et al., 2018). Для этого применяются методы, обеспечивающие формирование конфигурируемых устройств хемометрики и биотехнологии, обеспечивающие реакционную инженерию сложных систем (Kitson et al., 2012) с интегрированными элементами подвода и вывода проб (Comina et al., 2015; Gong et al., 2018); в том числе, методы пиропринтинга и вакуумного принтинга (Okkuno et al., 2002; Coppola et al., 2016). Технологии химического детектирования на подобных условиях предусматривают: Vis- и УФ-спектроскопию (Prince et al., 2015), спектрофлуориметрию (Zitka et al., 2015), фотометрию (Comina, 2017), бумажную сепарацию (Yafia et al., 2015), электрофорез, включая гель-электрофорез (Adamski et al., 2016; Walczak et al., 2017, 2018), а также прочие электрохимические методы, включая анализ с использованием квантовых точек (Krejcova et al., 2014).

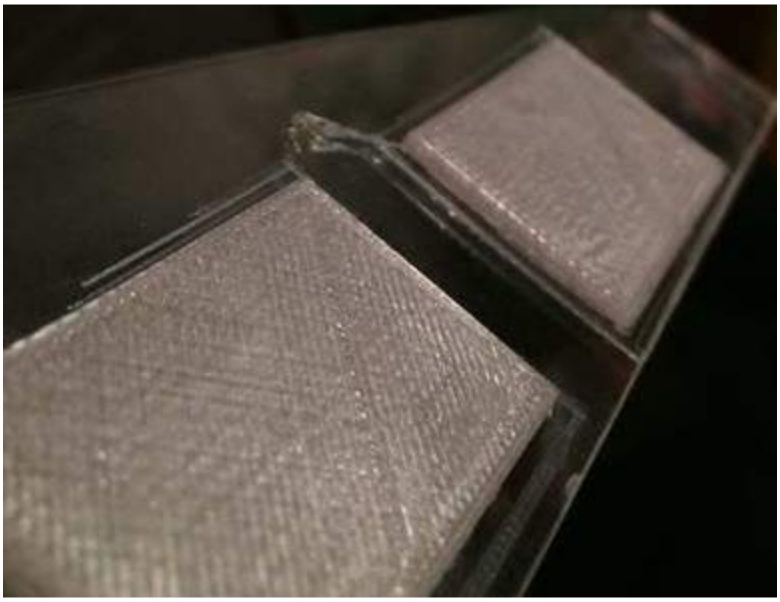
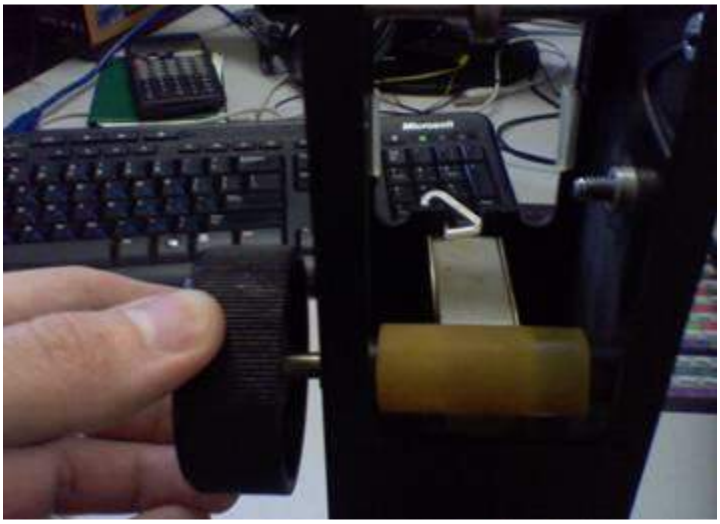


Рис. 7. Мезофлюидные многоячеистые полимерные чипы-прототипы на «конвейерной ленте» из акриловой смолы – полиметилметакрилата. Гнущаяся плекс-лента может быть изготовлена из данного состава любой трейд-марки (лимакрил, плазкрил, акрилекс, акрилайт, акрипласт, метаплекс, люцит, акрилит, перспекс, новаттро, плексима и т.д.)

Для биологических применений – таких, как микроваскулярные микроанатомические работы – в 3D-принтированных чипах часто используют гидрогелевые слои (Yang et al., 2016), делая акцент на биосовместимость чипов (Takenaga et al., 2015; Credi et al., 2018). Биоструктуры на чипе поддерживаются разного уровня сложности – от временной фиксации (цитратный буфер, гепарин или альтернативные – в зависимости от потребностей) клеток крови (Plevniak et al., 2016), сосудистых элементов и процессов васкуляризации в гидрогелях (Yang et al., 2016) либо анализа гематологических барьеров на чипе (Harding et al., 2017), что сложно, но принципиально очевидно, до культур клеток и органов на чипе (Knowlton et al., 2016; Podwin et al., 2018; Miller, 2013): например, от почек (Sochol et al., 2016) до нервной системы (Johnson et al., 2016), включая девинатные формы и структуры, в частности – онкологические (Yi et al., 2017). Одной из задач данных работ является клеточный и тканевый скрининг для фармакологии и токсикологии (Zhan-ying et al., 2014; Tourlomousis et al., 2014). Микрофлора, как правило, не исследуется в автономных условиях, однако она изучается в лабораторных установках по отдельным дескрипторам: биоэнергетике или биохимическим каскадам микроорганизмов при ферментации (Podwin et al., 2016; Podwin and Dziuban, 2017), возможности детектирования патогенов с помощью ассоциированных с наночастицами агентов etc. (Chudobova et al., 2015).

а



б

Рис. 8. Прототип конвейерного фото-/денситометрического калибровочного устройства для считывания плекс-лент чипов: а – общий вид; б – узел вращения каретки / носителя

Таксономические возможности таких работ не ограничены техникой определения собственно бактерий, так как детектируются и простейшие (как пример можно привести малярийный плазмодий (Bauer and Kulinsky, 2018), вирусы и горизонтальный перенос генов (Krejcova et al., 2014; Brennan, 2017) и т.д. Собственно микроорганизмы могут быть от наиболее крупных, часть из которых возможно анализировать и на чипах – безлинзовых микроскопах, до малых, как микроплазма, детектируемых по UV-Vis-характеристикам, но без позиционной чувствительности (Devathasan et al., 2013). Помимо этого, на чипах подобного типа можно изучать кристаллизацию и градиентные типы концентрационных явлений, в том числе – реакционно-диффузионных по природе (Liang et al., 2017; Chen et al., 2016). Индикаторами и сигнальными преобразователями переменных могут быть различные «ониксные» схемы, такие, как основанные на плазмонике (Law et al., 2016), нанофотонике (Koos et al., 2017), планарно-антенной радиоэлектронике и т.д. Поэтому они совместимы и с нашей идеологией многоуровневого преобразования на поверхности чипа (Gradov, Jablokow, 2016). Однако: для обеспечения микробиологической и таксономической эффективности данного типа мультиплексирования на чипе необходимо обеспечить не только совместимость чипа с преобразователями, обеспечивающими мультиплексирование, но и совместимость чипа как ксеногенного объекта со средой и целевым аналитом – почвой и её микрофлорой, то есть, в денотате, его биосовместимость и «экосовместимость» (в т.ч. – «педосовместимость»). Этот аспект проблемы рассматривается в разделе 5 настоящей статьи, так как его разрешимость в целом является следствием физико-химических и структурных особенностей, рассмотрение которых входит в пределы проблемы, освещаемой предварительно в следующем разделе.

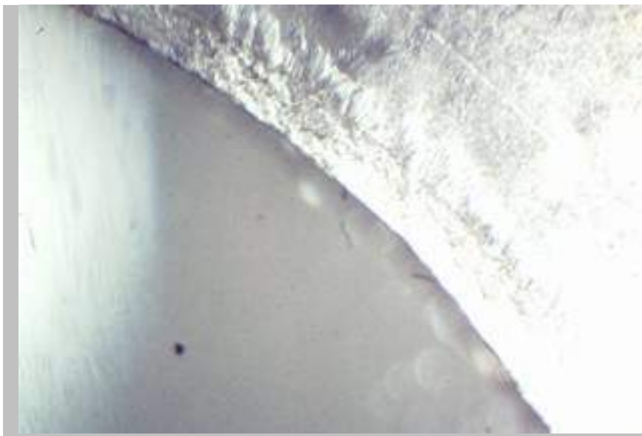
3. Результаты и обсуждение

Поверхность чипов и их структура. Микрографические исследования

Как было позиционировано выше, ключевым принципом для изготовления описываемых чипов – прототипов является совместимость поверхности и субповерхностной структуры чипов с естественной средой. Поэтому после изготовления чипов вышеописанным методом возникла очевидная проблема микроскопической характеризации как поверхности, так и субповерхностной структуры чипов. Были исследованы два типа чипов:

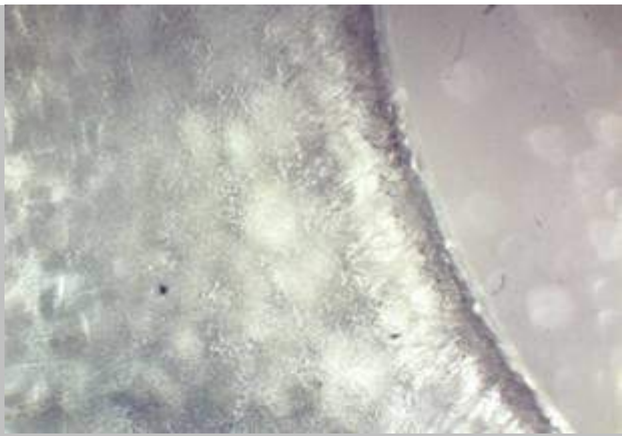
- Чип с ячейками, по геометрии подобный стандартному «геночипу» (Рис. 9). Исследование показало неоптимальность данной конфигурации для измерений (в силу плохой адгезии, формирования царапин и микротрещин, фаций-свилей и проникновения воздушных пузырей на границу подложки и полимера за счет наличия развитой системной перфорации (ячеек), понижающей надежность).
- Он же, но после введения флуоресцентных визуализирующих веществ (обычно – тетрапиррольных красителей), флуоресцирующих под действием лазеров, так как в работе предлагается «гибридизация» лазерно-флуоресцентного подхода (FRAP) с лазерной матрично-опосредованной десорбцией-ионизацией (MALDI). Флуоресцентная визуализация лунок / ячеек чипа при различных длинах волн, углах иллюминации и точках пространственного позиционирования лазерного пучка (при использовании цейтраферной оптоволоконной спектроскопии субнанометрового разрешения, данные которой приведены в Таблице 1) в районе лунок, с использованием полимерной матрицы (частично – как волновода или полупланарного оптического световода) показана на Рис. 10.
- Чип с полуячейками, сформированными путем 3D-принтинга в текстуре (класса волокнисто-тканых материалов) из стандартного субпрозрачного ABS-пластика (Рис. 11). Чип этого типа до исследований пористости структуры и сорбции (с использованием красителей типа родамин-6G) показал идеальные свойства: как по критерию удержания микроорганизмов, так и по развитости поверхности.
- Он же, но после введения флуоресцентных визуализирующих веществ (здесь это родамин-6G), флуоресцирующих под действием лазеров, так как нами в работе предлагается «гибридизация» лазерно-флуоресцентного подхода (FRAP, FLIP) с лазерной матрично-опосредованной десорбцией-ионизацией (MALDI и другими LDI). Несмотря на то, что данный тип чипов показал высокие метрологические и структурные характеристики на предварительном этапе, при введении в него красителя, индицирующего все

неоднородности поверхности и микроструктуры внутреннего полимерного слоя, являющиеся продуктом механики и алгоритмов 3D-принтинга (обеспечивающих «псевдо-тканую» структуру), обнаружил целый ряд структурных дефектов. Краситель попадал в них как диффузионным путём, так и через щели и топологические отверстия, вследствие чего использование на практике такого типа чипов привело бы к полному заселению его микрофлорой, а не только фиксации её в целевых областях ROI (Рис. 12).



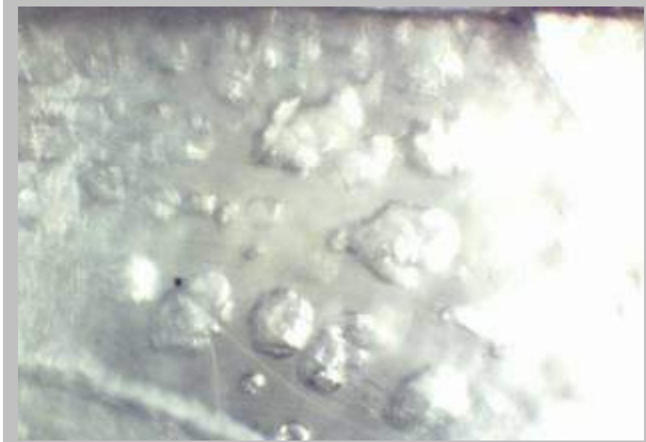
а

Интактная поверхность границы лунки чипа. Настройка освещения чипа – по Келлеру.



б

Граница лунки с полимерными границами, нарушенными воздушными пузырями.

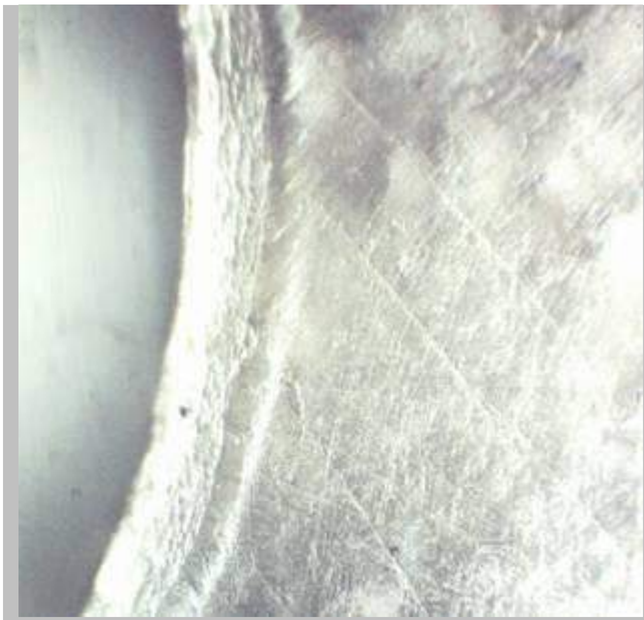


в

Воздушные пузыри в полимерном чипе под границей взаимодействия между подложкой и полимерным слоем после адгезии.

г

Воздушные пузыри в полимерном чипе на границе полимерной стенки лунки и среды, в которой находится детектируемый аналит.

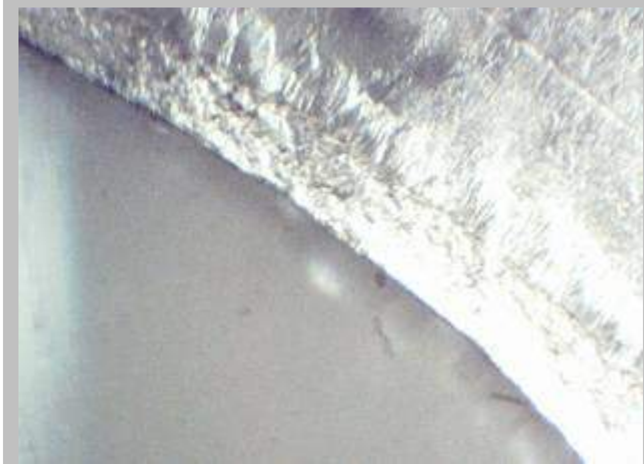


д

Микроцарапины / прекурсоры микротрещин на поверхности полимерного покрытия чипа в полимерном слое, не покрытом лаком или иным контр-абразивным покрытием.

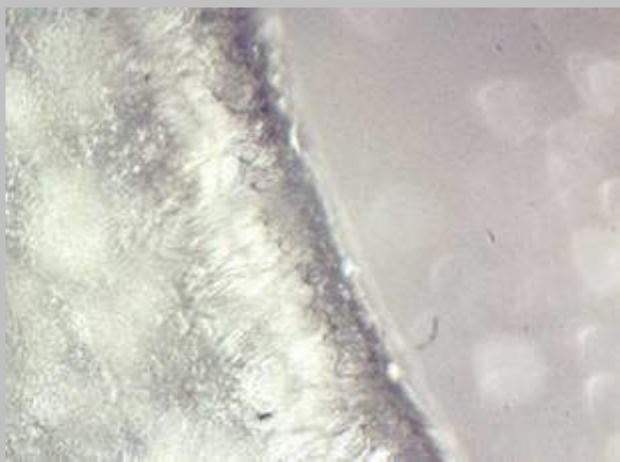
е

Наблюдение формирования групп и цепочек воздушных пузырей в полимерном слое чипа непосредственно под гладкой поверхностью, часть которой абразивно повреждена.



ж

Формирование сколов и фациальных свилей в приграничной зоне при прорезании чипа в одном из методов его подготовки к работе.



з

Формирование сколов и фациальных свилей в приграничной зоне при прорезании чипа. Видны также пузыри в зоне плохой адгезии.

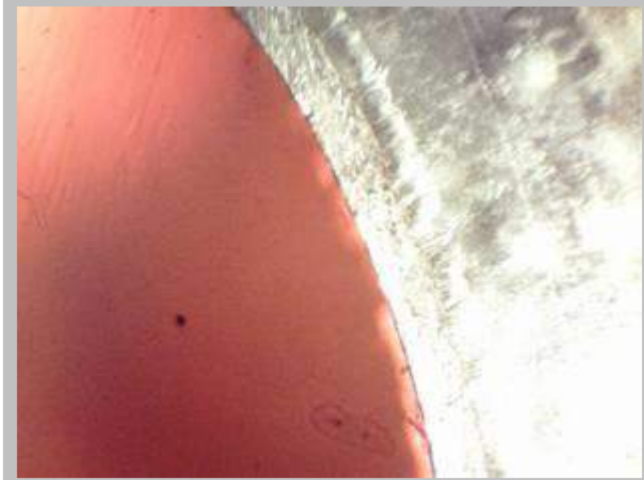
Рис. 9. Микроскопическое исследование чипа с ячейками.

Таблица 1. Примеры машинограмм (регистрограмм) спектров высокого разрешения

Дальний УФ-спектр высокого разрешения (фрагмент)		Ближний ИК-спектр высокого разрешения (фрагмент)	
293.510	528.000	993.900	688.000
293.670	527.000	994.120	656.000
293.840	536.000	994.340	679.000
294.010	529.000	994.550	661.000
294.180	530.000	994.770	676.000
294.350	525.000	994.990	681.000
294.510	520.000	995.210	683.000
294.680	537.000	995.430	660.000
294.850	528.000	995.640	707.000
295.020	547.000	995.860	672.000
295.190	541.000	996.080	690.000
295.350	528.000	996.300	679.000
295.520	522.000	996.510	705.000
295.690	540.000	996.730	675.000
295.860	528.000	996.950	694.000
296.030	532.000	997.170	688.000
296.190	525.000	997.380	706.000
296.360	528.000	997.600	664.000

Биолого-почвенная совместимость чипов

Отвечая на вопрос о возможности использования полимерных чипов для культивации микроорганизмов и экологически-совместимого мониторинга (environmental-friendly) почвенной микрофлоры, необходимо рассмотреть как пригодность данных полимерных сред для поддержания жизнедеятельности микроорганизмов, так и биодеградируемость, биорезорбируемость указанных субстанций. Ответ на вопрос о совместимости относится к биомиметической химии и теории внеклеточной полимеризации. «Что есть бактериальная среда внеклеточной полимеризации?» – такой вопрос задают Йост Вингендер, Том Нью и Ханс-Курт Флеминг (ныне – из центра биопленок Universität Duisburg-Essen, Fakultät für Chemie, Biofilm Centre) в главе классической коллективной монографии по экстрацеллюлярным полимерным субстанциям (Wingender et al., 1999). Ответ на него не так прост, как хотелось бы, поскольку полимеры, генерируемые живым и биокосным веществом, не остаются интактными, но вступают во взаимодействие с генерирующими их структурами, формируя, в результате, сложные композитные структуры биопленки. Хемосорбционные и адгезионные процессы усугубляют неспецифический характер процессов и продуктов их формирования. Многофазный характер описываемых структур обусловливается совмещением интерфейсных явлений (на границах воздух – почва, воздух – вода, вода – почва, почва – липиды живого вещества / вода – липиды живого вещества), действия катионов (изменяющих фазовый портрет биогенного и биокосного вещества), формирования коллоидных структур на принципах самоорганизации в столь комплексных градиентных условиях, не допускающих формирования гомогенной фазы на больших пространствах и стабильных временных интервалах, а также включением в процесс отличных по биохимической таксономии и, соответственно, биогеохимическим циклам микроорганизмов (бактерии, археи, микроводоросли, грибки), воздействующих на баланс EPS (Extracellular Polymer Substances), являясь прямыми матрицами / темплатами внеклеточной биогенно-индуцируемой полимеризации, а также прямыми генераторами полимеров, эджектируемых клетками в окружающую среду. Соответственно, нужно рассматривать не полимеризацию как таковую в отрыве от гетерогенной структуры градиентно-структуризуемой почвенной среды, но формирование композитной / биокомпозитной структуры в почве в процессе её формирования. Исходя из этого подхода, следует решать вопрос о совместимости полимерных чипов.



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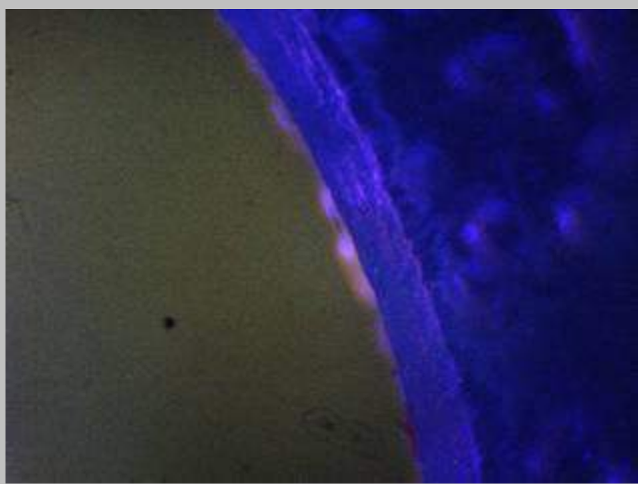

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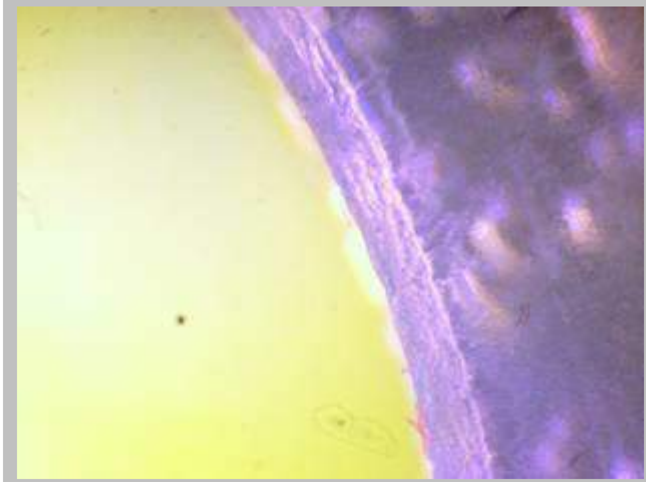

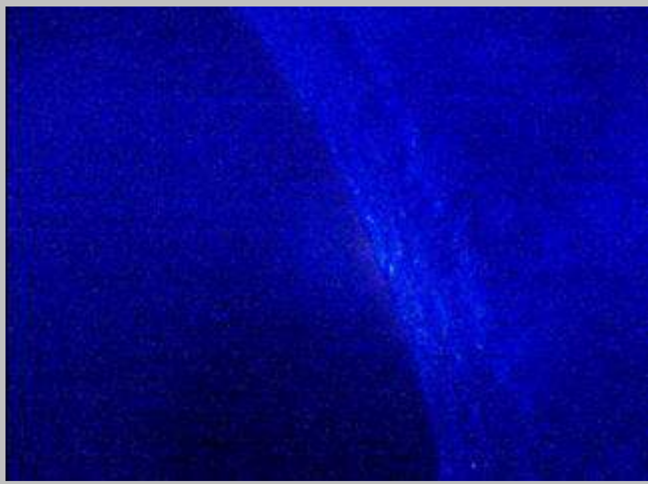

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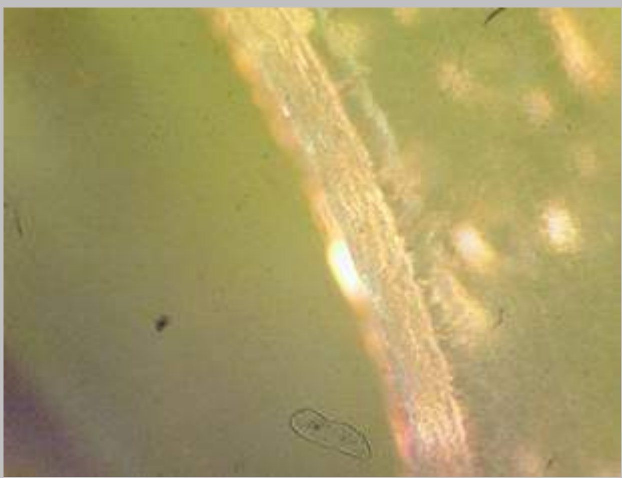

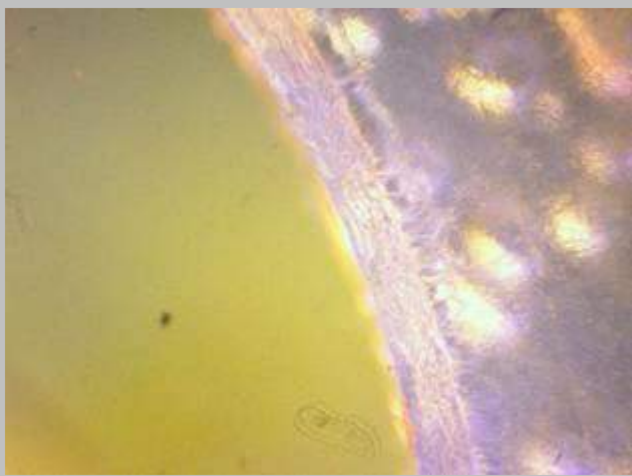

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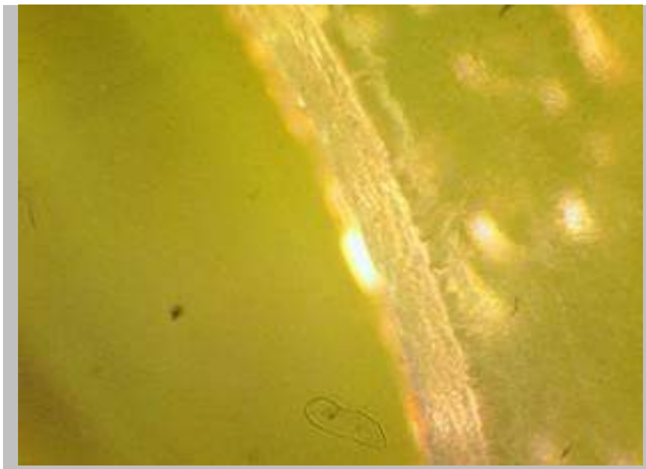

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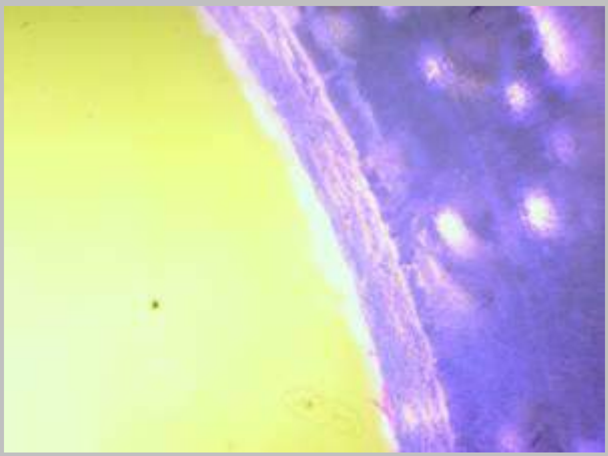

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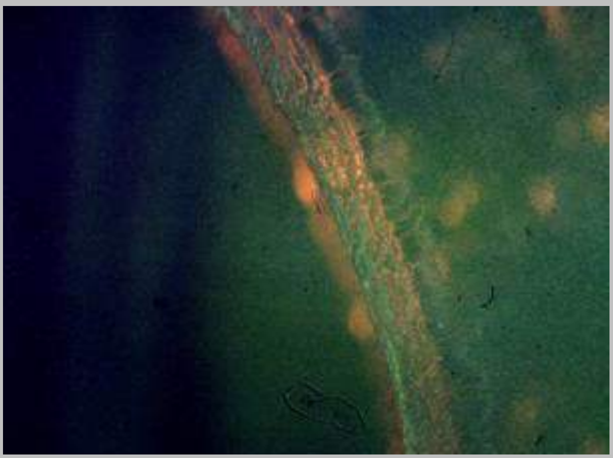

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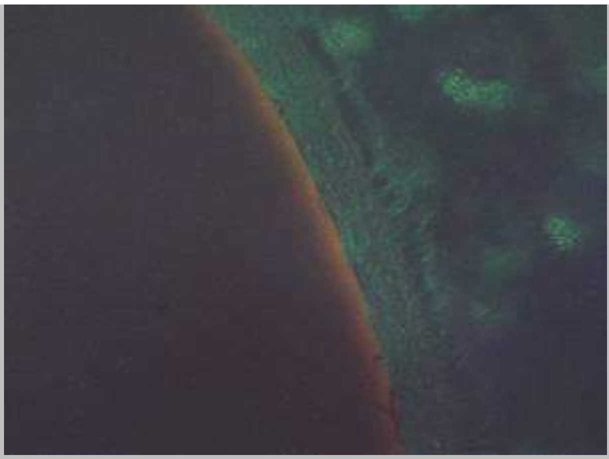

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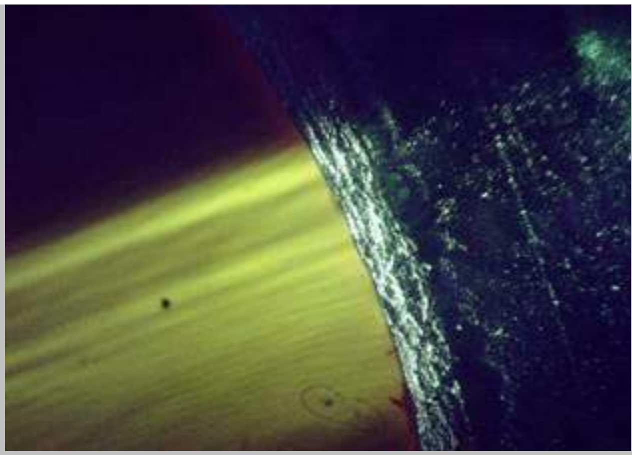

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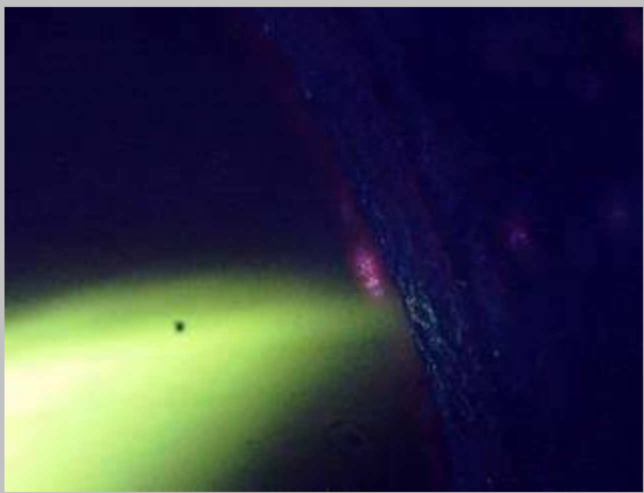

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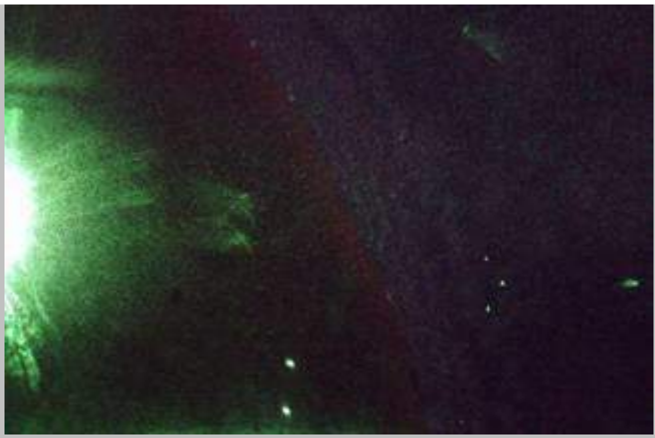

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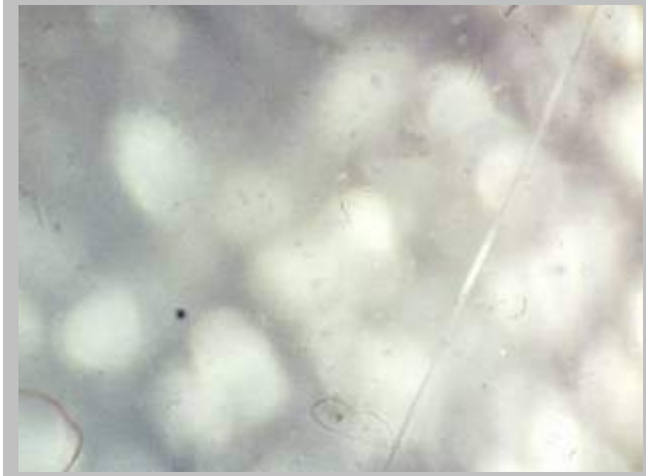
DPSS-лазер 532 нм.

Рис. 10. Флуоресцентная визуализация лунок / ячеек чипа при различных длинах волн, углах иллюминации и точках пространственного позиционирования лазерного пучка

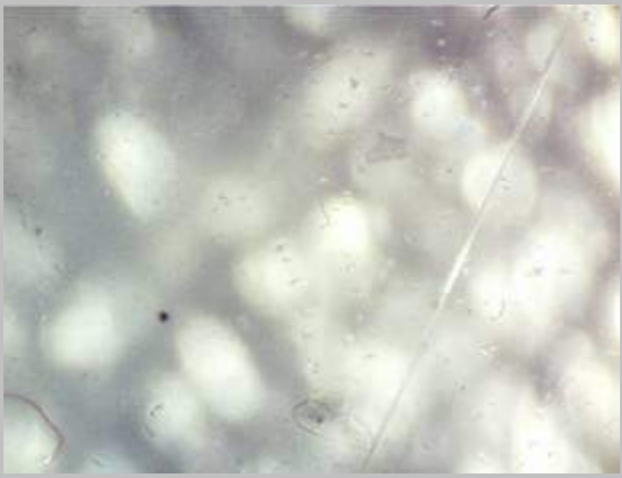
Бактериоспецифичные деградируемые полимеры, исходя из означенного принципа, могут быть изучены, исходя из специфичности синтетических или полусинтетических полимеров по критериям биомиметического синтеза либо эквифинальности с биологическим процессом. Так, для полиэфиров известны факты биосовместимости и биодеградируемости, обусловленной их синтезом с использованием метаболических путей микроорганизмов; принцип "plastics from bacteria and for bacteria" является одним из наиболее эмерджентных, т.е. экологически-прогрессивных подходов, несмотря на то, что хронологически он возник достаточно давно (Brandl et al., 1990). Этот принцип воспроизводят во многих работах по настоящее время (Lenz, 2007). Простейшим примером в области биосовместимости бактериогенных полимеров является целлюлоза – точнее, бактериальная целлюлоза (Keshk, El-Kott, 2017). Биомедицинские полимеры, являющиеся биодеградируемыми, также часто являются по путям их синтеза бактериогенными (Sevastianov et al., 2001; Basnett et al., 2017), как и аналогичные биодеградируемые полимеры общего назначения (Ojumu et al., 2004). Зачастую для этого используются бактериальные изоляты (El-Kady, 2014). Возможна поточная бактериальная конверсия токсичных и хмически-контаминированных стоков в ряд биодеградируемых полимеров (Holowach et al., 2014).

При этом бактериальные реакции являются определяющими как в аспекте состава / химизма, так и в аспекте физико-химических свойств, в том числе – реологии, трибологии и механохимии полимерного вещества в почве (Kang et al., 2017).

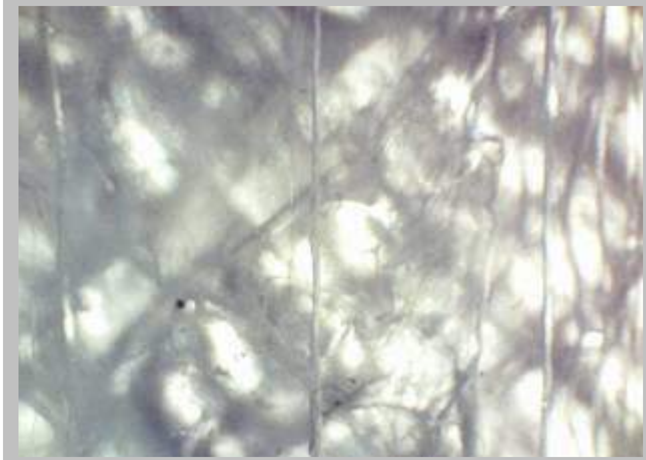
Следует отметить, что композиты в бактериологии и микробиологии, не исключая почвенную микробиологию, имеют достаточно большую историю внедрения. Первым «нано-композитным» полимерным методом в указанных областях являлся, по-видимому, электрофорез в агарозно-акриламидном геле композитного строения для исследования бактериальных рибосом (Dahlberg et al., 1969). В аспекте синергетического совмещения эффектов композитной матрицы / среды и бактериальных структур, вероятно, первой работой в этом направлении является работа Стюарта с соавторами, утверждающая, что факт формирования пигментных желчных камней обусловлен «биокомпозитным» взаимодействием бактериальных микроколоний и твердых веществ пигмента в составе гетерогенного и мультифазового комплекса (Stewart et al., 1987).



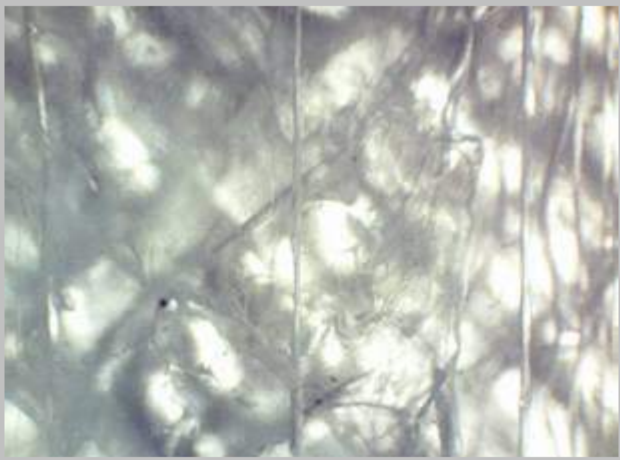
1-й слой фокусирования столом с ЧПУ



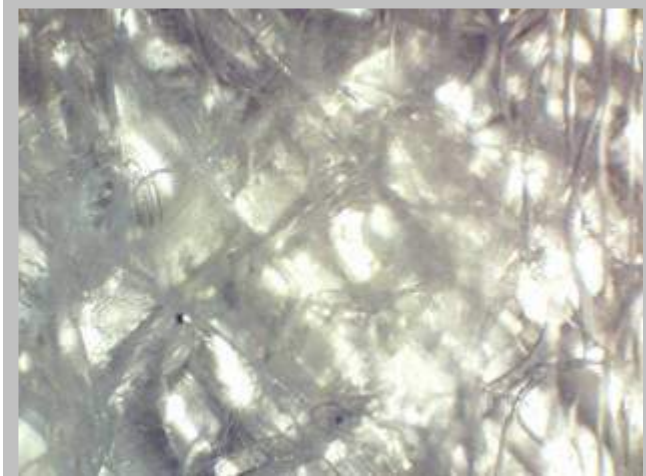
2-й слой фокусирования столом с ЧПУ



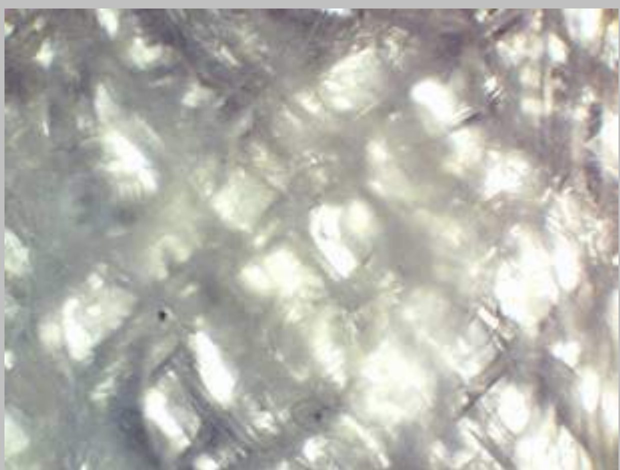
3-й слой фокусирования столом с ЧПУ



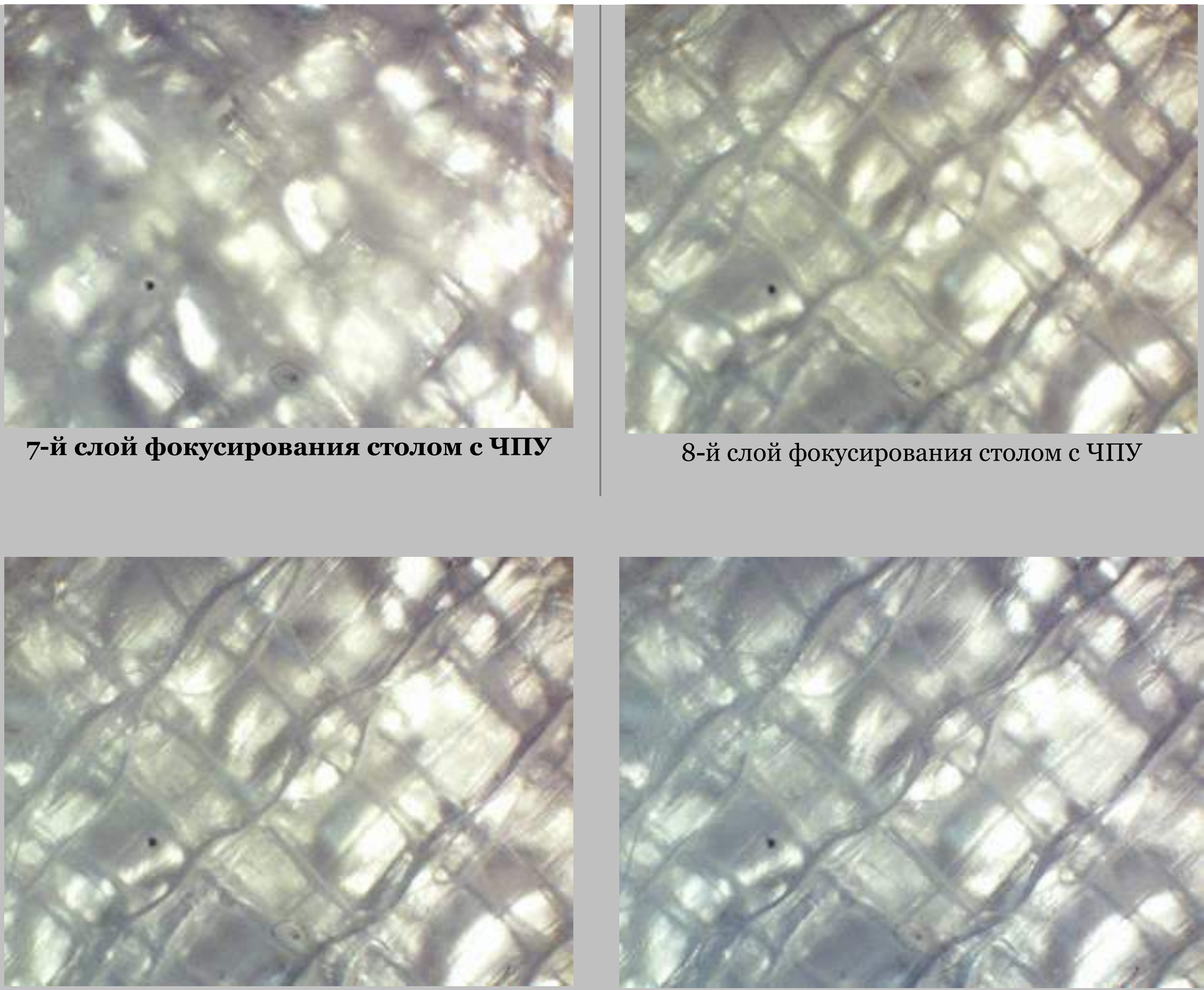
4-й слой фокусирования столом с ЧПУ



5-й слой фокусирования столом с ЧПУ



6-й слой фокусирования столом с ЧПУ



7-й слой фокусирования столом с ЧПУ

8-й слой фокусирования столом с ЧПУ

9-й слой фокусирования столом с ЧПУ

10-й слой фокусирования столом с ЧПУ

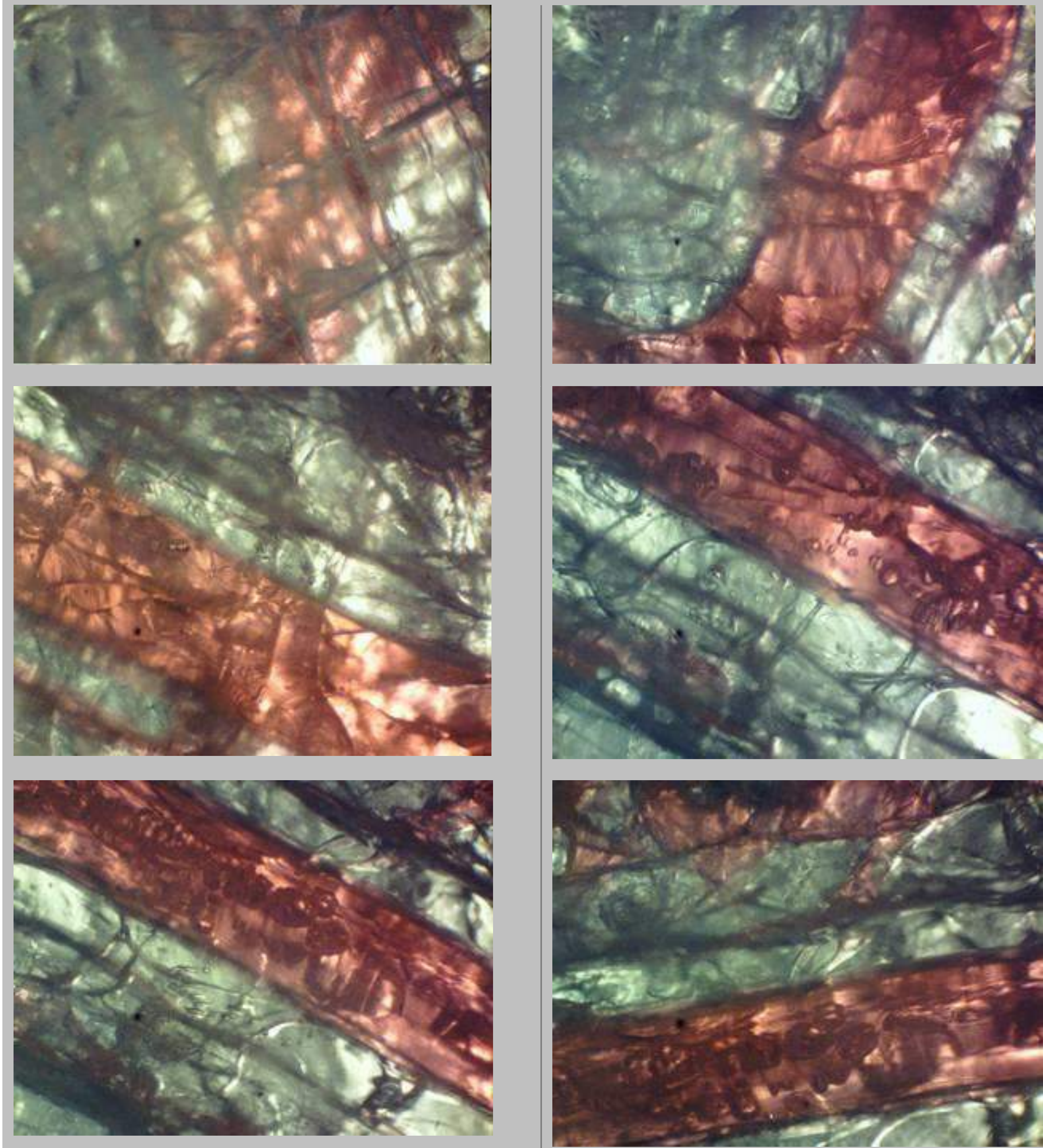
Рис. 11. Микроскопическое исследование чипа с полуячейками, сформированнымипутем 3D-принтинга в текстуре (класса волокнисто-тканых материалов) из стандартного субпрозрачного ABS-пластика

На данный момент исследования по синтезу матриц с микроорганизмами ведут в нескольких десятках центров, в том числе – специализирующихся на фазовой структуре биопленок, но композитными структурами называют биоструктуру в почве сравнительно редко, чем определяется относительно малые «индексы релевантности» данного сочетания в сетевых запросах (но в немецком и ряде иных языков, склонных к расширенному словообразованию, аналоги данного терминологического ряда встречаются более часто). Тем не менее, термины с данным содержанием часто применяются по отношению к надмолекулярным комплексам биогенных полимеров или биополимеров с внешними агентами / матрицами; например, только для бактериальной целлюлозы описана сотня с лишним вариантов композитов – система бактериальная целлюлоза / хитозан (Ciechanska, 2004; Kim et al., 2011), бактериальная целлюлоза / фосфаты (Barud et al., 2007), бактериальная целлюлоза / ПЭГ (Cai and Kim, 2010); бактериальная целлюлоза / экстракт *Aloe sp.* (Saibuatong and Phisalaphong, 2010), бактериальная целлюлоза / наночастицы серебра (Barud et al., 2011; Yang et al., 2012), бактериальная целлюлоза / коллаген (в тот же класс могут быть интродуцированы GIM – матриксы или комплексы с желатиновой иммобилизацией) (Zhijiang and Guang, 2011), etc.

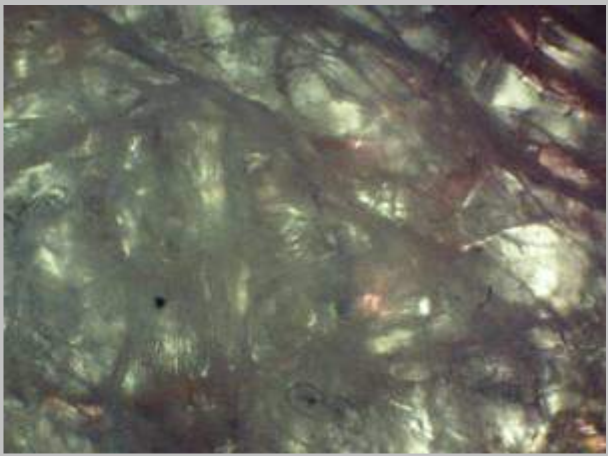
Визуализация мезофлюидных каналов прототипов почвенных чипов

с использованием красителя родамин-6G.

а

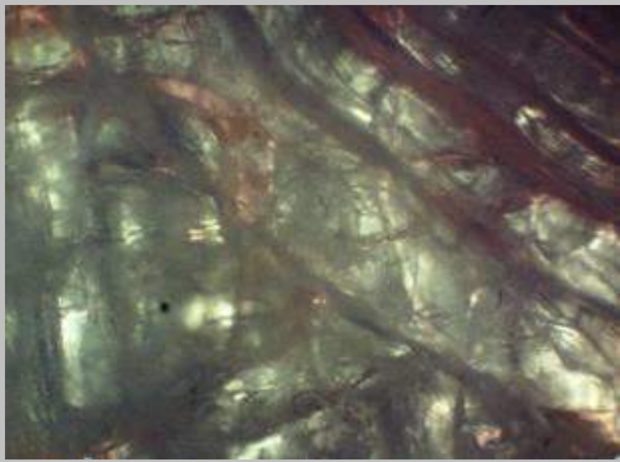


Проникновение красителя в микроструктуру чипа.



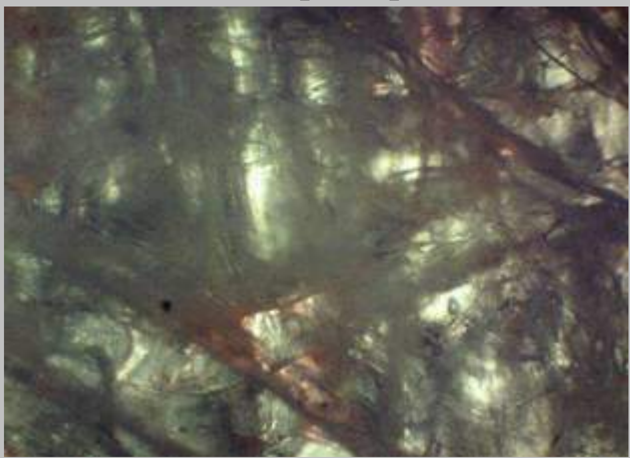
б

Взаимодействие с фибриллярной текстурой псевдо-тканого 3D-принтированного чипа.



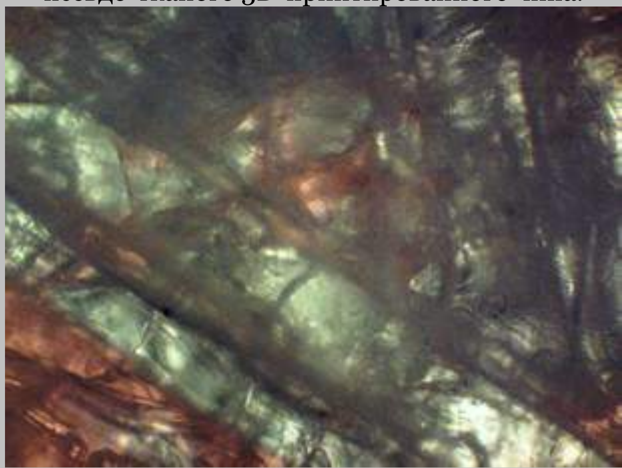
в

Взаимодействие с фибриллярной текстурой псевдо-тканого 3D-принтированного чипа.



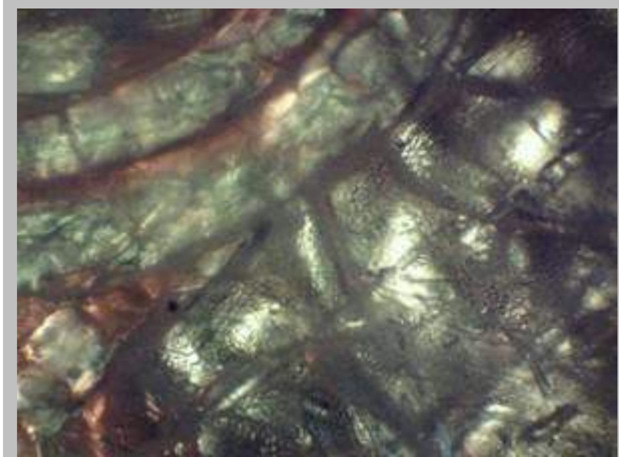
г

Взаимодействие с фибриллярной текстурой псевдо-тканого 3D-принтированного чипа.



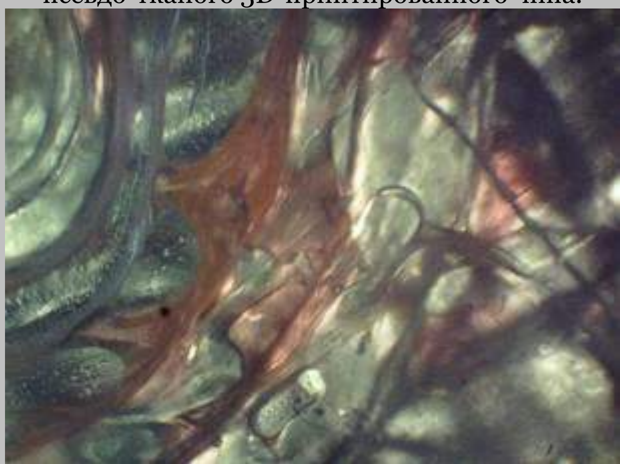
д

Взаимодействие с фибриллярной текстурой псевдо-тканого 3D-принтированного чипа.



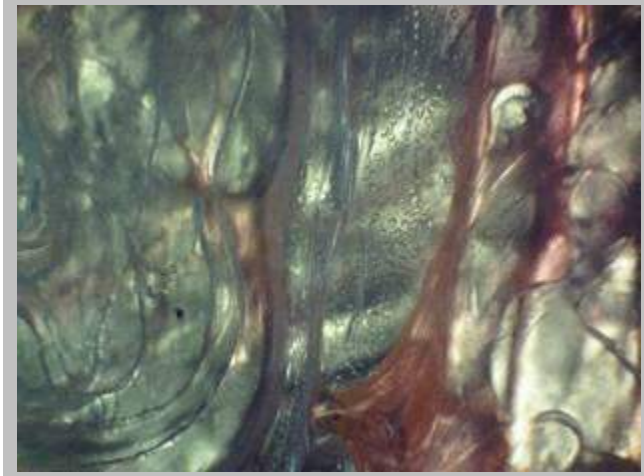
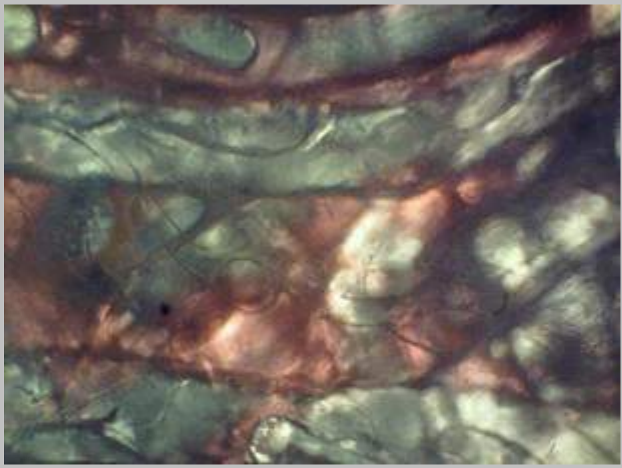
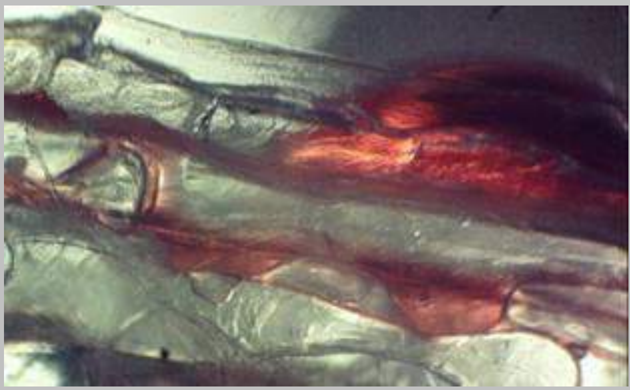
е

Помимо проникновения красителя можно наблюдать эффекты взаимодействия чипа с пузырьками воздуха.

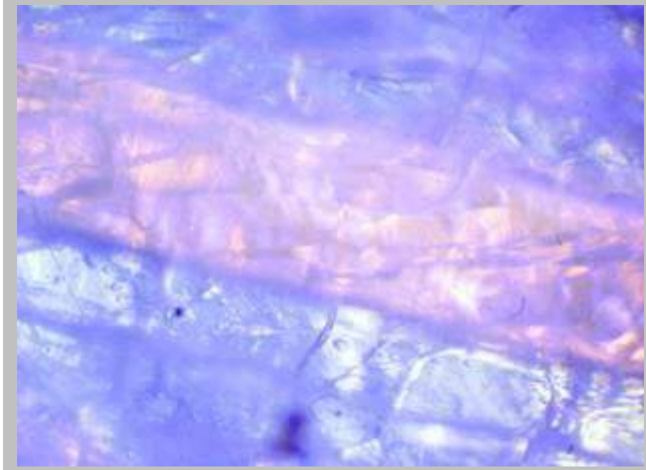


ж

Помимо проникновения красителя можно наблюдать эффекты взаимодействия чипа с пузырьками воздуха.

	
з	и
Помимо проникновения красителя можно наблюдать эффекты взаимодействия чипа с пузырьками воздуха.	Помимо проникновения красителя можно наблюдать эффекты взаимодействия чипа с пузырьками воздуха.
	
к	л
Помимо проникновения красителя можно наблюдать эффекты взаимодействия чипа с пузырьками воздуха.	Помимо проникновения красителя можно наблюдать эффекты взаимодействия чипа с пузырьками воздуха.

Лазерные флуоресцентные и лазерно-проекционные спекл-визуализации.

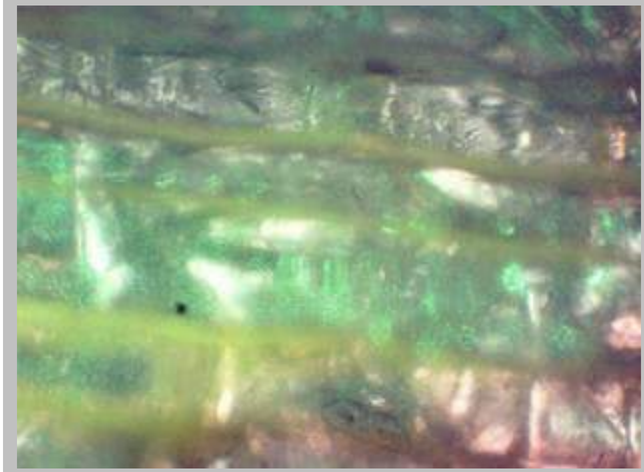


м

DPSS-лазер 405 нм. Флуоресценция.

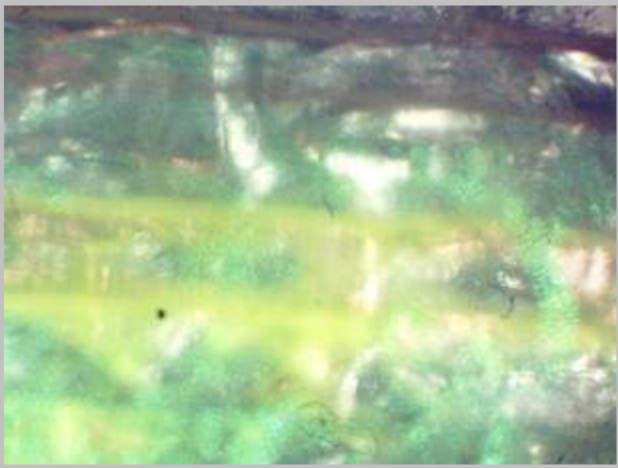
н

DPSS-лазер 532 нм. Флуоресценция.



о

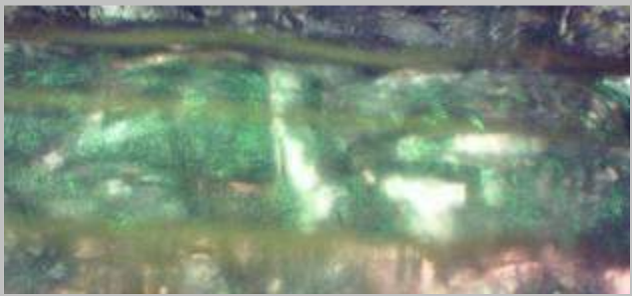
DPSS-лазер 532 нм. Флуоресценция и элементы спекл-структры.



п

DPSS-лазер 532 нм. Флуоресценция и элементы спекл-структры.

р
DPSS-лазер 532 нм. Флуоресценция и элементы спекл-структры.



с
DPSS-лазер 532 нм. Флуоресценция и элементы спекл-структры.

Рис. 12. Индикация структуры псевдо-тканого чипа с помощью красителя и DPSSL

В то же время, использование композитных материалов для мониторинга микроорганизмов и их культивации – сравнительно распространенная форма применения композитов в клеточной, микро-популяционной и экологической микробиологии: виды клостридий успешно культивируются на композитных PDMS-мембранах (Van Hecke et al., 2012), бифидобактерии могут работать на композитных фильтрах, как это делается на многих промпредприятиях, и т.д. В принципе, с точки зрения биофизики, это мало чем отличается от методов / технологий культивации клеток растений (Ikezawa et al., 1998) или животных на композитах / композитных скафолдах и гидрогелях (Bhowmick et al., 2016; Vikhrov et al., 1998; Popriadukhin et al., 2011; Xioxia et al., 2008; Li et al., 2009; Kremer et al., 2000). Значительно более распространены, впрочем, стандарты культивации клеток на обычных полимерных (не композитных) носителях с программируемыми свойствами. Как правило, речь идёт о животных клетках (Slavnaia et al., 1974; Arnold et al., 2002; Sternberg et al., 2004; Pierkes et al., 2004; Giselbrecht et al., 2005; Sudha et al., 2006; Shved et al., 2006, 2011; Haraguchi et al., 2006; Sitalakshmi et al., 2006, 2008; Vacik et al., 2008; Ivanov et al., 2010; Yasuda et al., 2011; Dolgikh et al., 2011; Matsunaga et al., 2012; Bauermeister et al., 2014; Imagawa, 2018), но известны прецеденты работы с полимерами для культивации водорослей (Kibok et al., 2013; Kerrison et al., 2017), растений (Hooker and Lee, 1990; Ilieva et al., 1995), простейших (Goto and Nakajima, 2015), грибов (Hayashi et al., 1976) и микроорганизмов (Dissing and Mattiasson, 1994; Khanna and Srivastava, 2010). Поэтому сама постановка проблемы имплантируемых в почву полимерных чипов для контролируемой культивации или псевдоинвитрального мониторинга микрофлоры, очевидно, имеет смысл. Учитывая, что для лабораторных условий используются чипы с микрофлюидной конфигурацией (Funfak et al., 2009) для контроля pH. Eh, pX и ряда других релевантных биоэлектрохимических показателей (Chagas et al., 2014), рационально контролировать на вводимых в почву полимерных либо композитных / биокомпозитных чипах и эту параметрику. Это архиважно, в том числе, с точки зрения анализа продукционных свойств биокосного, а не только живого вещества. Не затрагивая сотни работ, имеющихся по данной тематике на зарубежных языках, отметим, что в России это направление уже более 40 лет развивается В.И. Савичем и затем – школой В.И. Савича – ныне профессора ТСХА (Савич, 1974; Кауричев и др., 1975; Savich et al., 2004; Савич и др., 1979, 1993). Помимо редокс-характеристик почвы ими изучены роль комплексообразования (Савич, Никиточкин, 2012; Седых и др., 2012) в плодородном слое, произведена оценка состояния системы почва-растение по содержанию и соотношению положительно и отрицательно заряженных соединений (Савич, Трубицина, 1987; Савич и др., 1990а). В рамках построений данной школы объективно существующие электрохимические и концентрационные поля в почве являются факторами плодородия почв и почвообразования (Савич и др., 1989; Савич и др., 1990б; Савич и др., 2009). Различия эффектов положительно и отрицательно заряженных агентов при этом достаточно очевидны (Савич и др., 1990а). Таким образом, помимо локального колориметрического или спектроколориметрического анализа почв и ROI колониеобразующих единиц (Савич и др., 2004; Байбеков и др., 2007), системе на чипе можно делегировать редоксметрические функции.

Нами был осуществлен анализ поверхности одного из типов чипов, использовавшихся нами – с желатиновой подложкой из фотоэмульсии (рассмотрение этого типа чипов будет в одной из следующих частей статьи) ядерного назначения – на предмет наличия в подложке / на подложке микроорганизмов после гибридного эмульсионно-жидкостного экспонирования без последующей обработки. Этот тип чипов является оптимальным для задач темновой работы на больших глубинах почв, где агентами экспонирования слоя могут являться лишь химические факторы (как в хемоавтографии) и автофлуоресценция микрофлоры. Результат исследования для одного из ROI на пассивном чипе приведен на Рис. 13.

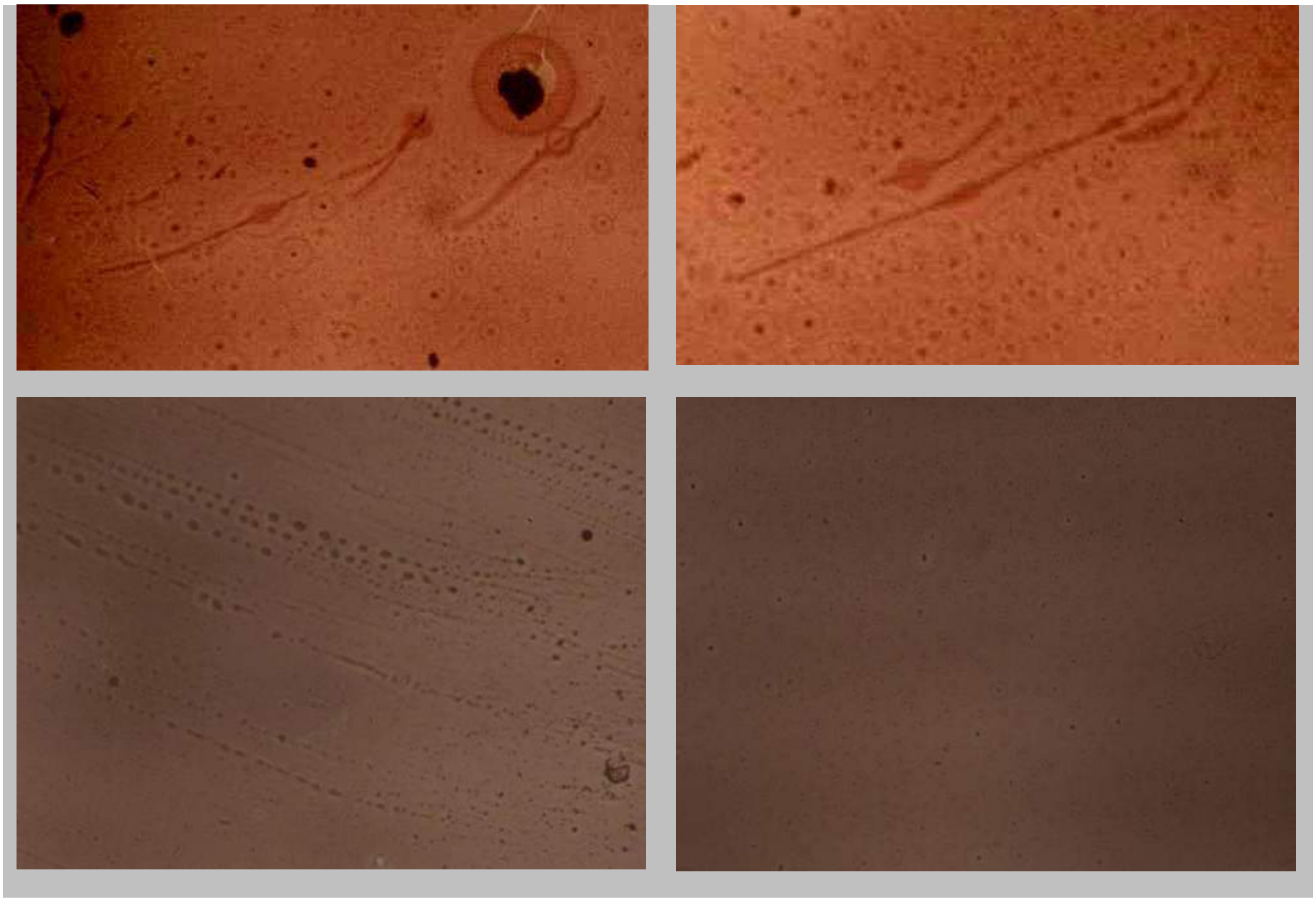


Рис. 13. Структуры естественной микрофлоры на почвенном GIM-биочипе

Нами, кроме того, были исследованы около 40 сэмплов, однако их подробный физико-биохимический анализ является предметом отдельных работ. Приведенные микрофотографии показывают, что на планарной подложке формируются ярко выраженные структуры контаминации. По данным консультации с микробиологами из РНИМУ, предположено наличие гетероморфных форм *Acromobacter sp.* – они строго аэробные и содержатся в воде и почвах; идентифицировать их как культуральные контаминанты также возможно. Из-за отсутствия возможности окраски в почве *in vivo* стандартными методами, определить оксифильность или базофильность их в режиме реального времени не представляется возможным. Однако соответствующие методы будут рассмотрены в одной из следующих частей настоящего цикла.

4. Заключение

Таким образом, по ключевым проблемам для решения задач комплексирования ряда методов MALDI, FRAP и FLIP на чипе, погружаемом для экспонирования в почвенную среду, получены следующие решения:

1) <u>Наличие/отсутствие специализированных чипов для почвенно-биологических задач (фенотипического и биогеохимического плана) реального времени</u>. – Наличие чипов для почвенно-биологических и биогеохимических задач, основанных на применении генетических маркеров и стандартных генетических методов, очевидно, не может как типичная секвеномная техника считаться средством исследования in situ / в реальном времени; поэтому, несмотря на наличие высококачественных чипов типа “GeoChip” и аналогичных им филочипов, потребность в создании чипов для биопочвенных задач сохраняется.

2) <u>Возможность быстрого прототипирования чипов</u>. – Возможность прототипирования, в том числе – быстрого прототипирования, почвенных чипов кастомизированного (с целью использования для конкретной задачи) уровня в условиях

лабораторий может быть достигнута с помощью бюджетных 3D-принтеров, изготовление которых может быть выполнено за несколько дней при затратах в несколько сотен у.е. либо заказано за суммы в несколько тысяч у.е. Прототипирование может осуществляться на основе биосовместимых или биоразлагаемых полимеров (см ниже), которые могут являться, в частности, частью подложки матрицы.

3) Совместимость полимерных подложек или покрытий чипов с почвенной биотой. – В случае GIM-чипов идеальная совместимость доказана материалами раздела 4, особо – таблицей 6. Теоретически, возможности биосовместимых полимеров обеспечивают широкий спектр характеристик и градаций биовосместимости, вплоть до частичной / грануляционной биоразлагаемости, рассматриваемой в одной из следующих частей в настоящем цикле работ.

4) Наличие полимерных подложек, красителей *in vivo* и матриц, совместимых с MALDI. – Принципиальная возможность использования красителей, совместимых с MALDI, продемонстрирована в табл. 3 и 5. Принципиальная возможность использования для этого ряда полимерных подложек – там же, а также в таблицах 1, 4. Анализ проблемы MALDI-совместимости материала почвенных чипов является отдельным предметом одной из следующих частей настоящего цикла работ.

5) Возможность исследования чипов с данными текстурами флуоресцентным методом. – Принципиальная возможность использования красителей, совместимых с техникой FRAP (Fluorescence Recovery After Photobleaching), а также FLIP (Fluorescence Loss in Photobleaching) и аналогичными флуоресцентно-кинетическими техниками частично продемонстрирована в табл. 3 и 5. Как следствие этого, возможно предполагать, что их использование будет рациональным для гибридизации MALDI и FRAP, MALDI и FLIP и т.д. (учитывая применимость их и в MALDI-матрицах). Текстура полимерного чипа достаточно прозрачна и не препятствует проникновению сигнала на детектор, как это показано в приведенных выше таблицах.

В ближайших выпусках журнала вопросы, означенные в пунктах 4 и 5, будут освещены более подробно.

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"MALDI-FLIP-on-a-chip" и "MALDI-FRAP-on-a-flap": новые технологии для почвенной микробиологии и биогеохимии. Часть 2: Прототипирование полимерного чипа

(Приглашенный материал)

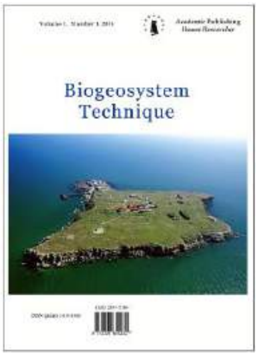
А.Г. Яблоков [a,*], Ф.А. Насиров [a], Ф.К. Орехов [b], О.В. Градов [a,b]

[a] Институт энергетических проблем химической физики им. В.Л. Тальрозе Российской Академии Наук, Российская Федерация
[b] Институт химической физики им. Н.Н. Семенова Российской Академии Наук, Российская Федерация

Аннотация. В предшествовующей статье данного цикла были рассмотрены возможности создания аналитического чипа для почвенно-биологических задач как концепта фингерпринтируемого устройства с т.н. «несуррогатным ключом», то есть

* Корреспондирующий автор
Адреса электронной почты: retromicroscopy@gmail.com (А.Г. Яблоков)

локально-телеметрируемого программно-аппаратного комплекса почвенного заложения, идентификация в котором производится на основе собственных физико-химических параметров идентифицируемых объектов. На данный момент препятствиями к массовому внедрению данного подходя являются материаловедческие проблемы – проблемы биосовместимости, биокоррозии и биоразложения тех элементов конструкции, которые при массовой закладке способны не только исказить своим вводом результаты эксперимента на локальных диффузионных промежутках, но и сместить дескрипторы биогеохимического и экологического равновесия. Поэтому вторая часть статьи посвящается, в существенной части, именно проблемам выбора материалов для изготовления чипа и их практической апробации. Кроме того, в силу телеметрического характера сбора данных и вполне формально математизируемого способа идентификации на «фенотипических» и биогеохимических «обменно-метаболических» чипах данного типа, необходимо, в первую очередь, проиллюстрировать главное преимущество чипов разрабатываемого нами типа – возможность синхронного анализа в реальном времени, недоступную другим чипам с возможностью обработки последовательностей и идентификации организмов, в том числе – с матрично-опосредованной десорбцией-ионизацией при масс-спектрометрическом секвенировании, а именно – генетическим чипам для анализа почвенной микробиоты. Если наш чип не обладает преимуществами данного типа, а современные активно распространяемые и используемые почвенные генетические чипы типа «GeoChip» способны делать то же самое, то смысла в разработке, очевидно, нет. Если наши чипы могут выдавать лучший метрологический результат, но токсичны для среды – это тоже не выход. Если применяемые полимеры и другие среды нейтральны для почвы и микробиоты, но не выдают флуоресцентного и матрично-опосредованного масс-спектрометрического сигнала – аналогично, прикладной и отраслевой смысл в работе отсутствуют, ибо она не выйдет за пределы лаборатории. Итого, ключевыми проблемами для решения задачи комплексирования MALDI (что уже аннотировалось выше – матрично-опосредованная десорбция и ионизация при разных видах масс-спектрометрии, начиная от времяпролетной, заканчивая орбитальными ловушками» и ловушками с динамической гармонизацией, масс-спектрометрами ионного циклотронного резонанса и т.д.), FRAP (метод исследования кинетики флуоресценции после фотообесцвечивания / фотовыжигания – см. предыдущую статью) и FLIP (установление уровня затухания и потерь флуоресценции в процессе фотообесцечивания / фотовыжигания) на чипе, погружаемом для экспонирования в почвенную среду, являются в комплексе: а) Наличие/отсутствие специализированных чипов для почвенно-биологических задач (фенотипического и биогеохимического плана) реального времени; б) Если нет – возможность быстрого 3D-прототипирования чипов данного назначения на тех материалах, которые оптимальны для данных задач (см. п. 3, п. 4 ниже); в) Совместимость полимерных подложек или покрытий чипов с почвенной биотой; г) Наличие полимерных подложек, красителей *in vivo* и матриц, совместимых с MALDI; д) Возможность исследования чипов с данными текстурами флуоресцентным методом. Эти вопросы подробно рассматриваются в настоящей статье.



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Silica Microphorms in Soils Affected by Hydrothermal Activity on the Yellowstone Volcanic Plateau, USA

Galina V. Kharitonova [a], Fyodor S. Kot [b, *], Valeria O. Krutikova [a, c], Evgeny V. Kharitonov [a], Victor Kislovsky [d]

[a] Institute of Water and Ecological Problems, Far East Branch of the Russian Academy of Sciences, Khabarovsk, Russian Federation
[b] Faculty of Civil and Environmental Engineering, Technion–Israel Institute of Technology, Haifa, Israel
[c] Institute for Tectonics and Geophysics, Far East Branch of the Russian Academy of Sciences, Khabarovsk, Russian Federation
[d] Faculty of Mechanical Engineering, Technion–Israel Institute of Technology, Haifa, Israel

Abstract

Many prominent ecological characteristics of Yellowstone derive from its hotspot-induced uplift, including the moderate- to high-elevation terrain, the cool climate and deep snowfall. Heat from the hotspot rises upward and drives Yellowstone's famed geysers, hot springs and mud pots. The major soil-forming factors in the area are volcanic parent rocks – rhyolite and andesite – and lake sediments overlying rhyolite, cold temperatures, and deep snow in winter and low precipitation in summer. The purpose of the work was to study the effect of hydrothermal activity – geysers, hot springs and mud pots on the Yellowstone Plateau post-volcanic soils, developed on rhyolite, lake sediments and andesite. The sampling sites were chosen in areas both affected by hydrothermal activity – by mud pots, active geysers and the field of thermal waters and off the direct hydrothermal effect – Hayden Valley and the Lamar River Valley. Chemical weathering was a major feature of the affected soils. Near active mud pots, at pH 5.1–5.6, active rhyolite weathering resulted in abundant amorphous silica production and sequential thriving of diverse diatom algae. In soils on the lake sediments and close to the geysers, at low pH values (< 4), weathering was moderate and biogenic silica was presented mostly by shells of testate amoebae. The content of diatoms in these soils corresponded with that in the parent lake sediments. Similar conditions were observed for the soils on andesite in the Lamar River Valley. Biogenic silica was also found in the form of phytoliths, well-preserved in the productive grassland of the Hayden Valley, but significantly affected in the soils near active hydrotherms. Hydrothermal activity was a driving force of silicate mineral weathering and resulted in the thriving of diatoms on the plateau.

Keywords: soils, hydrothermal weathering, opal-A, diatoms, testate amoebae, phytoliths, Yellowstone.

* Corresponding author
E-mail addresses: fskot@tx.technion.ac.il (F.S. Kot)

1. Introduction

Investigations of relatively simple natural systems may contribute significantly to our understanding of more complex phenomena. In soil science, an example of such systems can be a soil developed in extreme environments such as cold ultra-arid oases of Antarctica, hot ultra-arid deserts – Atacama, soils on recent volcanic rocks – Hawaii, Kamchatka (Karpachevskiy, 1965; Amundson et al., 2008; Mergelov et al., 2012; Targulian et al., 2017) and specific post-volcanic soils of the Yellowstone Plateau, affected by hydrothermal activity (Pierce et al., 2007).

At an elevation of about 2,500 m, the Yellowstone Plateau forms the core area of the Yellowstone National Park. The area is one of the last remaining large, nearly intact ecosystems in the northern temperate zone of the Earth and one of the world's foremost natural laboratories in landscape ecology and Holocene geology (Schullery, 2004; Hansen, 2006; Pierce et al., 2007).

The plateau was built up by extrusion of volcanic rocks – siliceous rhyolitic lavas and caldera-forming rhyolite tuffs produced by hotspot volcanism (Fournier, 1989; Christiansen, 2001). In contrast to the rhyolite plateau, which extends over much of Yellowstone, the northeastern part and the Absaroka Range are characterised by steep topography and high relief, primarily on friable Eocene volcanic clastic andesitic rocks (Prostka et al., 1975; Meyer, 2001).

Heat from the hotspot rises upward and drives Yellowstone's famed geysers, hot springs and mud pots. The thermal waters are specialised, primitive ecosystems, rich in algae and bacteria (Pierce et al., 2007). Neutral to alkaline, the thermal waters are mostly chloride- and silica-rich (300–500 and 230–676 mg L^{-1}, correspondingly). Acid-sulphate (-Fe-rich) boiling pools and mud pots occur in some of the geyser basins.

Many prominent ecological characteristics of Yellowstone derive from its hotspot-induced uplift, including the moderate- to high-elevation terrain and the associated cool climate and deep snowfall. The rhyolite plateau is remarkably gently sloping.

Climate

The region is characterised by cold continental climate, although influenced by elevation, latitude and broad-scale air masses. The two main features of the weather in Yellowstone are low temperatures and heavy snowfall. The nearest reporting station is at West Yellowstone, and this is usually the coldest place in the USA for several days every month of the year. Spatial extrapolations from meteorological stations indicate annual average temperatures from 6 to 7°C, a growing season longer than 5 months and average annual precipitation from 240 to 480 mm. Solar radiation is relatively high due to lower cloud cover, while the vapour pressure deficit is high due to low humidity and high temperatures. In the summer days, daytime temperatures range between +21 and +27°C, while night-time temperatures often fall below +4°C. Spring and autumn are transitional seasons between the long, cold winter and the short, mild summer. Winter air masses from the northern Pacific Ocean traverse the topographic low of the Snake River Plain to where orographic rise onto the Yellowstone Plateau and adjacent mountains produce deep snow (Hansen, 2006; Pierce et al., 2007).

Soils

The major soil-forming factors in the area are volcanic parent rocks – rhyolite and andesite – and lake sediments overlying rhyolite, cold temperatures, deep snow in winter and low precipitation in summer. Rhyolite is rich in silica (typically > 69%) and poor in plant nutrients and forms sandy, well-drained soils that support the monotonous, fire-adapted lodgepole pine (*Pinus contorta*) forests of the Yellowstone Plateau. As the summer progresses, the upland rhyolite areas and their lodgepole forests generally become dry earlier than the adjacent areas on non-rhyolite substrates. During the Pleistocene, glaciers sculpted the bedrock and produced glacial moraines that are both forested and woodless, sand and gravel of ice-marginal streams that are commonly covered with sagebrush-grassland as well as silty lake sediments that are commonly covered by lush, highly productive grassland, such as the Hayden Valley in the northwest (Marcus et al., 2012).

Most of the plateau soils fall into three orders, of which we sampled two: (1) Inceptisols (Cryepts, the cold Inceptisols of high mountains or high latitudes, corresponding approximately to Entisols) and (2) Mollisols (Cryols). Inceptisols are the most common soil order in Yellowstone and dominate the central and southwestern parts of the National Park. They have a poorly developed soil profile. Cryepts, representing the most common suborder, are formed under extremely cold conditions. Mollisols have relatively thick, dark surface horizons rich in organic matter and are usually associated with grassland in Yellowstone. The suborder Cryols is the most common type here.

One can presume that the soils in Yellowstone are subject to impacts from salt aerosols generated by hot geysers, geothermal springs and basins as well as mud pots. Hot-water systems are characterised by the dominance of a liquid-phase flow and carry solutes that stay in solution during boiling, such as Cl^-, SiO_2 and major cations (Ca^{2+}, Mg^{2+}, Na^+ and K^+), sometimes enriched with Fe. Vapour-dominated systems may contain H_2S (Nordstrom et al., 2005; Lowenstern and Hurwitz, 2008). Klages and Hsieh (1975) found that in the suspended matter of streams draining the area, quartz gave the dominant diffraction peak in the silt fraction.

The purpose of the present work was to study the effect of hydrothermal activity – geysers, hot springs and mud pots on the development of the post-volcanic soils of the Yellowstone Plateau.

2. Materials and Methods

Study area and soils

The study was focused on soils of the Yellowstone Plateau within the Yellowstone National Park. The soil samples were taken from the soil surface (0-10 cm layer) in August 2017. Sampling sites no. 1–5 were located on Inceptisols, the most common soils on the Yellowstone Plateau. Sampling sites no. 6, 7 were on grassland Mollisols with a relatively thick, dark surface horizon rich in organic matter. The sampling sites are briefly characterised in Table 1 and are shown on a schematic chart (Fig. 1).

Table 1. Brief characteristics of the sampled sites in Yellowstone National Park

No.	Site	Description	Soil, parent rocks
		Near sites of active hydrothermal activity	
1	Biscuit Basin East	Several tens of m from bubbling thermal springs	Inceptisol on rhyolite
2	Ibid	About 50 m from site 1	
3	Fountain Flat Drive	100-200 m from thermal waters, Celestine Pool, and about 400 m from an erupting geyser	
4	By Old Faithfull geyser	A forested spot, about 1 km from Old Faithfull geyser and acid-sulphate (-Fe) boiling pools and mud pots	Inceptisol on lake sediments
		Far from sites of hydrothermal activity	
5	Haiden Valley	A grassland enclave on fine-grained lake sediments surrounded by lodgepole pine forests on rhyolite	Inceptisol on lake sediments
6	Lamar, dry spot	High bank of the Lamar River	Mollisol on andesite
7	Ibid, meadow	Low bank of the Lamar River, about 30 m to site no. 6, grassland	

Fig. 1. A schematic map showing sample site locations

The sampling sites were chosen in areas both (1) affected by hydrothermal activity – by mud pots, active geysers and the field of thermal waters and (2) off the direct hydrothermal effect – Hayden Valley and the Lamar River Valley (Fig. 2).

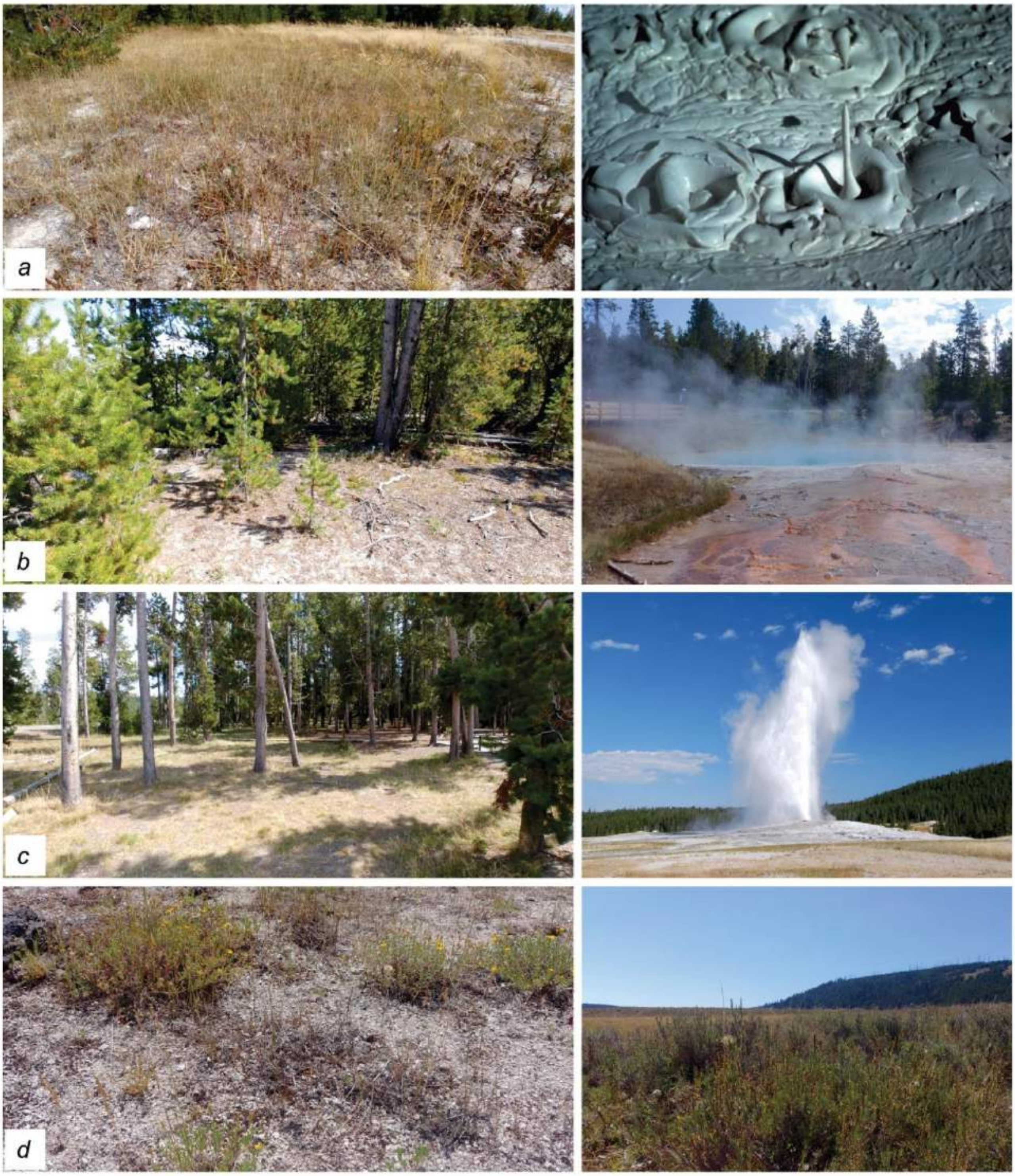

Fig. 2. Typical landscapes of the sampling site: *a* – Biscuit Basin East, mud pots, *b* – field of thermal water, Celestine Pool; *c* – forested spot near the large Old Faithfull geyser, *d* – grassland area next to the Lamar River. (Field picture *b* is from Pierce et al., 2007)

A brief description of selected physical and chemical properties of the soils is given in Table 2.

Table 2. Selected physical and chemical properties of surface soils (0–10 cm)

No.	pH	EC, mS cm^{-1}	Granulometric composition, %		Gross composition of fine earth, %						
			< 2 μm	> 50μm	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	K_2O
Soils on rhyolite											
1	5.08	0.214	2.2	55.5	91.8	4.4	1.5	0.6	0.4	0.5	0.5
2	5.35	0.056	0	83.5	96.4	1.7	0.6	0.2	0.3	0.3	0.4
3	5.62	0.267	3.2	57.3	92.6	3.3	1.1	0.7	0.4	0.6	0.8
Soils on lake sediments											
4	3.86	0.170	1.0	56.4	74.3	12.2	4.6	1.1	1.0	2.4	2.9
5	5.19	0.274	1.1	56.0	74.1	11.9	3.7	1.8	1.0	3.1	3.2
Soils on andesite											
6	7.33	0.227	1.0	56.4	60.3	13.9	9.2	6.1	3.4	3.0	2.1
7	9.56	0.391	0.7	61.7	59.0	12.8	8.9	7.6	4.4	3.3	2.1

Methodology

The major methods were granulometric and gross analyses and scanning electron microscopy (SEM). The SEM analysis was carried out using VEGA 3 LMH (TESCAN, Czech Republic). For the analysis, the samples, after grinding and sieving through a 2-mm sieve, were prepared via pouring, Pt-spraying and magnification of up to 20,000. A backscattered electron detector (BSE detector) was used for the analysis of phases with a high atomic number. When images are acquired using a BSE detector, phases with a high average atomic number are reflected in contrast more vividly than those with a lower atomic number. The X-max 80 energy-dispersive spectrometer (Oxford Instruments, UK) was used to analyse the elemental composition of the most representative regions. The capture area of the microanalysis was about 1 μm in diameter. If a smaller object was scanned, the result was distorted due to the influence of the surrounding matrix or the carbon table of the device. The granulometric composition determined a particle size distribution from 0.01 to 2,000 μm with the laser diffraction method on a particle size analyser SALD-2300 (SHIMADZU, Japan) (Rawle, 2017; Wolform, 2011). The contents of selected chemical elements were determined via the X-ray fluorescence method (XRF) (Pioneer S4, Bruker AXS, Germany), using the silicate technique. The SEM and XRF analyses were carried out in the Analytical Centre at the Institute for Tectonics and Geophysics, Khabarovsk, Far East Branch of the Russian Academy of Sciences.

3. Results and Discussion

The results of the chemical analysis (Table 2) showed that the soil composition was inherited from the parent rocks. The soils on rhyolite (no. 1–3) were characterised by a high concentration of SiO_2 (92–97 %) and a weak acid reaction at pH 5.1 to 5.6. The soils on andesite (no. 6, 7) were neutral to alkaline at pH 7.3 to 9.6 due to the high content of alkali and alkaline-earth metals and the relatively low content of SiO_2 (59–60 %). The soils on lake sediments (no. 4, 5) had intermediate characteristics. Site no. 4, however, showed an acid reaction at pH 3.9, the lowest value among all the studied soils, most likely due to the impact from the waters influenced by Old Faithfull geyser. All soils had low electroconductivity, ranging from 0.056 to 0.391 mV cm^{-1}, most likely due to effective drainage patterns and intensive leaching during spring snowmelt; no salt accumulations could be detected with SEM.

Despite the difference in chemical composition, the soils, however, were similar in granulometric characteristics and all showed a medium silt-loam texture. This is a rather paradoxical fact because usually particle sizes are closely related to the chemical composition (van Genuchten et al., 1999; Buurman et al, 2001; Eshel et al., 2004; Pachepsky, Rawls, 2004). This phenomenon could be explained by similar physical weathering conditions in the soils. The main granulometric fraction ranged from 20 to 200 μм. The maximum peaks of differential curves of particle-size distribution (PSD) (Fig. 3) corresponded to the low limit of physical weathering of quartz (~100 μм). In site no. 2, the high sand content (> 80 %) could be explained by eructation of the mud pots.

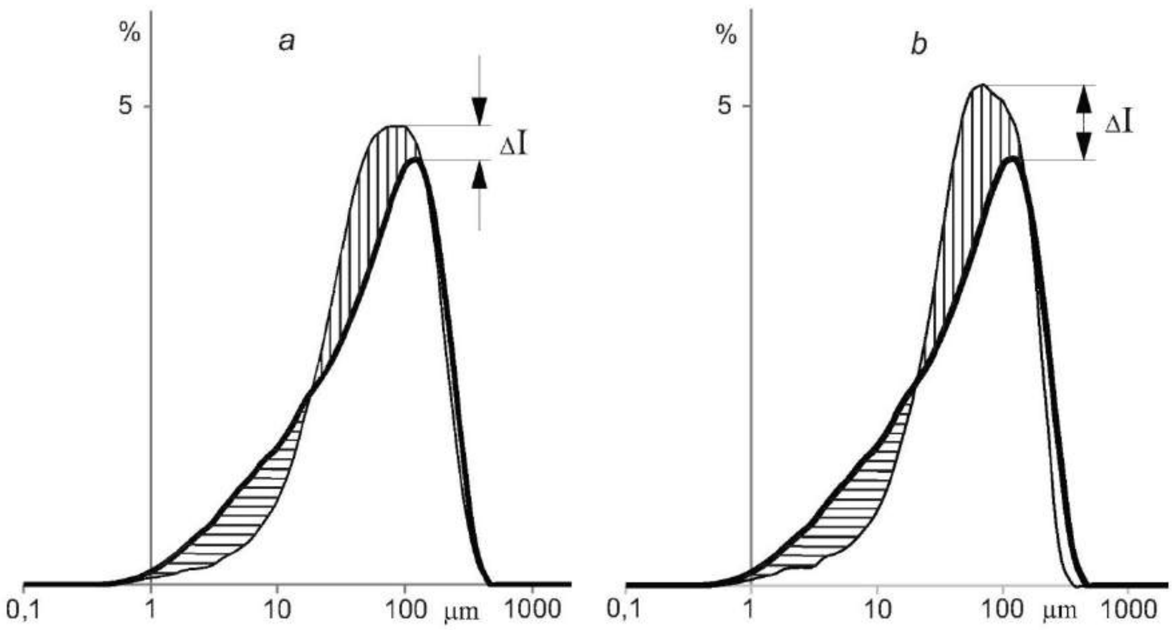

Fig. 3. Particle-size distribution in soils, differential curves: thick line – site 1(a, b), thin line – site 5(a), site 6(a), ΔI – peak intensity difference

Being similar in terms of granulometry, the soils, however, varied in particle size distribution. Mineral particles of soils on lake sediments and andesite were sorted more evenly compared to the soils on rhyolite. The former had differential PSD curves with intensive, narrow symmetrical peaks of the basic fraction compared to the soils on rhyolite. The latter had a left peak asymmetry with a long tale in the area of fine particles < 20 μм. Considering the 'more quartzite' composition of rhyolite soils, the effect of mud pots on their chemical weathering can be presumed.

According to SEM analysis, the effect of mud pots on chemical weathering of the rhyolite soils resulted in the formation of amorphous silica (Fig. 4). For example, in site no. 1, both clastic quartz (Fig. 4*a*) and 'acicular quartz' of varying degrees of amorphisation (Fig. 4*b*–*d*) were determined as, most probably, the result of hydrothermal weathering of feldspars. Similar acicular crystals of Na-feldspars have been found in sand granular soils in Mongolia (Shein et al., 2017). Semi-quantitative EDS-analysis of the elemental composition of 'acicular quartz' evidenced its mono-silica composition. This allows hypothesising the formation of both kinds of quartz, i.e. amorphous and acicular from clastic quartz. In site no. 2, the formation of amorphous silica (Fig. 4*e*–*f*) from rhyolitic tuff (together with silicified plant litter) (Fig. 4*h*) could be observed. Silicification of plant litter (Fig. 4*h*) and anomalously high concentrations of biogenic silica in the form of diatoms (Fig. 5) in the soils indicate the effective dissolution of amorphous silica.

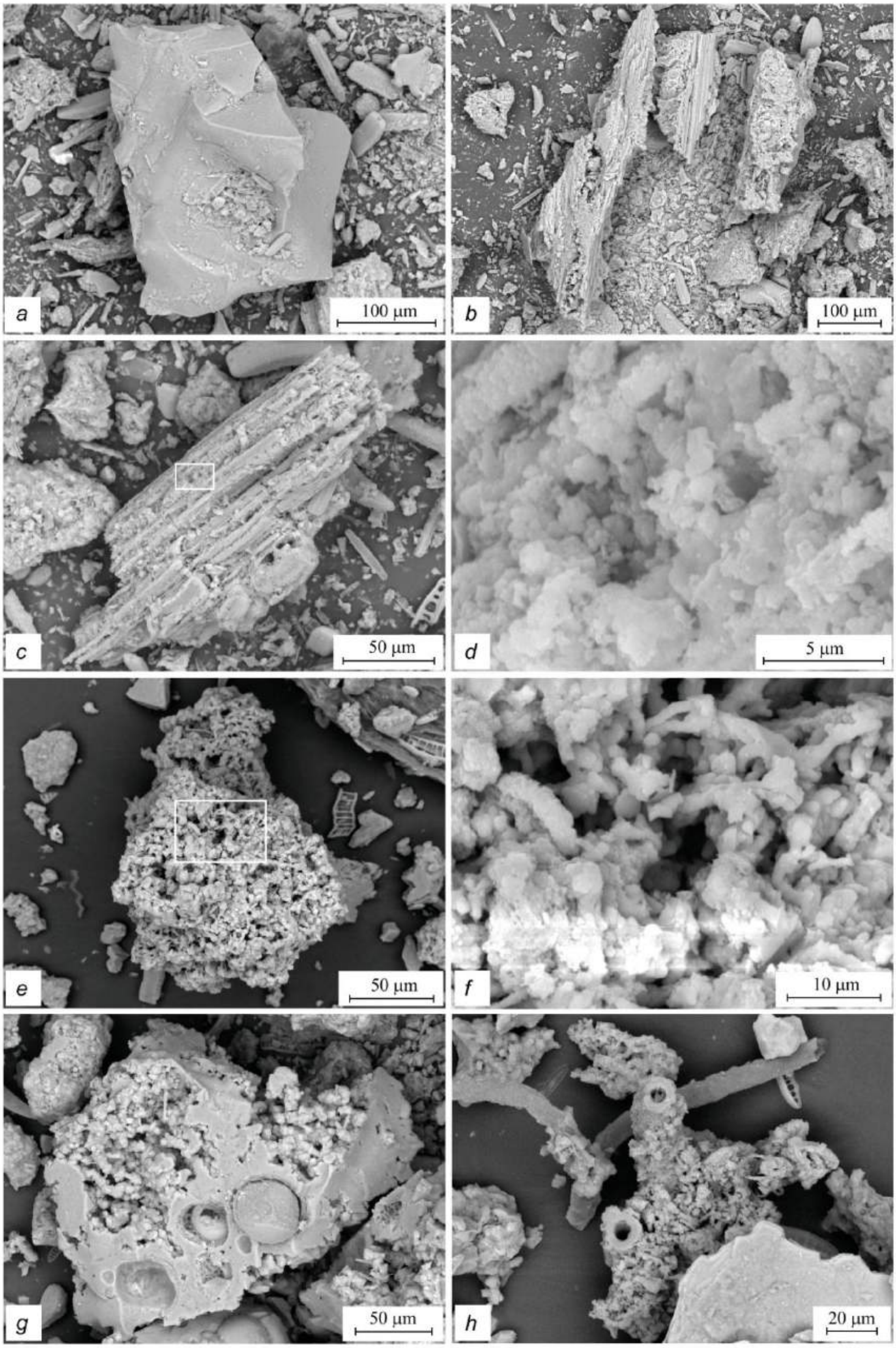

Fig. 4. Micrographs of SiO_2-formations in sampling sites no. 1 (a–d) and 2 (e–h): a – clastic quartz; b, c – 'acicular quartz'; d – bordered area in Fig. 4c is amorphous silica; e – new formations of amorphous silica, f – bordered area in Fig 4e; g – weathered rhyolitic tuff; h – silicified detritus (SEM, BSE-detector)

Biogenic opal (opal-A) in the form of diatoms can be found in small quantities in some soils (Lynn et al., 2008). Whilst aquatic diatoms have been studied extensively (Soininen, 2007), investigations of terrestrial diatom communities and diatom assemblages that can be found on the soil surface are scarce (Antonelli et al., 2017). However, special methods of maceration have been developed (Gol'yeva, 2008), and standards on diatom content have been established (European Committee for Standardization, 2003). However, for this preliminary work, we did not use special methods of diatom concentrating, mainly because the direct detection of diatoms in bulk soil samples is evidence of abnormal high contents in the soil. The commonly found diatoms (*Bacillariophyta*) are presented in Fig. 5. We found all three classes: *Fragilariophyceae* (Fig. 5*a*–*d*), *Bacillariophyceae* (Fig. 5*e*–*g*) and *Coscinophyceae* (Fig. 5*h*), showing high species variety in the soils from the mud pots, site no. 1. Diatoms were not identified to the genus and species levels.

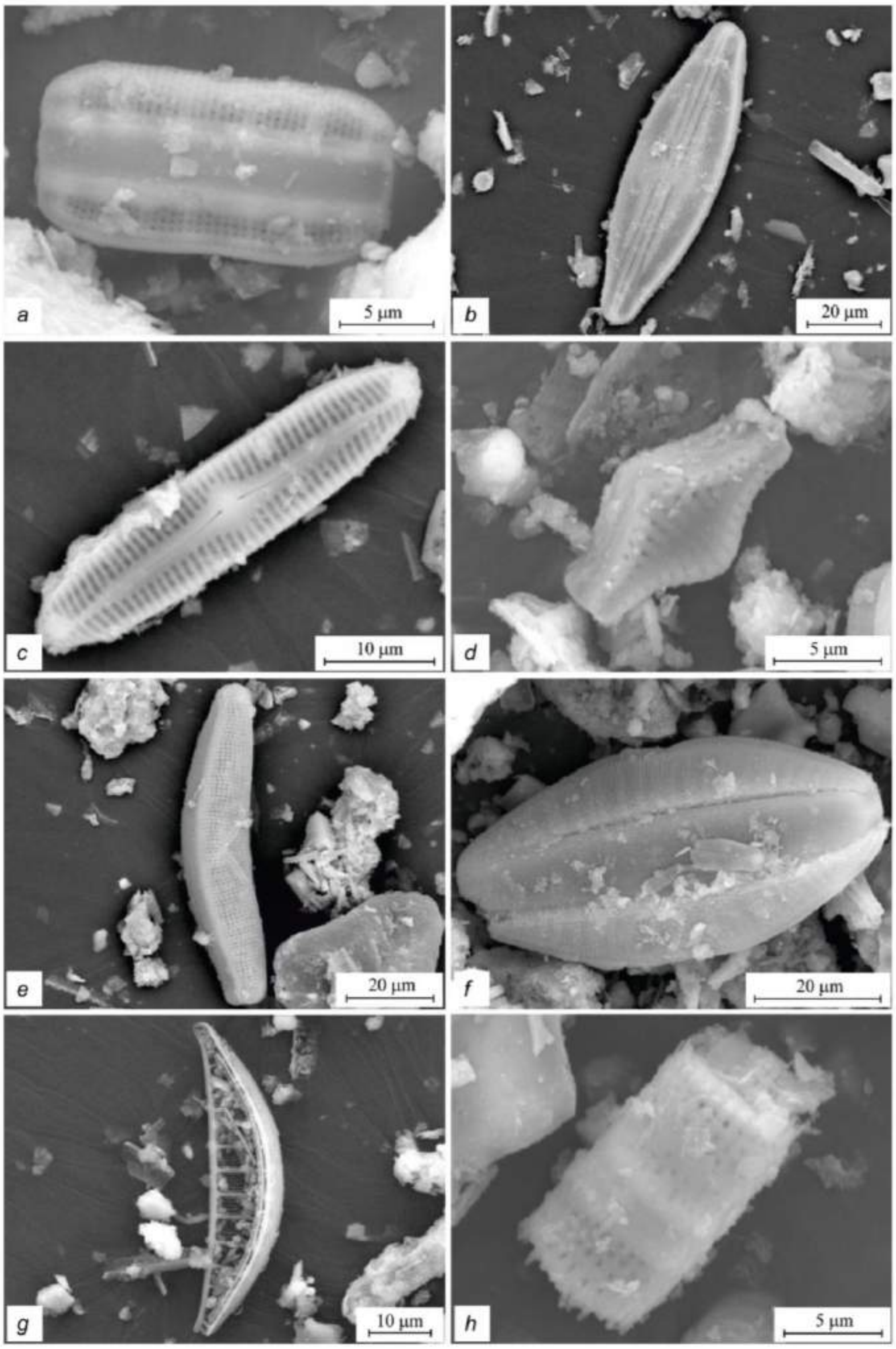

Fig. 5. Micrographs of the most representative species of diatoms, site no. 1: *a*–*d* – Class *Fragilariophyceae*; *e*–*g* – Class *Bacillariophyceae*; *h* – Class *Coscinophyceae*; SEM, BSE-detector

In site no. 3, near the field of the thermal waters and about 400 m from an erupting geyser (Fig. 6), chemical weathering was more moderate compared to site no. 1. 'Acicular silica' could not be found, and amorphous silica was only observed in small quantities of cemented and covered microaggregates of primary clastic grains (Fig. 6*a–b*). Diatoms were represented by closely related species, albeit in smaller quantities than in site no. 1 (Fig. 6*c–g*). In both cases (sites no. 1 and 3), diatoms formed microaggregates in the soils (Fig. 6*h*). Such aggregation occurs mainly through the production of muco- and polysaccharide gels (Zimmermann-Timm, 2002; Keil and Mayer, 2014). It is likely that in hydrothermally affected soils, organic matter produced by diatoms can contribute significantly to the primary soil organic matter. According to Brzezinski (2008), diatoms account for about 20 % of the carbon fixed through photosynthesis, whilst the contribution of other microbiota species is significantly lower.

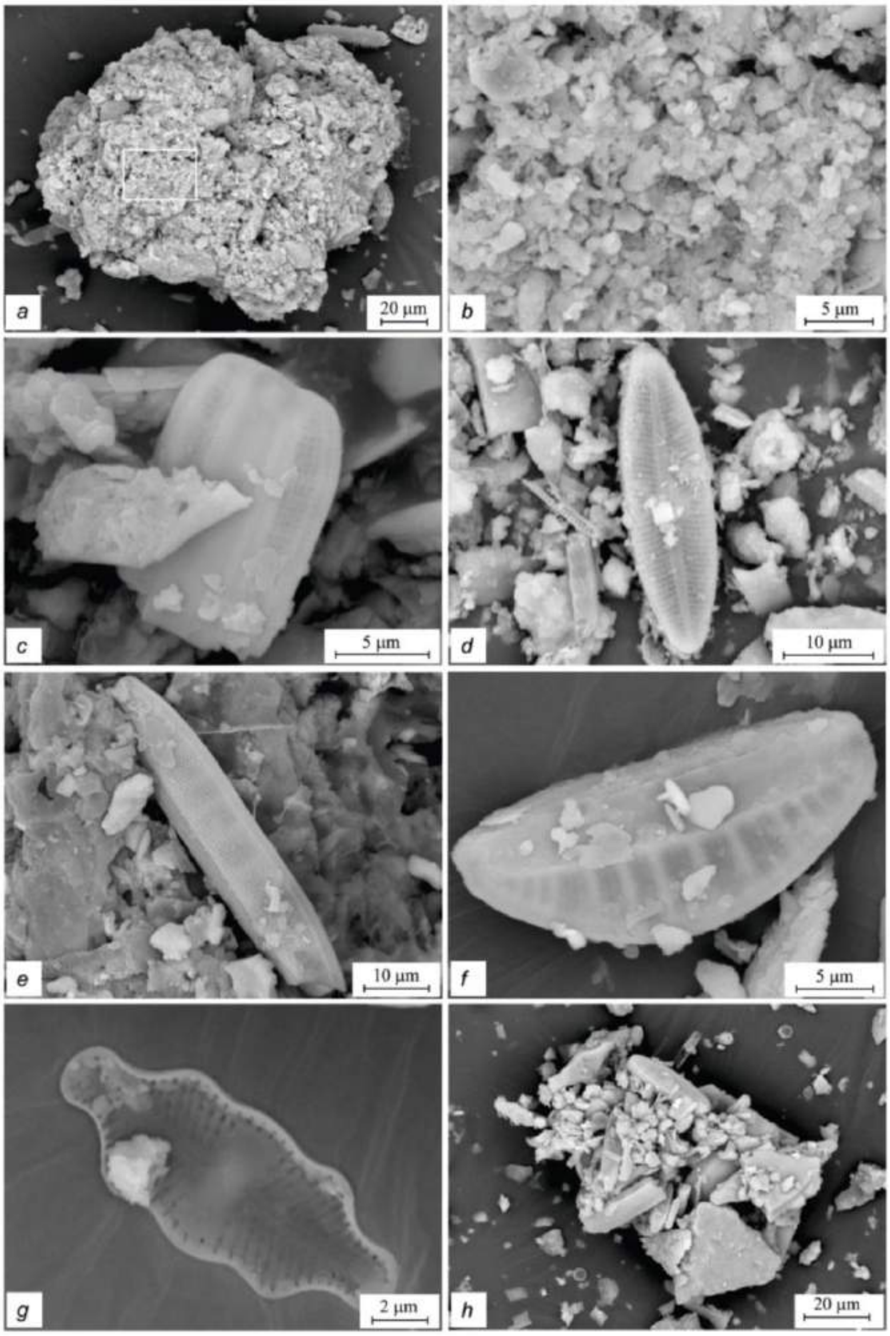

Fig. 6. Micrographs of diatoms, site no. 3: a – microaggregate with amorphous silica; b – bordered is the area (a) with amorphous silica; c–g – most representative diatom species; h – microaggregate with diatoms (SEM, BSE-detector)

In sites on lake sediments, sites no. 4 and 5, no weathered amorphous silica could be determined. Quartz was significantly less abundant than in the rhyolite sites, represented in the forms of both clastic and rounded grains; the latter can be related to glaciation. According to bulk chemical analysis, feldspar minerals dominated here. The content of diatoms was similar to that in the lake sediments (Fig. 7). It should be noted that in the acid soils of site no. 4, near Old Faithfull geyser, testate amoebae dominated over silica microbiophorms (Fig. 7*a*, *b*). Testate amoebae inhabit raw humus litter and promote the decomposition of organic matter. Their presence indicates a low rate of bioprocesses and an acid milieu (Corbet, 1973). These organisms use dissolved silica for their shell plates, fastened with protein, which disintegrate rapidly after the death of the organisms. The preservation of testate amoebae shells is lower compared to that of diatom shells, about tens and millions years, respectively (Gol'yeva, 2008). Therefore, the high amount of shells found on the sites indicates recent formation.

In the soil of site no. 5, Fe-Mn concretions were a product of diagenesis in lake sediments (Fig. 7*e*). In addition, frustules of centric diatoms were found in this site (Fig. 7*f*), presumably from *Stephanodiscus yellowstonensis*, an endemic species of Yellowstone Lake.

In the soils on andesite of the Lamar River Valley, no formation of amorphous silica could be determined, with low diatom contents.

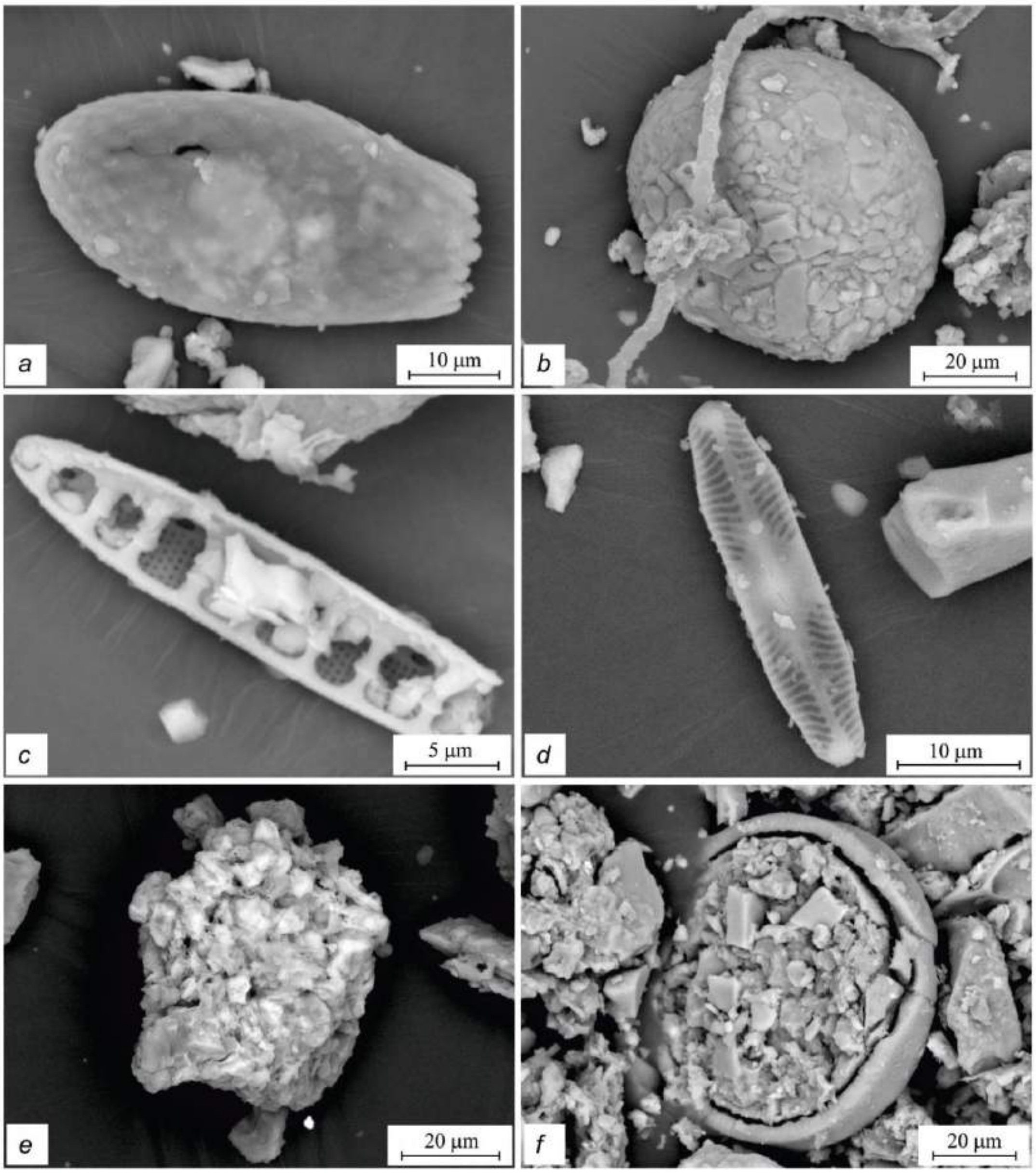

Fig. 7. Micrographs of testate amoebae (a, b), diatoms (c, d, f) and Fe-Mn concretions (e): a–c –site no. 4; d–f, site no. 5; SEM, BSE-detector

In addition to diatoms and testate amoebae, another of biogenic silica, phytoliths, could be detected (Fig. 8). They are formed during intracellular silica precipitation and follow the shape of the cell. Phytoliths are indicative of phytocenosis, a major soil formation factor (Gol'yeva, 2008). An abundance of well-preserved phytoliths was determined in the Hayden Valley (site no. 5), a highly productive grassland ecosystem (Fig. 8*a*, *b*). In the soils of the Lamar River Valley, phytoliths are represented by close associations. In less productive forest ecosystems on rhyolite, next to the field of the thermal waters of Fountain Flat Drive and about 400 m from an erupting geyser (site no. 3), grass and pine-tree phytoliths were found (Fig. 8c, *d*), although less abundant and of average preservation. Least preserved phytoliths were determined in site no. 1, Biscuit Basin East, several tens of m from the field of bubbling thermal springs (Fig. 8*e*, *f*), probably as a result of effective weathering. Similar processes have been observed, for example, in highly weathered podbel (whitened) horizons of meadow podbel soils of the middle Amur River Valley (Kharitonova et al., 2013). According to a previous study, the weathering of grass phytoliths resulted in the formation of fine spherical particles of opal silica of about 150–300 ηm (Chizhikova, 2013). No evidence of chemical weathering of the two other forms of biogenic silica, i.e. diatomic and testate amoebae skeletons, could be found in this active hydrothermal spot due to their high resistance, but we found evidence of mechanical destruction.

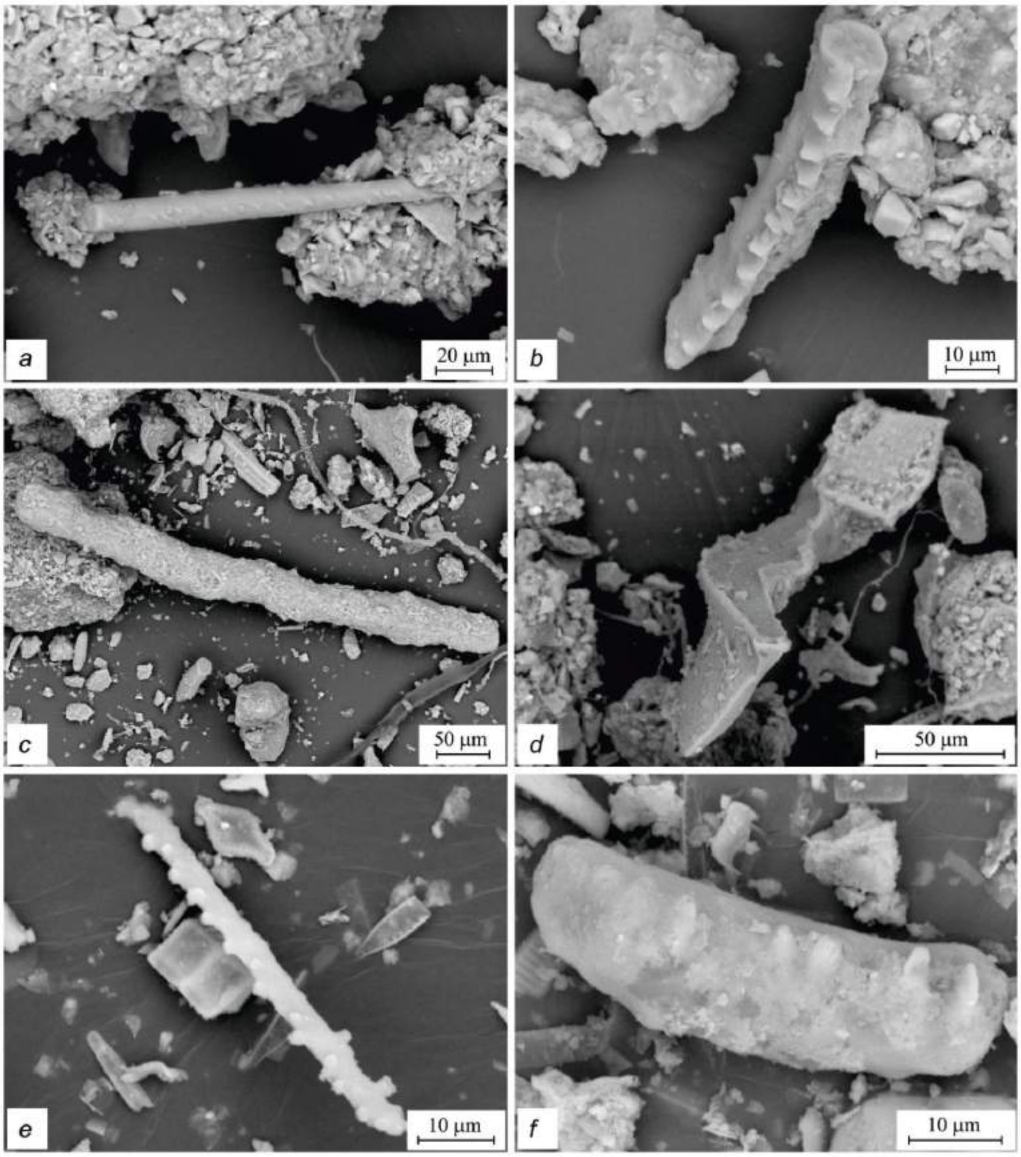

Fig. 8. Micrographs of phytoliths: a, b – site no. 5; c, d – site no. 3; e, f – site no. 1; SEM, BSE-detector

4. Conclusion

Soils of the Yellowstone volcanic plateau, developed on rhyolite, lake sediments and andesite, were investigated. The soils near and beyond hydrothermal phenomena varied significantly in their chemical, biotic and mineral patterns. In the area of mud pots, at pH values from 5.1 to 5.6, effective chemical weathering of rhyolite resulted in the formation of abundant amorphous silica and the sequential thriving of diatoms of high species diversity. In the soils on lake sediments near the active geysers and with low pH values (< 4), chemical weathering was moderate and biogenic silica was mostly represented by shells of testate amoebae. Similar contents of diatoms were found in the soils and parent lake sediments of the Lamar River Valley, not directly influenced by hydrothermal effects. Additionally, soil biogenic silica in the form of phytoliths was abundant in lake sediments of the productive grassland of the Hayden Valley. These phytoliths were more susceptible to hydrothermal weathering compared to the more stable shells and frustules of diatoms and testate amoebae.

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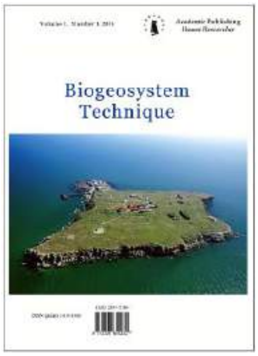

The Infrastructure of Land Management in the Post–Antique Agrolandscapes of Crimea

Fedor N. Lisetskii [a, *], Zhanna A. Buryak [a], Evgenia Ya. Zelenskaya [a]

[a] Belgorod State National Research University, Russian Federation

Abstract

Ancient extended microforms of the relief are widely represented in a number of regions of the Crimea, where post-agricultural landscapes are found. These microforms are analogues of ground hydraulic engineering structures on arable land, which are used for the purposes of regulating surface runoff of water and controlling the intensity of erosion processes in erosion hazardous agro landscapes. The results of comprehensive studies of ancient land management and land use near the archaeological sites (settlements of farmers and pastoralists) of ancient times in the North-Western Crimea are presented in the article. Relict elements of land use in the form of trees and ditches, which were found in the ancient agricultural regions of the Crimea, are formed as a result of agrotechnical features of soil treatment within the allotments and by creating external borders of land plots to secure the rights of land users. The infrastructural elements of the antique land surveying systems were identified using remote sensing data and field methods. Features of soil cultivation in land allotments for perennial plantations and crops have been established. The physicochemical features of the soils showed that the marginal ridges in the post-ancient landscapes were uncultivated. Using the results of a detailed geodetic survey, the universal morphometric parameters of earth structures of various types were first obtained. With the help of remote sensing data and field methods was determined the topological structure of antique land surveying systems, and also were identified features of soil cultivation in land allotments for perennial plantations and grain crops. The classification of surveying rampart by their morphometric parameters has established three main types, which determines the prospects for further reconstruction of the technologies for their creation. According to the results of soil-chronological assessments, it was determined that surveying systems relate to the period of ancient land management (IV century BC – I century AD). Adaptive decisions in land use, which to some extent implement the principles of "geonics", have a similarity both in creating regulatory boundaries (groundwater hydraulic structures on arable land) in modern land management projects, and in ancient agricultural practices.

Keywords: ancient land management, land use relics, surveying system, geomorphology, soil age, Crimea.

1. Введение

Характеризуя современные проблемы в землепользовании, отмечают (Kalinitchenko, 2016), что вместо гармонии природы и технологии диагностирован их перманентный конфликт, для преодоления последствий которого необходимо долгое время для

[*] Corresponding author
E-mail addresses: liset@bsu.edu.ru (F.N. Lisetskii)

воспроизводства ресурсных и экологических функций природно-территориальных комплексов после антропогенных воздействий. При значительной доле склоновых земель в агроландшафтах противоэрозионная подсистема эколого-ландшафтных систем земледелия должна включать инфраструктурные элементы постоянного действия. Даже при наличии агротехнических мероприятий на пашне для более эффективного регулирования остаточного поверхностного стока необходимы противоэрозионные гидротехнические сооружения. При этом принципиально важно реализовать комплексный подход к организации всей территории водосбора, начиная от водораздела и заканчивая руслом малой реки, тальвегом балки (Лисецкий и др., 2015), что позволяет учесть сложившиеся горизонтальные связи ландшафтной структуры, траектории потоков вещества и энергии. Для почвоводоохранного и экологического обустройства агроландшафтов в земледельческой зоне России наиболее действенным шагом можно считать осуществляемый в последние 25 лет переход от внедрения отдельных почвозащитных приемов к конструированию почвозащитных систем контурно-мелиоративного земледелия на ландшафтной основе. На пашне к противоэрозионной инфраструктуре относятся разнообразные гидротехнические сооружения, которые направлены на выполнение водозадерживающих функций (валы-канавы, валы-лиманы, валы-террасы, валы-дороги) и водонаправляющих функций (напашные валы, валы-канавы, валы-террасы, валы-дороги, валы-распылители, залуженные водосбросы). Они становятся инфраструктурными элементами агроландшафта стокорегулирующего и противоэрозионного действия.

В древнеземледельческих районах обнаруживаются реликтовые элементы землепользования в виде земляных валов и рвов, возникновение которых обусловлено как особенностями обработки почвы (нецелевые в почвозащитном отношении элементы инфраструктуры), так и необходимостью закрепления участка размежеванного земельного массива внешними границами для закрепления прав землепользователя (Лисецкий и др., 2017). В отличие от других районов Северного Причерноморья в Северо-Западном Крыму до сих пор сохранились обширные территории, нетронутые современной распашкой, где на поверхности хорошо заметны следы древнего землеустройства и землепользования (Смекалова, 2010). Результаты исследований в Северо-Западном Крыму (Смекалова, 2011; Смекалова, Чудин, 2012; Смекалова и др., 2015) показали, что границами полей эпохи поздней бронзы были только валики, а существенная особенность полей раннего железного века заключается в наличии параллельных валиков с ровиками посередине. Это отличие объясняется применением разных орудий обработки почвы по мере эволюции земледельческих практик. В постагрогенных ландшафтах выявляются также микротопографические изменения, вызванные использованием в древности дорог (Plue et al., 2009).

В современном земледелии последних десятилетий активно развивается адаптивный подход, который в землеустроительном проектировании предполагает максимальную согласованность пространственной организации агроландшафта с природными условиями, чтобы обеспечить агроэкологическую однородность полей севооборота. При нарезке полей прямоугольной формы на склонах субпараллельная (контурная) организация землепользований предполагает размещение длинных сторон в направлении, близком к направлению горизонталей (изогипс), что дает возможность осуществлять обработку почвы и другие технологические операции поперек склона, то есть перпендикулярно линиям тока воды. Такая организация землепользования эффективна в противоэрозионном отношении, и в античном земледелии она практиковалась при создании систем землеустройства под севообороты с полевыми культурами, где изученные земли с размежеванием были преимущественно размещены на склонах северо-западной экспозиции (Лисецкий, 2015). В условиях южной и сухой степи это наиболее увлажненные склоны, где, при субпараллельной ориентации длинных сторон прямоугольников земельных наделов, удается перехватить поверхностный сток, осуществляя "сухую" мелиорацию (Лисецкий, 2015).

Наблюдения в природе за эффективностью преград при формировании процессов поверхностного стекания водных масс и транспорта наносов могли быть перенесены в практику мелиоративной трансформации геосистем, в частности путем преобразования наиболее консервативного их компонента – рельефа, что допускает возможность рассматривать подобные решения как использование подхода, называемого геоникой.

В условиях склонового земледелия, где закономерно складывается дефицит продуктивной влаги, использование обвалования пашни для закрепления границ уже является агротехническим приемом. В осознанном применении этого принципа для мелиоративных целей имеется и своя предыстория. В конце XIX в. для решения проблемы регулирования интенсивности стока талых вод была установлена эффективность таких простейших гидротехнических сооружений, создаваемых по горизонталям рельефа, как земляные валики высотой 15–30 см. В 1891 г. П. Янковский установил, что "разбивкою бассейна оврага на участки, ограниченные земляными валиками (дамбами), проведенными по горизонталям местности, вполне прекращается поверхностный сбег воды, выпадающей на площадь бассейна"* (Янковский, 1902: 348-349). Кроме регулирования поверхностного стока воды, а соответственно и смыва почвы, при создании обвалованных рабочих участков осуществляли, вероятно, неосознанно, "сухую" мелиорацию. Анализируя результаты агрономических опытов Адамова 1893 г., Янковский выполнил расчеты, которые показали, что степень увлажнения слоя почвы 0–100 см между валиками (при расстоянии между ними 19,2–42,7 м и высоте 16 см) больше на 23 относительных %, чем вне валиков (Янковский, 1902: 349).

Первоначальный облик положительных форм микрорельефа (валиков), фиксирующих границы наделов древнего землеустройства и землепользования, претерпел за тысячи лет геоморфологическую трансформацию под действием денудационных процессов: почвенный материал осел и переместился по общему уклону местности, контуры валиков сгладились, уменьшились их относительные высоты. Несмотря на то, что со временем проходила планация валиков, но одновременно за счет их зарастания степной растительностью проходил процесс гумусонакопления и, соответственно, воспроизводство гумусового профиля. Причем аппликативное развитие гумусового профиля на протяжении длительного времени имело различные скорости. На восходящей ветви изменения солнечной активности (от греческого минимума (2350 лет назад) к рубежу веков) сложились более благоприятные климатические условия, чем в предшествующую эпоху, что способствовало ускоренному воспроизводству степных почв (Иванов, Лисецкий, 1994).

В настоящее время пришло понимание того, насколько большим информационным потенциалом обладают почвы археологических памятников (Чендев, 2013), однако при этом остаются мало изученными древние земляные насыпи и напаши (фортификационные, межевые валы и т.п.), особенно из-за трудности датирования при отсутствии в них артефактов. Межевые валики это чаще "немые" в археологическом отношении объекты (без артефактов, синхронных сооружению), однако они могут быть датированы после проведения почвенно-генетических исследований и использования педохронологического метода датировки (Лисецкий и др., 2016). Всестороннее изучение реликтовой инфраструктуры землеустройства и землепользования и последующая ее датировка позволят понять, как эволюционировали системы земледелия в древности и была ли организационно-производственная структура землепользования, отраженная в доступных для изучения межевых системах, природосообразной.

2. Объекты и методы

На Тарханкутском полуострове, в 5 км к юго-востоку от городища Караджа расположен уникальный по сохранности земельный массив с ясными следами организованной системы землепользования в виде так называемых "длинных полей" и несколькими поселениями, одно из которых – "варварское" поселение № 502 эллинистического времени (Смекалова, 2010). Используя геоархеологический подход, были изучены в этом районе наиболее типичные валики в межевой системе: при их нумерации с севера на юг – третий по счету (R2), его продолжение к западу (на останце) – R3 и пятый валик (R10) (Рис. 1).

* В цитате сохранена орфография автора.

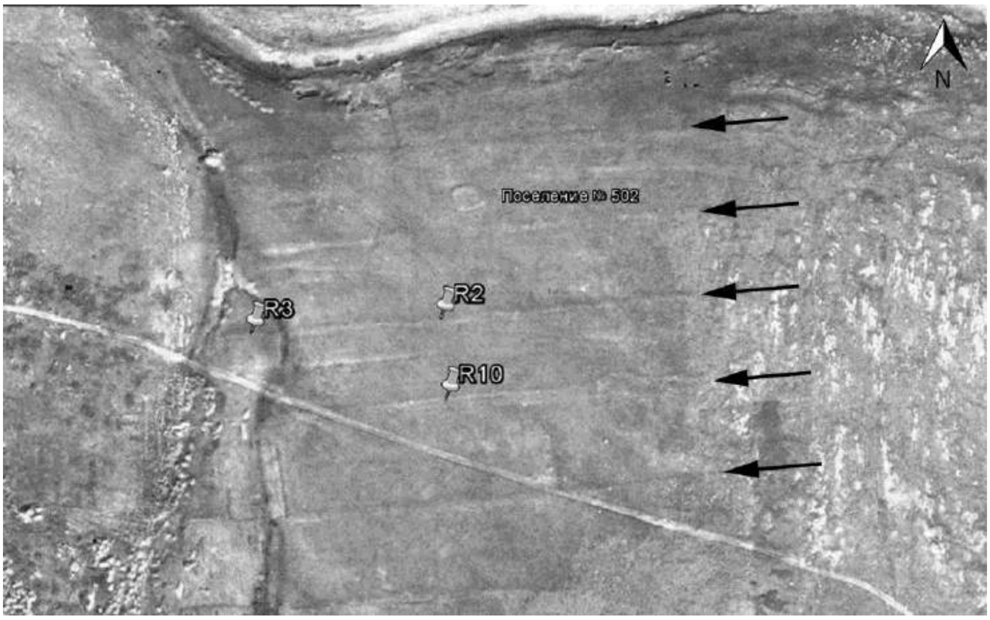

Рис. 1. Местоположение поперечных траншей (R2, R3, R10) в систем межевых валиков (указаны стрелками) в округе городища Караджа

Другая межевая система расположена к северо-востоку от Евпатории, на водосборе Тюменьской балки, вблизи поселения раннего железного века Тюмень 2. Участок представляет собой постантичную и современную залежь с хорошо видимыми и сейчас межевыми валиками (с относительной высотой до 19 см), чередующимися через 41–42 м (Рис. 2) (Смекалова и др., 2015). Три стратиграфические траншеи были заложены поперек основных межевых валиков.

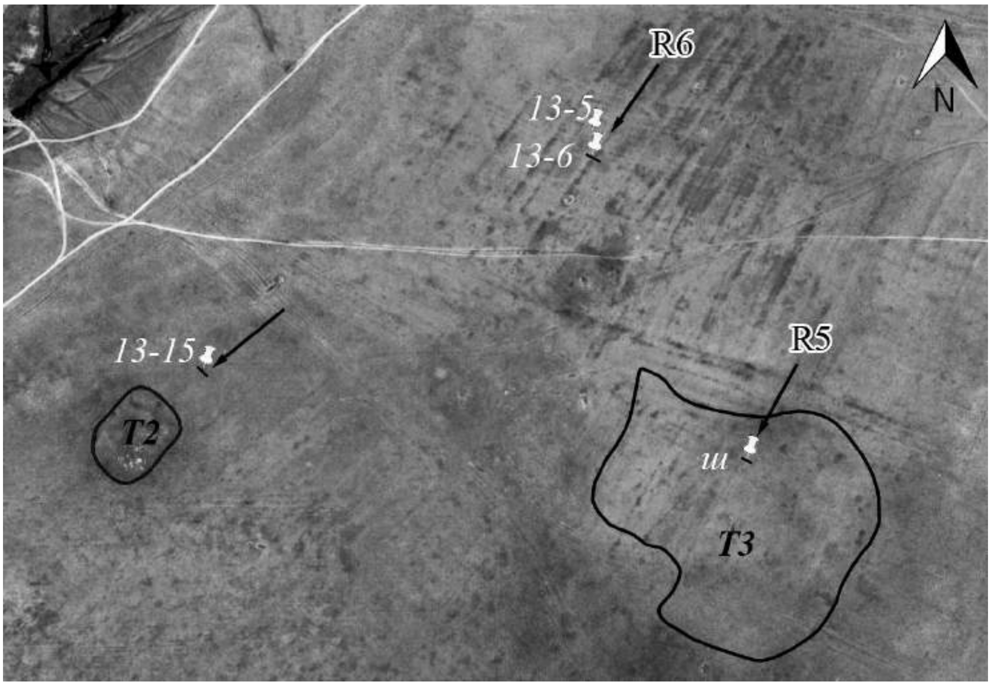

Рис. 2. Местоположение почвенного разреза в земельном наделе (13-5) и почвенных траншей (13-6, 13-15, ш) поперек межевых валиков (R6, R13-15, R5); поселения Тюмень 2 и Тюмень 3 (Т2 и Т3 соответственно)

Особый объект расположен на территории поселения Кельшейх 1 (R11, R12). Это два вала в пределах селитебной зоны, по своему назначению это, видимо, валы-ограды.

Первый для Таманского полуострова опыт междисциплинарного исследования древней межевой системы (Гарбузов и др., 2004) отличался дополнением полевой идентификации признаков землеустройства, выявленных дистанционно, изучением почвенно-генетическими методами стратиграфической траншеи, заложенной через межевой вал.

Почвенно-генетический метод исследования предполагает изучение морфологического строения почвы как последовательности генетических горизонтов со своими характерными временами развития. Почвенные горизонты помимо генетической соподчиненности в профиле имеют временную обусловленность и зависимые от времени почвенные свойства. Наиболее полное представление о развитии почв во времени может обеспечить синтез информации о морфоструктурной организация почвенного профиля и таких характеристик процессов почвообразования, которые наиболее чувствительны к временным изменениям (например, гумусонакопление, выщелачивание от карбонатов, геохимические характеристики и т. п.).

Используя результаты региональных геоархеологических исследований, калибровки по почвенно-хронологическим данным хронофункций почв появляется возможность применения метода почвенно-генетической хронологии (почвенно-хронологического метода). Этот метод датирования (Лисецкий и др., 2016) был успешно апробирован на нескольких “варварских” поселениях в Северо-Западном Крыму: Кельшейх 1, поселении № 502, Джангуль 1, Тюмень 2.

При землеустройстве в античное время обычно создавали два типа ровиков: 1) ровик посередине земельных наделов в системе так называемых “длинных полей”; 2) ровик, примыкающий к валику и сформированный при его создании (с помощью напаши, что наиболее вероятно, или насыпи). Сейчас валики, как правило, имеют высоту 10–20 см и, согласно геоморфологической классификации, относятся к мельчайшим формам рельефа, или топографической шероховатости (Пиотровский, 1977). Графически отобразить такие формы рельефа позволяет детальная геодезическая съемка. Кроме того, топологическую структуру землеустройства по микрорельефу можно выявить, используя данные дистанционного зондирования (ДДЗ) высокого разрешения или результаты магнитной съемки. По регистрограммам морфологического строения почв в межевой зоне (Рис. 3) можно изучить особенности обработки почвы в земельных наделах, что другими методами воссоздать практически невозможно.

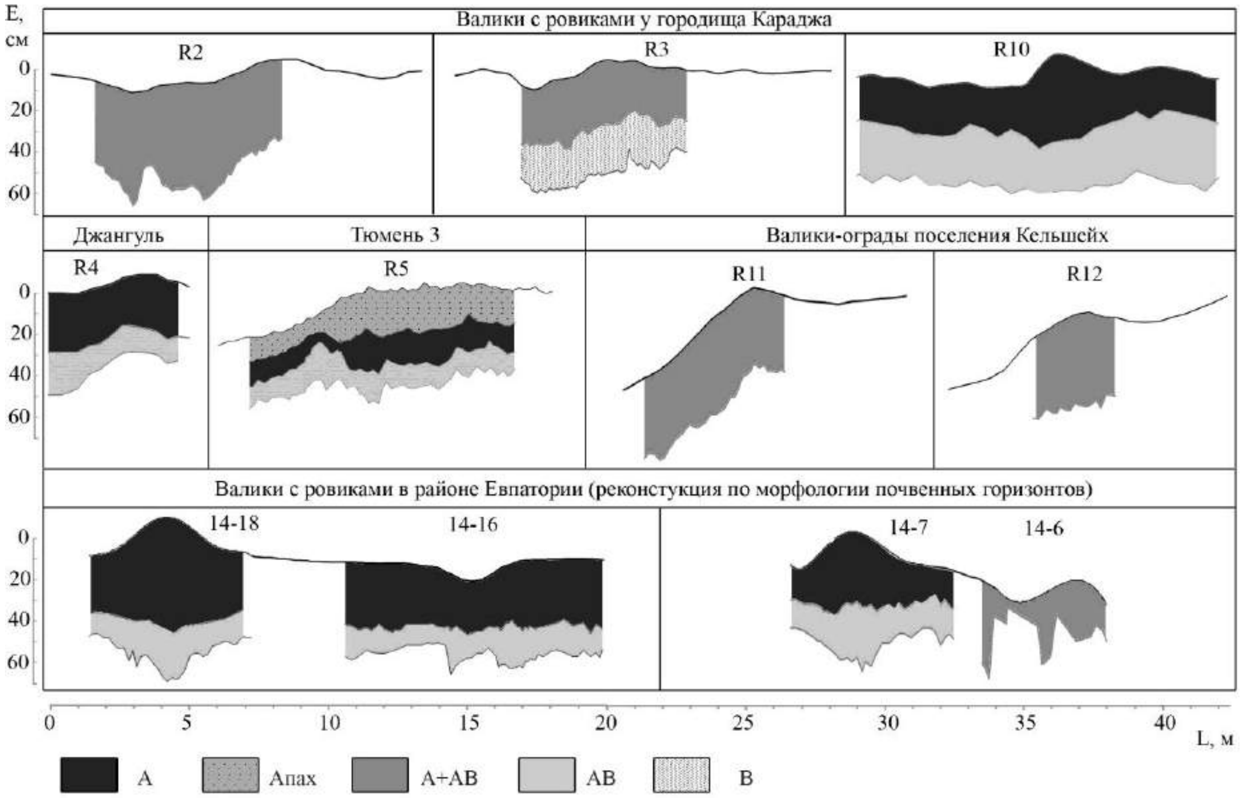

Рис. 3. Почвенно-геоморфологическое строение некоторых валиков и ровиков (Северо-Западный Крым). Соотношение осей X:Y – 1:63

Если поперечный профиль каждого валика представить с помощью упрощенной модели (Рис. 4), то можно определить универсальные морфометрические параметры земляных сооружений для их последующего сравнения.

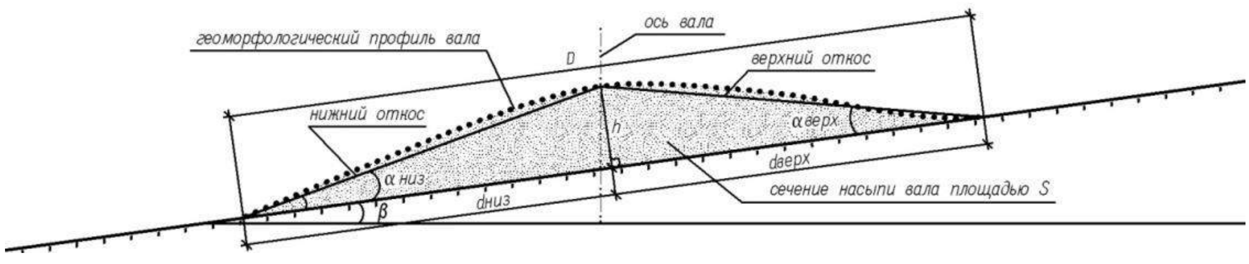

Рис. 4. Схема поперечного сечения валика для получения морфометрических параметров

При изучении почв окраска сухой почвы установлена по атласу цветов Манселла (Munsell Soil Color Charts). Углерод органического вещества, групповой состав гумуса, pH водный определены стандартными методами, подвижные формы фосфора и калия – по методу Мачигина (ГОСТ 26205-91).

Концентрацию макро- и микроэлементов определяли в порошковых пробах почвы на рентгеновском спектрометре "Спектроскан Макс-GV" по методике измерений массовой доли химических элементов. По результатам расчета основных геохимических соотношений и коэффициентов были отобраны (по величине коэффициента вариации) наиболее информативные из них. Коэффициент элювиирования ($К_э$) рассчитан по модифицированной формуле Liu et al. (2009): $К_э = Al_2O_3/(MnO+CaO+K_2O+MgO+Na_2O)$.

Геохимическое сходство объектов определяли путем интерпретации результатов кластерного анализа (метод Уорда, Евклидова дистанция, значения нормированы по среднеквадратическому отклонению) в программе *Statistica* 10.

3. Результаты исследования и их обсуждение

Вблизи семи археологических памятников, где были обнаружены межевые валики (Кутайсов, Смекалова, 2013), нами выполнена нивелировка относительных высот и построены гипсометрические профили. Это три валика у поселения Караджа, два валика у пос. Кельшейх 1 и по одному объекту у поселений Тюмень 2 и Джангуль 1. Кроме того, рассмотрены особенности морфометрического строения почв в ровиках.

Для каждого валика получены основные морфометрические параметры (Таблица 1): β – общий уклон местности, град.; $O(S)$ – экспозиция склона; $O(L)$ – ориентация длинных сторон земельных участков по ДДЗ.

Таблица 1. Параметры территориальной организации античных систем землеустройства в Северо-Западном Крыму

№№ п.п.	Местоположение объектов исследования	Памятник	Датировка памятника	Растительность	β	O(S)	O(L)
R2 R3 R10	Тарханкутский п-ов, западная часть	Караджа	посл. четв. IV – перв. треть III в. до н. э.	Разнотравно-ковыльная, ОПП 70 %	0,4 1,0 0,2	ССЗ	ВСВ-ЗЮЗ
R4	Там же	Джангуль 1	IV в. до н. э. – перв. треть III в. до н. э.	То же	0,4	ССЗ	ВСВ-ЗЮЗ
R5	Окрестности Евпатории, побережье оз. Сасык	Тюмень 2	IV в. до н. э. – II в. до н. э.	Разнотравно-злаковая, ОПП 60 %	1,0	ЗСЗ	СВ-ЮЗ
R11 R12	Тарханкутский п-ов, отвершек б. Кель-Шейх	Кельшейх 1	IV–III в. до н. э.	Разнотравно-злаковая, ОПП 30 %	2,4 2,6	СВ	С-Ю З-В
16-24 16-12 16-26	Белогорский р-н, предгорье Крымских гор	Борут-Хане	III в. до н. э. – I в. н. э.	Разнотравно-ковыльная, ОПП 90–95 %	2,3 2,6 1,7	СЗ	СВ-ЮЗ

В результате по каждому валику получили набор уникальных характеристик, сочетание которых может быть критерием объединения объектов в однородные группы. Группировку методом кластерного анализа проводили для валиков, имеющих агрогенное происхождение (т. е. кроме валиков-оград R11 и R12) (Рис. 5). Кластерный анализ был выполнен, используя следующие морфометрические параметры антропогенных микроформ рельефа: S – площадь сечения валика, м^2; h – высота валика, см; D – общая длина валика, м; $d_{верх}$ – длина верхнего откоса валика, м; $d_{низ}$ – длина нижнего откоса валика, м; $\alpha_{верх}$ – уклон верхнего откоса валика, град.; $\alpha_{низ}$ – уклон нижнего откоса валика, град.; $\alpha_{верх}/\alpha_{низ}$ и $d_{верх}/d_{низ}$ – соотношения уклонов и длин верхнего и нижнего откосов, отражающие характер планации валика.

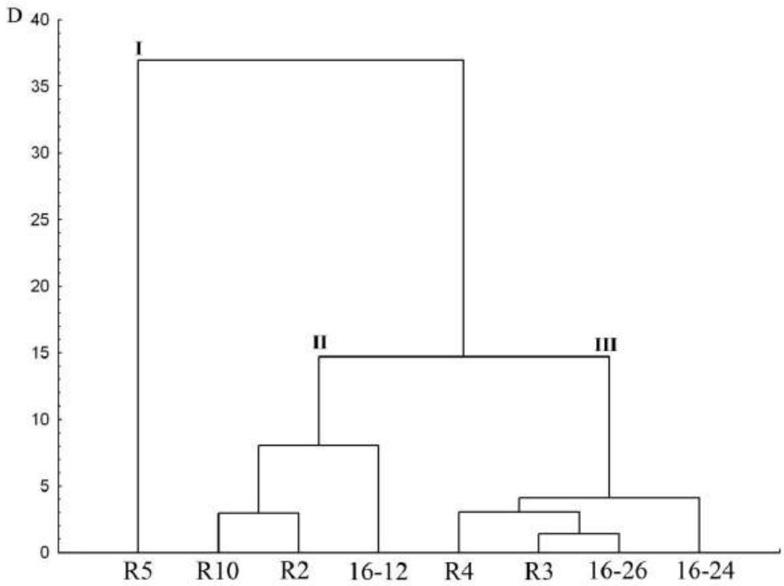

Рис. 5. Дендрограмма кластерного анализа межевых валиков по их морфометрическим особенностям (римскими цифрами обозначены выделенные типы)

Кластерный анализ по морфологическим параметрам межевых валиков показал наличие трех типов. Различия в параметрах земляных напашей (невысоких насыпей, выполненных несколькими последовательными проходами отвального плуга) можно объяснить их функциональным назначением (отраслевая специфика растениеводства, тип рубежа в системе межевания), разновременностью их создания, длительностью прямого использования, степенью сохранности из-за различий в истории землепользования на протяжении нового времени и, возможно, различиями в технологии их создания.

Валик в межевой системе “длинных полей” у поселения Тюмень 2 (R5) в наибольшей степени отличается от остальных: этот валик самый большой по высоте, протяженности и площади сечения. Его также отличает характер планации, выраженный через соотношение длин и уклонов верхней и нижней частей валика: в данном случае вершина валика не плавно сдвигалась вниз по склону, а со временем сместилась вниз по склону, что определило смещение вершины валика относительно первоначального положения.

В отличие от валика R5 объекты в двух других кластерах более близки между собой, однако между этими типами имеются различия. Валики кластера II более объемные и протяженные, чем в кластере III: площадь сечения у типа II 0.30–0.47 м², у типа III – 0.13–0.20 м².

На постантичной залежи у поселения Тюмень 2 (к СВ от валика R5) выполнено комплексное почвенно-генетическое исследование внутринадельного ровика в шурфе длиной 10 м и соседнего с ним валика в шурфе длиной 6.5 м. Реконструкция по морфологическому строению гумусового профиля почв показала, что первоначальная высота межевых валиков составляла 15 см, а относительная глубина ровика – не более 5–6 см. Осевая зона ровика до сих пор визуально просматривается большую часть вегетационного периода по зелёным многолетним растениям из-за лучшего увлажнения почвы в локальной депрессии рельефа.

Межевая система в окрестностях городища Караджа

Три валика у поселения № 502, несмотря на различия морфометрических параметров (см. Таблица 1), в общей морфологии профиля имеют схожие черты, что проявляется при совмещении их гипсометрических профилей (Рис. 6).

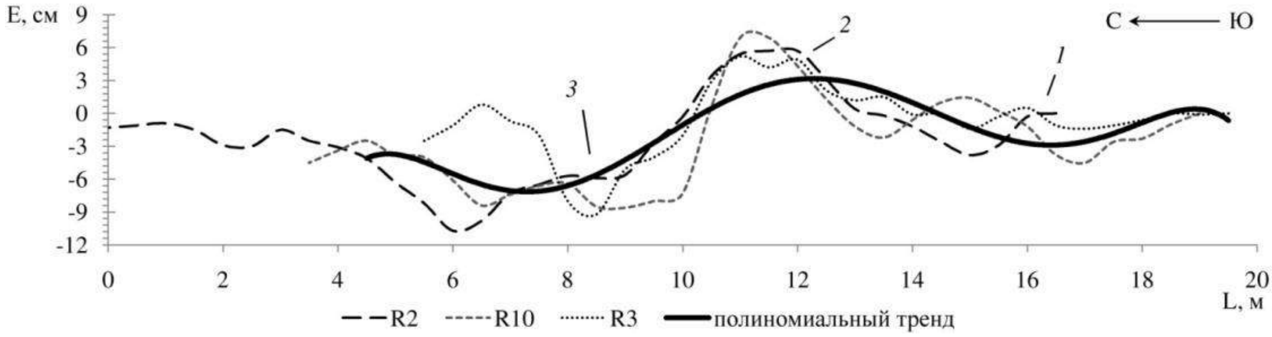

Рис. 6. Совмещение гипсометрических профилей трех межевых валиков в округе городища Караджа (*E* – относительное превышение по длине (*L*) шурфа)

Результирующий гипсометрический тренд для трех валиков в межевой системе выявляет следующую последовательность микроформ рельефа вниз по природному склону Караджинской балки (см. Рис. 6): 1) ровик шириной около 2 м; 2) собственно валик высотой 9–12 см, имеющий более длинный нижний откос; 3) глубокая западина за нижним откосом валика.

Гумусовый материал для создания валиков был задействован с южной стороны в полосе шириной 4.6 м, вероятно, путем оборота пласта в два прохода. Перед валиком фактически получился стокорегулирующий и противоэрозионный ровик (современная глубина – 15 см), который перехватывал сток воды и наносов с вышележащих элементов рельефа, то есть выполнял стокорегулирующие и противоэрозионные функции. Трудно сказать, был ли этот замысел осознанным, но с позиций современной практики организации почвоводоохранного земледелия в районах недостаточного увлажнения этот вариант землеустройства следует рассматривать как пример природосообразного решения.

Так как в стратиграфических траншеях было обнаружено, что под вершиной валиков с глубины 56–63 см залегали плитки известняка, можно предположить, что при первоначальном размежевании земельного массива на наделы этими плитками маркировали оси будущих межевых валиков, чтобы обеспечить равновеликие площади наделов.

На одной мезоформе рельефа находятся соседние валики (R2 и R10), что отличает их от R3, который расположен на межбалочном “останце”. По замыслу кадастровой системы валик R3 – логичное продолжение валика R2, расположенного на пологом склоне древнего суходола. В древности (через несколько десятилетий после распашки целинных склонов Караджинской балки) стали формироваться овраги, которые отрезали от валика R2 его оконечность (R3). Он заметно (на 2,0–2,5 м) короче других валиков и ниже. Но у него лучше видна конфигурация ровика. По условиям рельефа уже априорно можно было сказать, что валик R3 никак не мог быть создан в земледельческий период (XVIII–XX вв.), а имеет более ранний возраст, отнесенный ко времени первичного размежевания земель. Другие изученные валики (R2, R10) и их аналоги на склоне Караджинской балки в процессе земледельческой эксплуатации участка могли подновляться при использовании наделов. У валика R3 лучше сохранился первоначальный профиль – уклон нижнего откоса в 1,2–1,5 раз больше (см. Рис. 6).

Межевые валики были сформированы на целинных почвах. Исключением стал валик R3, что делает необходимым дать более подробную характеристику стратиграфии шурфа. В осевой зоне валика выделены три контрастных горизонта: 1) новообразованная почва (0–33 см); 2) гумусированная толща насыпи (или, возможно, напаши) валика (от 33 до 48–52 см); 3) слой, перекрывающий хозяйственную яму (с 53–58 см). Впервые керамика встретилась с глубины 67 см, на глубине 99 см обнаружена ручка амфоры с желобком посередине (синопская, IV–III вв. до н. э. – определения В.А. Кутайсова), со 106 см стали появляться кости животных. Таким образом, во время землеустройства на этом месте у оси валика уже была каменная наброска с керамикой – перекрытие хозяйственной ямы. А при создании межевых валиков яма была перекрыта слоем суглинка и гумусированным материалом, либо это было сделано в момент землеустройства.

По результатам микронивелирования рубежей землепользования у городища Караджи (по Табл. 1 – R2) установлено, что общая ширина сооружения (валик-ровик) на природном рельефе составляет 9,6 м. Современная ширина валика у основания – 3,7 м, относительная высота – 16–17 см. Используя почвенно-морфологический метод, удалось провести реконструкцию первоначальных параметров земляных валиков. Полоса антропогенных земляных нарушений составляет 4,5–4,9 м. Ширина валика составляет у основания 4,1–4,7 м. В привершинной части первоначальная высота валиков была около 20 см.

Природная денудация валиков как микроформ рельефа происходила не столько за счет действия длины и крутизны микросклонов, совместное действие которых выражается рельефной функцией (Лисецкий, Половинко, 2012), а сколько под действием ударной силы капель. Хотя эрозионно-аккумулятивный процесс проявляется на первичном масштабном уровне, но из-за того, что эрозия наблюдается на вершине и микросклонах, а аккумуляция наносов в ровиках, всё это геоморфологическое сопряжение, вслед за (Ollier, 1976), можно назвать эрозионной микрокатеной. Результаты действия этих процессов хорошо отражают регистрограммы почвенного покрова, получаемые при закладке длинных траншей поперек межевых границ.

В Таблице 2 представлены результаты химико-аналитических исследований почв в межевой системе у городища Караджи.

Таблица 2. Химические свойства почв в межевой системе у городища Караджи

№	Слой, см	Цвет (сух.) по Манселлу	pH (H_2O)	$CaCO_3$, %	Обменные основания, ммоль/дм³ в 100 г: Ca^{++}	Mg^{++}	Na^{+}	P_2O_5, мг/кг	K_2O, мг/кг	$P_{вал}$, %	C/N	$C_{гк}/C_{фк}$
Дерново-карбонатная почва на элювии известняков (целина)												
15	0–24	10YR 7/3	8,3	59,5	10,2	2,2	0,8	117	458,4	0,24	9,4	0,6
	24–32	10YR 6/6	8,6	60,9	5,9	2,0	2,0	62	615,6	0,26	7,8	0,6
Почва на вершине межевого валика R2												
1	0–20.5	10YR 6/3	8,5	31,0	17,0	1,4	0,6	140	423,6	0,16	12,9	–
	30–33	10YR 5/3	8,4	20,8	22,1	3,6	0,9	47	182,5	0,13	8,7	0,2
	33–36	10YR 5/4	8,4	19,1	21,3	4,0	0,9	37	164,1	0,12	6,7	0,3
Почва на вершине межевого валика R3												
2	0–13	10YR 5/3	8,3	3,2	23,6	3,0	0,9	16	228,4	0,12	9,6	1,0
	13–33	10YR 5/2	8,4	0,7	25,2	2,8	0,9	8	184,5	0,12	7,4	1,3
	33–48	10YR 5/4	8,6	8,6	20,8	3,9	0,8	19	140,5	0,13	3,8	0,5

Сопоставление новообразованной почвы (0–21 см) и погребенного гумусированного слоя (33–36 см) на вершине межевого валика R2 показало их существенное различие: верхняя часть профиля по всем показателям характеризует более плодородную почву. Исходя из этого, можно сделать предположение, что валики в античное время были необрабатываемыми.

С помощью почвенно-хронологического метода датирования определено время прекращения жизнедеятельности на поселении № 502 – около 270 г. до н. э. Нахождение этого поселения на размежеванной территории, по-видимому, свидетельствует о его более раннем возникновении, чем изученной системы землеустройства. По совокупности имеющихся данных, полагаем, что в 5,25 км к юго-востоку от Караджинского городища земельный массив площадью 73,7 тыс. м² был размежеван по строго регулярной сетке на 11 земельных наделов шириной 23,5 м в третьей четверти IV в. до н. э. или на рубеже IV–III вв. до н. э. Землепользование длилось не более полувека и больше эта территория в обработку не вовлекалась, так как по совокупности почвенно-генетических данных уверенно можно говорить о сохранности здесь постантичной залежи.

Поселение Джангуль находится на северо-западной части полуострова Тарханкут, в 3,5 км к северу от п. Оленевка (Смекалова, Кутайсов, 2013). Изучение почв в шурфе, заложенном поперек валика, ограничивающего одно из “длинных полей”, у поселения

Джангуль 1 (R4), показало, что первоначально он при ширине 1,6 м имел высоту 20–21 см. На гребне валика зафиксирована мощность гумусового горизонта почвы (A+AB) 385 мм. По результатам замеров на археологической бровке постройки (В3) (раскопки Т.Н. Смекаловой) определена средняя мощность A+AB, которая составила 384,5 мм, что по результатам расчета педохронологическим методом соответствует возрасту почвы, сформировавшейся после IV в. до н. э. (Лисецкий, 2015) (см. номограмму, Рис. 7). Это позволяет синхронизировать время жизнедеятельности на поселении Джангуль 1 с функционированием примыкающей к нему межевой системы.

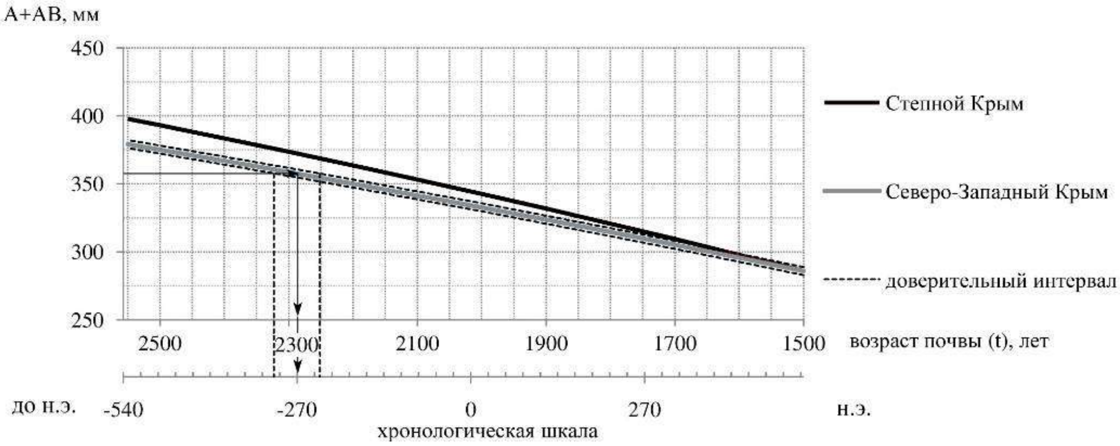

Рис. 7. Пример использования номограммы для определения возраста почв Крыма по мощности гумусированной части их профиля (A+AB)

Межевая система под полевые культуры (вблизи усадеб Тюменьской балки)

Земельный надел у поселения Тюмень 2 (Смекалова и др., 2015) расположен в пределах слабонаклоненного затяжного склона южной экспозиции. Эти условия при распашке земель способствовали развитию поверхностного смыва почвы. Участок в настоящее время находится в постагрогенном режиме.

Верхний слой почвы на вершине валика R-6 подвержен денудации, о чем свидетельствует более высокое содержание SiO_2, *Ti*, *Mn*, *As* (Табл. 3). Материал насыпи валика может в определенной мере характеризовать палеоклиматические условия, которые предшествовали античности. Однако только как тенденцию можно отметить особенности биогеохимической обстановки в предантичный период, которые способствовали аккумуляции *Mn*, *Fe*, *Al*. Почвы в ареале древнего земледелия, сохранили остаточные свидетельства агрогенеза: более низкое содержание *Ca* и *Sr* (Лисецкий, Воробьева, 2016).

Результаты анализа морфологического строения почв на валике R5 (на поселении поздней бронзы Тюмень 3) позволили определить первоначальную ширину валика у основания – 2,8 м. По результатам нивелирования современная относительная высота напашного валика по отношению к зоне отвала составляет около 30 см. Межевой валик R5 асимметричен, с юго-восточного края хорошо выражена зона шириной 3 м, откуда верхний слой почвы был перемещен к оси создаваемого напашного валика. Это обусловлено тем, что в начале 60-х гг. XX в. рассматриваемая территория была кратковременно вовлечена в пашню. Мощность горизонта А увеличивается от вершины валика к северо-западу, составляя в среднем 343±37 мм. Максимальная мощность гумусового горизонта (A+AB) отмечена в приоровочной части современной оси валика, где она превышает на 14,7 см среднее значение в пределах траншеи, заложенной поперек валика.

Таблица 3. Химические и геохимические показатели в почвенных разрезах на межевых валиках

Химические элементы и геохимические показатели	Размерность	Валик R6, разрез 13-6 к востоку от пос. Тюмень 2					Валик R5, разрез "ш" на пос. Тюмень 3		
		глубина, см							
		0–20	20–31	31–49.5	49.5–60	78	0–21	21–39	39–54
SiO_2	%	42,4	38,91	40,75	40,32	44,73	38,31	47,91	39,95
CaO	%	6,26	6,53	6,78	8,37	14,51	17,66	18,56	23,09
Al_2O_3	%	10,20	9,59	10,03	9,30	9,84	8,66	10,16	9,22
Fe_2O_3	%	3,11	3,08	3,12	3,04	2,71	2,69	2,68	2,40
MgO	%	1,13	1,12	1,16	1,20	1,70	2,23	2,51	2,72
TiO_2	%	0,80	0,77	0,77	0,72	0,60	0,59	0,58	0,51
P_2O_5	%	0,11	0,12	0,12	0,12	0,10	0,21	0,24	0,22
V	мг/кг	94,58	93,38	94,29	94,32	82,52	74,94	75,03	68,6
Cr	мг/кг	92,00	87,41	85,69	86,92	80,63	82,54	80,09	75,36
MnO	мг/кг	0,13	0,12	0,12	0,12	0,08	0,11	0,10	0,08
Co	мг/кг	19,08	15,22	14,69	16,36	11,06	14,12	9,95	8,06
Ni	мг/кг	51,61	51,03	53,44	51,82	43,48	40,57	40,37	39,89
Cu	мг/кг	52,44	56,6	57,73	51,3	37,81	33,62	32,4	26,9
Zn	мг/кг	74,2	73,58	73,89	72,36	74,29	65,66	75,56	74,88
As	мг/кг	42,08	34,13	37,28	34,82	27,77	9,02	10,41	8,25
Sr	мг/кг	117,5	113,5	114,1	127,9	168,2	225,3	184,0	182,9
Pb	мг/кг	22,61	21,46	18,68	20,36	13,19	17,75	13,33	13,47
Si/(Al+Mn+Fe)	безразм.	3,16	3,04	3,07	3,24	3,54	3,34	3,70	3,41
Ca/Ti	безразм.	7,83	8,48	8,81	11,63	24,18	29,93	32,00	45,27
(Ca+Mg)/Al	безразм.	0,72	0,80	0,79	1,03	1,65	2,30	2,07	2,80
Σ (Ni, As, Cr, Cu, Pb, V)	безразм.	355,3	344,0	347,1	339,5	285,4	258,4	251,6	232,5
Кэ	безразм.	4,70	4,22	4,26	3,62	2,53	1,79	2,11	1,47

Предполагать, что в древности применяли мелкую обработку почвы, можно уверенно, поскольку даже в конце XIX в. в степной зоне Украины (Постников, 1891) глубина основной обработки обычно не превышала 12–15 см. Ориентировочные расчеты показывают, что для формирования одного погонного метра межевого валика было необходимо 0,68 т почвы, что соответствует 6,4 м^2 обработанной плугом поверхности (при глубине обработки 12 см).

4. Заключение

С помощью педохронологического метода датировки показано, что система землеустройства в округе городища Караджа относится к античному времени (валик R2 датирован первой половиной III в. до н. э., валик R3 – IV в. до н. э.). Земляные валики межевой системы под полевые культуры у позднескифского поселения Тюмень 2 (R5) оказались синхронны финальному этапу формирования культурного слоя на этом поселении и датированы II в. до н. э. По мощности новообразованной почвы земляной вал-ограда на поселении Кельшейх 1 (R11), который по конструктивным особенностям и назначению отнесен нами к противопаводочному типу, датирован первой половиной III в. до н. э. К более позднему времени относится обширная межевая система в Предгорном Крыму, у пос. Борут-Хане (III в. до н. э. – I в. н. э.). Таким образом, возможные сомнения о неантичном происхождении межевых систем в Северо-Западном и Предгорном Крыму с топологическими особенностями землеустройства и морфометрией границ наделов, которые были установлены на изученных объектах, могут быть отвергнуты.

На основе выполненной классификации межевых валиков по морфометрическим параметрам установлены их три основных типа, что определяет перспективы дальнейших реконструкций технологий создания землеустроительной инфраструктуры античного времени.

Установленные отличия параметров земляных насыпей (напашей) можно объяснить возможными различиями в технологии и времени их создания, особенностями функционального назначения (в зависимости от отрасли растениеводства, роли рубежа в системе межевания), длительностью прямого использования, степенью сохранности из-за различий в истории землепользования на протяжении нового времени.

При формировании противоэрозионной подсистемы агроландшафтов созданием рубежей регулирования разных порядков в современных проектах землеустройства и, по всей видимости, неосознанно в землепользовании древности, в определенной степени реализованы принципы “геоники”. А природосообразные решения в землепользовании и адаптивном землеустройстве могут рассматриваться как составная часть более общего подхода – биогеосистемотехники, предполагающей внедрение трансцендентальных технических решений и технологий управления биогеохимическим циклом вещества в газообразной, жидкой, твердой фазе для экологически безопасного рециклинга вещества в почвах, прироста ресурсов и продовольствия, непротиворечивого решения производственных и экологических проблем ноосферы в едином технологическом цикле (Калиниченко, 2012).

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Инфраструктура землеустройства в постантичных агроландшафтах Крыма

Фёдор Николаевич Лисецкий [a, *], Жанна Аркадьевна Буряк [a], Евгения Яковлевна Зеленская [a]

[a] Белгородский государственный национальный исследовательский университет, Российская Федерация

Аннотация. Древние протяженные микроформы рельефа широко представлены в ряде районов Крыма, где обнаружены постантичные агроландшафты. Эти микроформы являются аналогами земляных гидротехнических сооружений на пашне, которые используются для целей регулирования поверхностного стока воды и контроля интенсивности эрозионных процессов в эрозионно-опасных агроландшафтах. В статье представлены результаты комплексных исследований древнего землеустройства и землепользования вблизи археологических памятников (поселений земледельцев и скотоводов) античного времени в Северо-Западном Крыму. Реликтовые элементы землепользования в виде валов и рвов, которые были обнаружены в древнеземледельческих районах Крыма, сформированы в результате агротехнических особенностей обработки почв внутри наделов и путем создания внешних границ земельных участков для закрепления прав землепользователей. С помощью данных дистанционного зондирования Земли и полевых методов выявлены инфраструктурные элементы античных межевых систем, а также установлены особенности обработки почвы в земельных наделах под многолетние насаждения и зерновые культуры. Физико-химические особенности почв показали, что межевые валики в постантичных ландшафтах были необрабатываемые. Используя результаты детальной геодезической съемки, впервые получены универсальные морфометрические параметры земляных сооружений различных типов. Классификацией межевых валиков по морфометрическим параметрам установлены три основных типа, что определяет перспективы дальнейших реконструкций технологий их создания. По результатам почвенно-хронологических оценок было определено, что все исследуемые межевые системы относятся ко времени античного землеустройства (IV в. до н. э. – I в. н. э.). Установлено, что природосообразные решения в землепользовании, которые в определенной степени реализуют принципы “геоники”, имеют сходство как при создании рубежей регулирования (земляных гидротехнических сооружениях на пашне) в современных проектах землеустройства, так и в древнеземледельческих практиках.

Ключевые слова: античное землеустройство, реликты землепользования, межевые системы, геоморфология, возраст почв, Крым.

* Корреспондирующий автор
Адреса электронной почты: liset@bsu.edu.ru (Ф.Н. Лисецкий)

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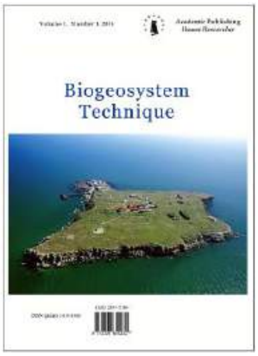

Considerations on Sustainable Land Use:
A Contribution to the Movir's 50th Anniversary

Jan Diek Van Mansvelt [a, *]

[a] Down2Earth foundation, Wageningen, Netherlands

Abstract

In this paper I reflect on plant breeding in the context of agriculture, agriculture in the context of society and society in the context of culture. Altogether a whole, created by interacting and interdependent humans, on a precious and limited globe. In order to rationally reflect on this whole, I use not only natural science [beta] notions, but refer to socio-economic [gamma] and cultural [alpha] science notions as well, in their mutual interactions. This to emphasise their interweavennes in all appliances of (agro and other) disciplinary sciences. Clarity on this interweavennes helps to understand the considerable problems that nature, environment, human health, animal welfare, crops and soil-ecosystems, labour conditions etc. are faced with by today's industrial, chemotechnical agriculture.

Keywords: agriculture, environment, human health, animal welfare, crops and soil-ecosystems.

Introduction

In this paper I try to see plant breeding in the framework of agricultural production, and agriculture in its social context. Society somehow always has the agriculture it demands. Only when groups in society shift their demands towards products from a socially fair and ecologically sound way of production, can producers changes their production accordingly. But at the same time: only when some producers start to take the risk of introducing products on the market that are produced in a way that complies with sustainable development worldwide, can consumers support that way of production by buying those products.

Today, looking back at 50 years of MOVIR research, it is clear how society's changing demands have changed the position and the strategies of plant breeding, compliant to the changes in demand form agriculture. Among the changing demands, the need for a more clearly elaborated focus on sustainable development – in Bruntland's sense – is paramount.

In plant breeding, this shifts the focus of high external input agriculture towards low external input; from soluble mineral fertilisers toward carbon-rich organic manures, from growth only to full ripening, from full single disease resistant lines to multiple diseases resilient lines and last but not least, from lines that do reasonably well in many places to lines that produce very well in their specific region (optimal soil/climate adaptation) (Brundtland, 1987).

In my opinion, over the last decades MOVIR has done very well to live up to the sustainable development demands. And so under the sub-optimal research conditions – to say the least – as they have emerged over those last decades. Great sacrifices have been made by the researchers and

* Corresponding author
E-mail addresses: jandiek@vanmansvelt.nl (J.D. Van Mansvelt)

their staff allowing continuation of the work started in the earlier decades of MOVIR, when agricultural research was still prominent on the national research agenda and budget. I herewith like to express my sincere appreciation and even admiration for that performance. Therein, I guess, the directorship of Dr. Sulukhan Temirbekova will be remembered as a crucial contribution of MOVIR's survival in the early 21st century. Let me finish these words by congratulating the Vavilov Institute of Plant Industry with the 50th anniversary of its younger sister, its Moscow branch: MOVIR. As a foreign member of the Russian Academy of Agricultural Science, I feel honoured to be invited to participate in this 50 years of MOVIR symposium.

Summary. Guidline for the reader

In this paper I invite you to look at plant breeding in the wider context of science in our today's society, wherein the latter always figure within a more or less explicit perception of man and nature: a world view, episteme or paradigm. Within those paradigms, social and economic perceptions are decisive, particularly where political decisions are at stake. And: science, including plant breeding, thrives and flowers or suffers and vanishes according to political decisions: governmental as well as institutional.

I will start with my view on two bipolar perceptions: conservative versus progressive and reductionism versus holism.

I then proceed to some steps between facts and targets, criteria and parameters, exploring their degree of objectivity versus subjectivity. It elaborates the reductionism – holism opposition.

Then the question arises how to position the various scientific disciplines, with their often conflicting interests, in a way that facilitates their cooperation in favour of sustainable development.

Some words on the People, Planet, Profit approach are followed by the presentation of an adapted Maslow model, which from thereon is elaborated in the rest of the paper throughout various realms of sciences: environment, ecology, economy, sociology and alpha sciences. By consciously reflecting on this hierarchy, planning partners can clarify their own position as well as that of their partners, to facilitate fruitful cooperation amongst them. Such reflections will also help to find ways on how to position conflicting interests of the different disciplines

The relevance of that approach for Russian Agriculture in general and MOVIR's plant breeding in particular is provisionally specified, stepwise, per item.

Finally some words are dedicated to the work of Herman Daly (1989, 1996) and to an option I can see within the broad range of perspectives for MOVIR's development in the next half century.

Two bipolar perspectives in science and society

Whatever plans for landscape and or agricultural projects are tabled by whatever actor, two opposite tendencies can be found, both in looking backward and in looking forward.

First of all there is a certain balance between an innovative, entrepreneurial, daring and modern-times approach with a firm base in science, and a conservative, history oriented, respectful and classical-times approach, with a good portion of scepticism toward modern, technocratic solutions. In the scheme below the two horizontal arrows between conservatism and progressivism represent those two tendencies. Now in each of these two, two other tendencies can be found. One more and one less aware, conscious and motivated to think of long-time effects. Obviously, long-term effects regard all people affected by the new projects, plans or policies. In Scheme 1 I marked the tendencies with a + that are compliant with sustainable development, including social justice and cultural multiplicity. On the other hand, those tendencies that are primarily focused on people's own group's short time interest, competing with all others, are given a – in this scheme.

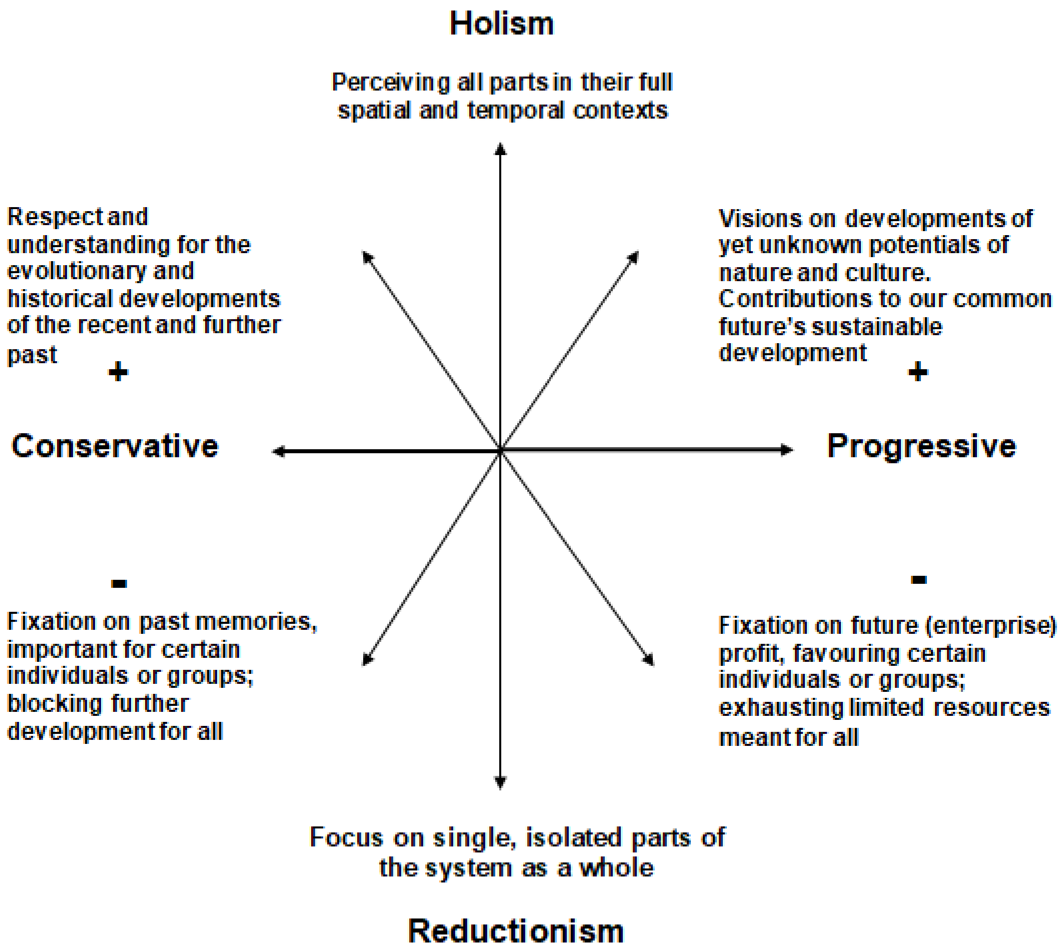

Fig. 1. Reduction and Holism in context

Figure 1 as presented here offers a nice tool for discussions on – for example – plant breeding, (agro-) landscape planning, food quality and the evaluation of their developments. It will be often noticed how easily people, in their presentations of plans or reports based on their particular expertises, tend to focus mainly on the + aspects of their own plans and on the – aspects of the opponent's. Each of those will do the same for their respective positions.

In the so-called SWAT analysis (Mind tools, 2018), this controversy can be nicely transferred, as therein, Strengths, Weaknesses, Opportunities and Threats of all expertises and options are made explicit and discussed. In my opinion, the above scheme nicely fits the SWAT approach.

Therein, by combining the strong points they have in common, and avoid their negative ones, they can altogether manage so called win-win strategies, wherein they focus on their common opportunities and take care to prevent the threats. Whereas the so called SWAT analysis is developed for business and personal consultancy, it works out fine for agricultural enterprises as well.

On Facts and Targets, Criteria and Parameters

In all discussions on agro-landscape design and management there is much to do about facts and targets, criteria and parameters, values, facts, intentions and so on. Quiet often arguments based on one of these get mixed up with such based on others, troubling clear and transparent decision making.

For example hard scientists, as specialists in their discipline, often focus on facts and figures, regarding everything else a waste of time. On the other hand, politically and sociologically aware generalists tend to stress goals and targets, values and opinions, leaving out thorough elaboration of relevant facts. In this often conflicting process, the specialists have implicit objectives and value systems, which are intrinsic in their discipline's way of thinking, observing and acting. On the other hand, the philosophers tend to presume that the implementation of their wonderful ideas will somehow bring about all relevant and needed facts and figures.

The scheme below offers my view on how I see the mentioned positions inevitably linked. By consciously reflecting on this hierarchy, planning partners can clarify their own position as well as that of their partners, to facilitate fruitful cooperation amongst them.

This scheme is based on the notion that people taking initiatives often start with a holistic notion of their objectives (see the upper left). These objectives are as such invisible for the outer eye, yet clearly visual as an idea or concept in peoples' minds. On the other hand, starting from the lower right with the empirical data, they are clearly visible, touchable, concrete, measurable, countable and thus experienced as ultimately reliable.

However, the relevance of the data is not intrinsic in those data themselves. Their relevance depends on what they mean to each of those involved in their application, in the objectives set by those who apply these facts and figures to reach their goals.

Interestingly, a critical screening of leading people involved in management, land-use and agriculture can nicely show how both sides and their intermediates have their roles in linking theory to practice and vice versa. On Figure 2 it looks like this:

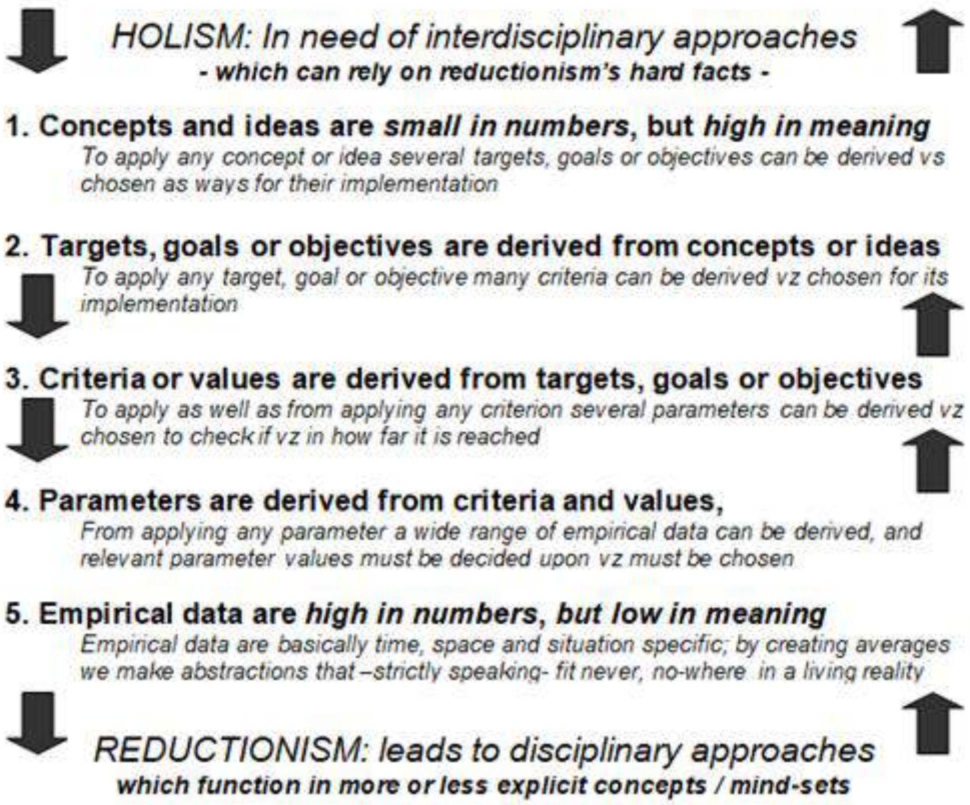

Fig. 2. Steps relating reductionism and holism

Starting with 5: when empirical data are presented in any discussion or publication, it is important to be quite clear: 4. what parameter they refer to, and then which criteria (3.) are chosen to be represented by that parameter (what does that parameter stand for), and 2. what values are represented by the criteria used and, finally, 1. what objectives are to be served by the criteria as chosen.

If you talk about hard facts such as inhabitants per surface unit, about production per labour unit or per large cattle unit, or about crop varieties' production or pests' resistance, you immediately can see the point of the crucial importance of figures. At the same time you are aware of their relative prediction value, as they are average figures, derived from various locations and times. The figures as such can be hard, but their meaning for the future of your investment (time, money) is less hard, or even useless. Seeing facts in their relevant – broader – context c.q. on a meta-level, is crucial for understanding of the object under research, and fruitful for the applications of that knowledge, minimising failures.

If you talk about nitrate per cubic meter of soil or water, about ammonia per cubic meter of air: the circumstantial conditions, including the actors' objectives on the one hand and the sampling-method on the other hand, make or brake the meaning of the figure. For figures on varieties' productivity or on bio-diversity it is the same story: where and how did you measure what type of crop, on what soil, in what season? The figure is of no real meaning for any specific location without sufficient knowledge of the places' past and future: its history and its perspectives in the eye of the researcher viz. the manager who ordered the research.

This is not to say that any of them would be basically misleading! Just being professionally biased, as any expert almost inevitably is, gives all fact-findings its particular colour, trend, perspective or how you name it.

Regarding perspectives of Russian Agriculture and Plant Breeding for the next decades, the contribution of various specialists is needed to cover its different dimensions and aspects: in depths as well as width, regionally as well as national, in international perspective. Here policy and science should discuss with industry, based on a common vision of agriculture's position in society at large, with respect for consumer's and the agro-landscape's health as a reference.

As for plant breeding in particular: contributing to consumers' and ecosystems' health seems of utmost importance: today as well as for the next decades.

In such a mixed group of planning experts it is important that the chair person makes sure those present do move full consciously from one down to five as a start and then from five up to one (see the above scheme). In my opinion, over and again going down-and-up, up-and-down in the above scheme, is an itinerary process that should be organised to go on during all research and evaluation in agriculture and land management. In this process that meets the demands of the initiative do stepwise, per round, become more and more clear as do the possible contributions that the experts can deliver.

How to position the conflicting interests of the different disciplines

In discussions as advocated here, regarding for example agricultural research, planning and management, quite often the problem is how to position economic interests compared with those of environment, or how to place nature conservation relative to regional development, or how to appreciate aesthetics with ethics and both related to the company's efficiency.

More or less metaphorically we can compare that question with this one: how to balance the key wants & needs for human survival in ourselves: the needs for physical survival, for socio-economical survival and for ethical survival (somewhat like food, money, status and religion).

A 2nd millennium industry management instrument to set a balance is the well know triplet: People, Planet and Profit (Elkington, 1999). This instrument is based on the idea that three key issues: > human well-being, > sustainable management of nature and > profitability of the industry, should all be taken into account and then well balanced. When all three give in a bit of their one-sided expert ideals, then everyone can be happy. Thus says a simplified version of the PPP policy.

Critics of the PPP say that, first of all, each of the three P's has a wide range of meanings, which should be clarified in sessions such as for example those recommended in the five-step-scheme mentioned above. Imagine that you see a) *the environment* as sufficiently served when sticking to today's national rulings of you country, versus b) the perception of *the environment* as being the producer of healthy air for breathing and water ready for human daily consumption (quantity & quality). And going for b): what do you mean with healthy air and water? What diseased do you want to take into account? How long should people be healthy? And: which susceptible groups of consumers are included in - or excluded from - the sample? Is the sample representing the average inhabitant of that area or city, or is the sample's health-level adapted to society's vulnerable groups in particular? I guess you can make a similar exercise thinking on what is meant with Profit. So for example: who's profit, in what percentage of the turnover, and on what time scale do you think about? And then, regarding People, it makes a huge difference in planning and management if you see people in a Darwinian way, genetically programmed to fight each other to make the fittest survive and thus improve the human race over many centuries of time, ór that you see yourself as a basically social being, living to serve and enjoy the presence of the other people. But if so, then the question still is: hów to serve one another, and how to enjoy. How to handle (un)equality? What are your perceptions of education's potentiality and people's career perspectives?

In my opinion the 3P's are a way to make people in industry start to consider the balance between man, environment and society. They are a simplified and distorted version of Maslow's 20th century model (Maslow, 1943).

Maslow developed an earlier, more challenging and humane view on the balance between human needs. He refers to human motivation, in a still quit valid and useful concept, which I will present here in a somewhat adapted version. He argued that humans' acts are driven by their motivations. His second idea was not to limit himself to the average human (a mathematical construct) but to include such people in his study society really appreciates, people that set a role model for their culture. Because such models are that what most people say and show to strive for during their lives. So he measured people not on what they lived for in the average, but on what they strived for to do as humans.

From that research Maslow found out that we as humans have roughly three levels of motivation: physical survival, social survival and psychological development. He differentiated the physical survival into water for drinking and food to eat as the most basic motivation. In times of disaster all people's energy is dedicated to staying alive as a living body. Vice versa, when people hate to live, a known policy is that they start starving themselves to death.

The second sub-motivation for physical survival is having shelter of some kind against cold, rain, heat, sun and other dangerous exposure to the environment. The moment the hungry person finds some food, he or she returns to the shelter, to eat and/or feed those dependent on their food finding.

On the level of social survival Maslow differentiates between having a position in society and getting appreciation for your contribution to society from the position you are in. 'Being seen' is a most basic form of appreciation. Having a family fits in here as well. Decoration with awards, being mentioned in papers, climbing the ladder in the firm and/or increase in salary: these are additional ways to express and experience appreciation of your social setting.

But then Maslow noticed that neither salary, position, political appreciation, housing, and the like, make humans completely happy on the long run. Only the feeling that they do realise their true inner being, that they manage to develop their inner potential, makes people really feel good on the long run. It is this potential inner-being that wakes people up, in the night, on a long walk or on an unforeseen moment, asking them: was this the life you were born for? Irrational as it may sound, when such a call for the essential had been implemented by a compatible change of job, position or way of life, people report to feel reborn, finally happy on their track to ongoing development. Learning as a way of life, realising their unknown self.

Now Maslow points to an interesting feature of human motivation. He points out that quite easily a failure to reach a next step in a way that meets the person's own ambition, is to compensate that failure by satisfying one of the more primary levels of motivation. Eating and or drinking away any failure in society is not an unknown phenomenon. But also job-addiction (working harder and harder), a car addiction (more and more expensive cars), a house addiction (more and larger houses in richer neighbourhoods), and social status addiction (number of employees, financial turnover and profit): on all levels the status quo blocks the ongoing development. And, at the same time, a hampering inner growth, transitions included, feeds the tendency to have more of the known instead of some of the unknown.

To make all this relevant for management in plant breeding, agriculture, landscape planning and research, I propose the hypothesis that not only plants and animals, but also farm fields, farms and landscapes can be seen as complex vital systems. Somewhat like living organisms. Each on their particular scale and stage of development.

On all such levels, they have a physical base, a developmental history (a kind of biography, relevant to the scale), a social structure and a position in the surrounding next larger system, up to the regional landscape. They also have an identity that makes them unique on their particular scale, yet they always also figure in some kind of socio-ecological setting on the next scale.

In that structured system of the landscape organism, humans as well as nature play their role, figuring as the material base and the changing agent respectively. Or, in other words, as the sculptor and as the material which that artist is using to express his or her ideas and thus, at the same time, expressing him or herself. Farms have an identity, rivers and mountains have an identity, estates, forests, meadows, bays and lakes: they all have their physical features, their history, their ecosystem and character as expressions of that identity.

Regarding Maslow's human triangle, it can 'mutatis mutandis' also be translated to groups of humans, teams, firms, societies and nations (Figure 3).

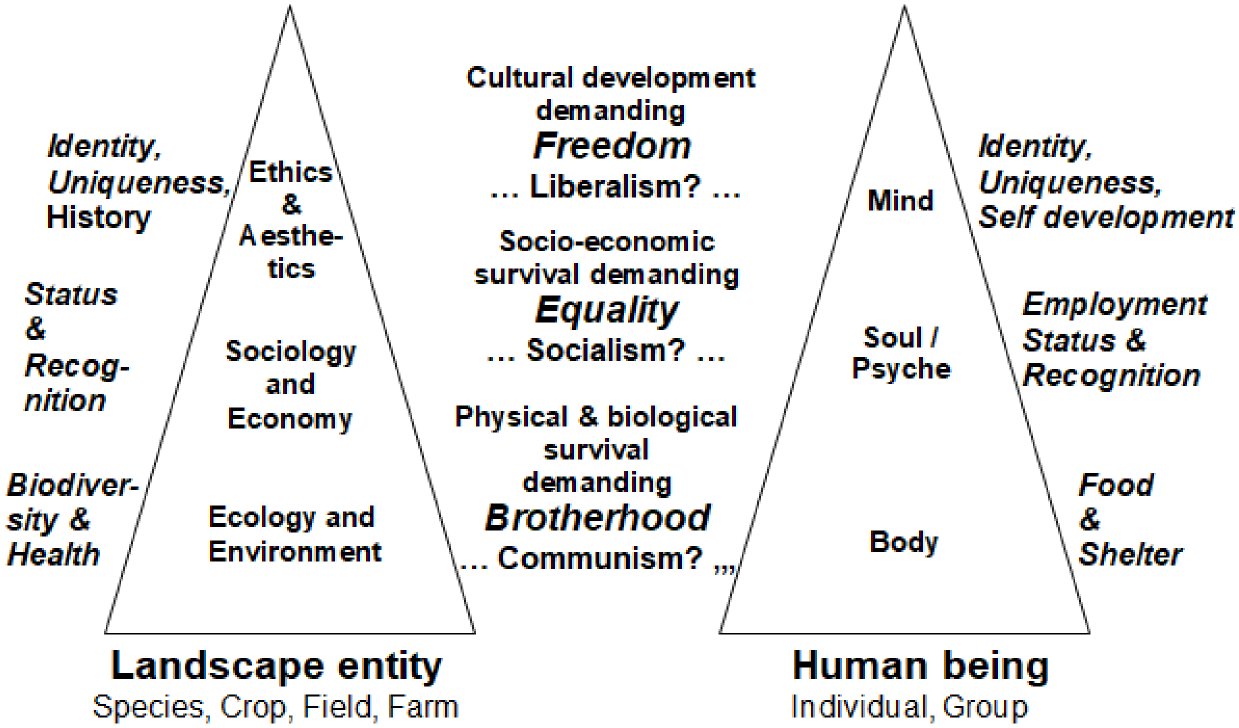

Fig. 3. Maslow adapted by the author.
Links between People's and (Farm) Landscape's organisms

As you will see, I have added three well known value-systems – Freedom, Equality and Brotherhood – in this scheme. Each fitting the most appropriate position in the hierarchy, referring to the different qualities relevant in each of them. These key values stem from the French Revolution and elaborated during the past century in the so-called movement for Social Three-folding.

To illustrate the relevance of presenting the three value systems in their optimal syntheses, I add a few words on their tendencies when separated from one another and/ or put in rigorous competition.

As for crop species and varieties, they also function on each of those levels with their anatomy, physiology and ecology on the body level, their socio-economic value on the market on the psychological level but also on the level of unique identity.

How different disciplines' subgroup-interests may trigger conflict and biases

When the hard facts-and-figures are believed to be the world's one and only essence, a radical materialism emerges. Some call the extreme of this position autistic: no serious communication is possible as man's egocentric genes and nerve systems decide all. In this worldview individual responsibility is a chimera. The natural sciences' paradigm tends to support this position. Unlimited measuring and calculating absorb people's energy for ever. Everyone has to fight everyone else in order to survive (Hobbes, 1620; Darwin, 1872; Huxley, 1932; Dawkins, 1976).

When on the other hand people choose to believe that spiritual goals and value systems are the ultimate essence of life on earth, the question is who has access to what man's goals and ultimate values really are. Here cultural sects and dictatorship lie on wait. Some would call this tendency hysteric, in contrast to the before mentioned autism. The knower with access to the unknown, invisible essence he and nobody else is aware of will inevitably be made into a kind of higher being by those admiring him or her. Here the cultural sciences' paradigm tends to paralyse people's individual responsibility by subordinating them.

When economy is perceived as internalising profits by externalising costs, the opportunism of social and political power is luring. References to facts and ideas are made instrumental to increasing wealth and power for few, disregarding their intrinsic values for all. Here Orwell's 'Animal Farm's line "all animals are equal but some animals are more equal then other animals" nicely fits. Leadership on all scales can be reviewed along this line of consideration.

A conscious awareness of how the natural, social and cultural sciences are basically complementary, having their roots in the human being as a whole, allows for optimal cooperation of the three in favour of a fair & sustainable development worldwide.

Within each of the larger science realms also the sub disciplines may be used to compete for recognition, influence, money, jobs etc. In plant production for example, soil science, fertilisation science, pest management, hydrology, plant breeding and mechanisation may fight for the 'most important' effect on the harvest: quantity and or quality. As said before, making one's expertise instrumental to cooperation for a commonly recognised goal facilitates optimal teamwork.

Applying Mansvelt's adapted Maslow triangle to land management

From hereon I'll elaborate what the Maslow (Maslow 1943) approach, when applied to land management (agriculture and environment in their socio-cultural settings) could mean in practice. This chapter more directly leans on van Mansvelt & van der Lubbe's (1999) book 'Checklist for sustainable landscape management'. For this MOVIR conference, I try to specify the relevance for its plant breeding strategies in the context of Russian Agriculture.

The Natural Science's Realm

I therein start with the respect for the site specific natural qualities, conditions and potentials. Environmental pollution and ecological degradation are here the main concerns. Improvement of the lively hood and the soil's production capacity is here the challenge.

1. Regarding the Environment the main criterion is its cleanness, meaning its long time availability for man and nature. They should be kept in or be returned to their status of being the clean basic material for life on earth.

Regarding Russian agriculture, the challenge is to revitalise the degraded (polluted, desertified and mineralised) lands all over the country, aiming at the restoration of lost soil fertility.

For plant breeding this could mean that – besides breeding for highest production under optimal conditions - also lines should be (re)developed that do well under poor conditions. Here such features as deep rooting, minimal nutrients requirement, drought resistance and the like are crucial, especially for grains and beans.

Generally speaking soils are the products of life processes in the recent or far-away past (flora and fauna in ecosystems of all dimensions interacting with weather/climate conditions and human management). Sustainable agriculture in general (organic/biodynamic agriculture in particular) relies on building the land (Land-bau, in German language). But also catching rains and buffering water, braking winds and making shadow are environmental functions of land management. As well the catching and storage of chemicals such as nitrogen (nitrates), carbon dioxide, and various minerals.

Thus land management in general, including forestry, wetlands and dry-lands, can be perceived and handled as a tool for a sustainable development. Making sure that next generations have water, food and shelter as needed.

In the same way (unpolluted) bio-topes inside the eco-topes should be managed in ways that allows for sustainable development.

The main target of a clean environment (a-biotic) can be defined as: the long term availability of resources, allowing for human and rural well being in a sustainable managed biosphere. In our view the ongoing development is an intrinsic part of well being.

1.1. For a fertile and resilient soil the target may be: prevention of soil degradation (pollution and loss of structure) and soil erosion; incentives for long term soil fertility improvement in rural or agro-sylvi-pastural production regions. Soil structure and fertility are the key issues of sustainable land-use. However, a minimal amount of natural erosion of the mineral soil is an intrinsic and appreciable aspect of the nutrient recycling within the biosphere. The natural erosion of the mineral underground, caused by natural weathering together with plant-root and edaphic activities, underlies the biosphere development per se.

Some parameters for a fertile and resilient soil are:

- Minimal soil pollution (heavy metals, pesticides etc.);
- Manure quality (C/N ratio);
- Stocking rate (SR) matching the soil and the carrying capacity of the system;
- Anti-erosive belts and contour tillage;
- Soil cover (winter or off-season);
- Crop rotation and crop mixture;
- Soil structure and organic matter content.

For Russia's degraded lands the challenge is to (re)build a fertile soil starting from the more or less degraded situation. In nature conservation areas at first a pioneer vegetation (ecosystem) should get established which then gradually should develop a humus' soil with its increasing resilience and fertility. Here the soil building policy must find a balance between focussing on a kind of sandy hydro cultures and the focus on long term soil building strategies.

The establishment of minimum external input systems of mixed (crops & husbandry) production seems quite sustainable, as many reports on organic agriculture clearly show. The exact implementation demands expertise on the regional potentials for specific plant and animal species (varieties), stocking rates and crop rotations. The regional species' appropriateness to be elaborated and improved with the expertise of MOVIR and the overall Vavilov Institute of Plant Industry.

1.2. For a clean and healthy fresh water (groundwater and surface water) the target may be: prevention of water pollution and water depletion, incentives for the long-time conservation of drinking water quality and water reserve volumes in the relevant rural or agro-sylvi-pastural regions. However, most water pollution has its source in flow emissions from polluted soils. In addition, wastewater effluents must be carefully considered.

Some parameters for clean and healthy water are:

- Cattle units per hectare (enough to fertilise the soil; minimal competition with human food production);
- Level and time of manuring (quantity per hectare per year);
- Waste water treatment;
- Bookkeeping of minerals and additives;
- Bookkeeping of other potential pollutants;
- Water use and management.

For Russia's degraded lands good stewardship is crucial. The production of healthy drinking water, good for fish, crawfish etc., should be set as a firm condition for agricultural production. Regional species' appropriateness to be elaborated and improved with the expertise of MOVIR and the overall Vavilov Institute of Plant Industry.

Most probably drought resistance and salinity resilience are crucial selection features for agriculture's future.

1.3. For a clean, fresh and healthy air in the countryside the target may be: prevention of bad smell emissions and volatile emissions of pesticides and residues, which affect human beings and the ecosystem. But also wind-control to prevent damage to soils, crops and livestock must be considered. However, much air pollution is generated from soil-emissions, manure or slurry, and surface water emissions like volatilisation. But also direct emissions from pesticide sprayings and husbandry housing are sources of air pollution. However, a certain *intrinsic emission* from animals like breathing and flatulency and also their excretions of urine and manure should not be regarded as unnatural, but appreciated in the context of ecological nutrient recycling. By limiting the number of cattle units per – well sheltered – surface unit, a natural ecosystem buff can be warranted. See also for the stocking rate matching the soil and carrying capacity of the system, the anti-erosive belts and contour tillage.

Some parameters for air quality are:
- Ammonia and other emissions;
- Wind-shelter belts's presence;
- Presence of lichens and other vulnerable species (bees, butterflies).

For Russia's degraded lands minimising wind erosion, creating shadow for livestock and warranting cover crops for soil protection seem to be key issues. Optimal use of straw and other natural sources of celluloses prevents NH_3, N_2O & CH_4 losses (air pollution) from liquid manure. Optimal use of roughage in feed minimises N losses from livestock.

As you all are aware, Yeltsin signed in 1991 Russia's first comprehensive environmental law, On Environmental Protection. Modelled after a similar Soviet law, it made many general statements about the environmental rights of citizens without setting any specific goals. The law also defined numerous environmental functions for every level of government as well as for citizens and nongovernmental organizations, and it specified environmental regulation of every aspect of society, from health resorts to electromagnetic radiation. The sheer inclusiveness of such provisions made practical enforcement impossible.

The Commission on Ecological Security went into operation in 1993 under the Security Council: with great fanfare but little funding. Its assignment was assessing the most serious environmental problems as they endanger national security.

It seems an interesting option to show regional and national policy how

the expertise of MOVIR et. al. can contribute to environmental protection by breeding specific lines for ecologically sound / organic and soil recovery purposes.

2. Regarding the ecosystem management, three main targets can be distinguished:
Bio-diversity 2.1.
Ecological coherence 2.2.
Animal welfare 2.3.

2.1. As for bio-diversity the main target may be to safeguard a sustainable development of the regional landscape biosphere diversity, within the context of a well-structured and well-cultivated regional and supra-regional network of ecosystems. Therein, the biosphere network of ecosystems has a number of functions towards the a-biotic environment, which it supports and depends on, like soil, water and air. It has also has a number of functions towards the human society, which it supports and depends on, like the socio-economic and cultural environment.

Species' diversity, bio-tope diversity, ecosystems diversity are aspects of bio-diversity. They cover plant as well as animal species: macro, meso and micro.

Some parameters to be used here are:
- Species diversity per bio-type and bio-tope;
- Targeted Plant Species Diversity (TPSD), Target Trees Index (TTI) and Target Shrubs Index (TSI);
- Plant Species Diversity (PSD) and Plant Species Distributions (PSDN);
- Minimum standards for bio-topes per farm type;
- Minimum standards for types, numbers and size of ecosystems per landscape and region;
- Multifunctional landscape management;
- Regional specifications on presence (quality) and abundance (quantity).

For Russia's degraded lands situation of today is diverse. There is a East – West gradient as well as the N-S gradient, with mountainous and lowland areas therein.

Regionally appropriate biotope variation in time and space is important, on scales relevant for optimal biodiversity, to balance seasonal climate effects and pest occurrence in the crop as well as in the herb, fruit and livestock production. Organic agriculture and Biodynamic agriculture are established all over the world as applied – food productive! - biodiversity. Thus soil life, aquatic life and airborne life are to be included here in the objectives of agriculture. Worms, ants and bees (wax & honey), for example, included.

For MOVIR et al this could mean the establishment of cooperation with honey bee conservation and breeding institutes, national as well as regional, as their common interest is so obvious.

2.2. As for Ecological coherence, it should be seen as complementary to the mentioned diversity of species, bio-topes and landscapes, as it implies that diversity is found within a unifying context. If not, unnatural sites like zoos, botanical gardens and mega cities would be the ultimate examples of successful bio-diversity management. However, diversity as a criterion for sustainability of landscapes, refers to an ecologically coherent diversity. Compliant to the before mentioned targets, the idea is that each species can only figure and function within an eco-system of other flora and fauna species. Such a system relies on and also contributes to the common environment of different species. Now it should be noted that technically, crops and animals can be kept or produced in "hors sol" and "off-season" conditions by supplying them with an artificial environment including nutrients and waste-management. However, such an artificial environment does not produce a sustainable ecosystem nor the appreciated landscape that our urbanising society increasingly demands. In order to warrant the sustainable management of the (agro-) landscape, a keen awareness of the various connections and links of species and bio-topes with one another and their environments must be generated or at least encouraged.

Some parameters to be used here are:

- Site specific indicator species;
- Site specific habitats and ecosystems;
- Species coherence;
- Habitat and eco-system coherence;
- Full lifecycles of species and systems;
- Seasons compliant management: availability of nectar for 'flower-insects';
- Seasons compliant management: timely differentiated hedge and woodland management;
- Seasons compliant management: timely management of water-bodies;
- Seasons compliant management, timely optimised management of permanent pastures;
- specifications on presence (quality) and abundance (quantity).

For Russia's degraded lands situation of today is diverse. There is a East – West gradient as well as the N-S gradient, with mountainous and lowland areas therein.

Regionally appropriate biotope variation in time and space is important, on scales relevant for optimal biodiversity, to balance seasonal climate effects and pest occurrence in the crop as well as in the herb, fruit and livestock production. Organic agriculture and Biodynamic agriculture are established all over the world as applied – food productive! - biodiversity. Thus soil life, aquatic life and airborne life are to be included here in the objectives of agriculture. Worms, ants and bees (wax & honey), for example, included.

For MOVIR et al this could mean the establishment of cooperation with honey bee conservation and breeding institutes, national as well as regional, as their common interest is so obvious.

2.3. As for animal welfare conditions, the target is to respect their species' intrinsic behaviour's demands. In view of sustainable landscape management, or in other words, the landscape's welfare, the most important aspects for husbandry are:

- Cattle and husbandry should fit and respect the carrying capacity of the regional ecosystem (see environment);
- Cattle and husbandry should be instrumental to the landscape management. Intensive, 'hors-sol' and outdoor livestock production systems do not fit into this concept.

However, at the moment, society may largely prefer other concepts and priorities, such as cheap meat and high returns on investments. Examples of landscape management with livestock are dairy farms in the mountains of Switzerland, Austria, and Norway and nature conservation areas with sheep and 'wild' horses or ruminants. Obviously, these parameters are to be specified, per species and per region, with local experts.

Some parameters for animal welfare conditions are:

- Space for natural behaviour (that is: living in a sufficiently species specific merger of free-range & appropriate housing);
- Shelter against adverse weather in the free-range conditions;
- Preventive health care (to come away from sickening livestock conditions covered by cyclical or even permanent medication).

For Russia's degraded lands the challenge is to make crop production and animal husbandry in their widest sense instrumental to the creation of locally adapted, coherent and bio-diverse agro productive ecosystems. They are to provide for a fertile soil (manure) as well as grains, greens, meat and dairy products for human consumption. The use of roughage and crop residues not fit for human consumption is included in this function. Relaxed lives for animals – free range husbandry - surely makes the food they produce less stressful for the consumer than meat, eggs and dairy from intensive animal production systems (battery breeding).

Here it becomes clear that for MOVIR et al, not only the requirements for human nutrition are at stake, but also those of the performance of specie's breeding lines performance as roughage for animal production and manure.

3. The social sciences realm: economy and sociology

In the development of sustainable agro-sylvi-pastural (rural) landscapes, norms, attitudes and processes of the socio-economic sphere are crucial. All decisions made by whoever are based on some kind of prioritising: in whatever group(s) in charge for whatever part of the landscape: its people and industries. Although the agro-landscape is usually seen as an object of either natural ('hard'/quantifiable) or anthropological ('soft'/arguing) sciences, underlying all decisions that earlier or later sort visible effects, deliberations on values, feasibility, profits, and interests have been weighed and traded out in some sort of transparency (see scheme 2). People take decisions on all kind of aspects with all kinds of arguments and these decisions take place within social structures.

In the end, these decisions are reflected in the biosphere, agricultural production and quality of the people's nutrition. Decisions within and on plant breeding fully fit within the before said.

Here, the social realm, representing the qualities of the social environment, has been subdivided into economy (goods, money and services) and sociology (power and access to responsibility for decision-making) see schemes 2 and 3. The argument to start here with economy is that it focuses more on quantifiable issues/parameters than sociology.

The economic and social criteria represent the trade-out area between the human (and society's) physical survival and its ethical survival (individual development). That trade out area can also referred to as between the human and the humane.

Here way people and societies decide to spend their money and to participate in socio-political activities is at stake. It reflects their empathic coherence with the sources of the purchased product or service and or the ideals pursued in the political or other social actions they support.

From this point of view, buying (all purchases) and political decision-making are both phenomena of implemented *sympathy* or *antipathy*, or in other words phenomena of implemented *engagement* or *alienation*. This can be found clearly reflected in the original market status, where the acts of meeting (socialising) were at least as important as those of the trading (economising), with the bargaining as an expression of the quality of that personal meeting.

Although it is now widely perceived that these days most decisions are made on economic grounds, the identity of actual beneficiaries of those economic considerations may not be always very clear to the public. For example, few actors are fully aware that economic "laws" are the reflections of historical and regional habits, attitudes and the appreciation of societies. Theoretically they are open for modification by societies: if they want to.

For example, perceiving the farmer's income as necessarily based on the sales of their food, feed and fibre production, and the landscape as an issue of public services, is a socio-political choice with considerable impact. On the other hand, perceiving farmers as the major managers of landscape's ecosystems and the environment, with the food and fibre production as main products but landscape as an inseparable side product to be fairly remunerated, is another one view. Moreover. calculations on the economic effects of such decisions fully depend on the factors included in the calculations as well as on the number of factors which are kept fixed ("business as usual") versus factors allowed to be changed in compliance with the new policy to be proposed. The political dilemma on all relevant levels – firms' management policy as well as political policy – is to generate sufficient changes to reach the targets as they have been set without, however, changing too much of vested interests' positions and prospective. Opting for win-win solutions, to be reached within an acceptable time-span, seems the only way-out. This means here that the challenge is to make ecosystems as well as cultural systems gain a benefit from all socio-economic actions (Daly, 1996; Daly, Cobb, 1994; Appendix).

The importance of parameters as presented below, soft or hard as they may be, is that they facilitate more a clear and conscious decision making. Here we presume that the more trade-offs are known, the less surprises or unforeseen side effects will occur on the medium or long run.

In this study, equity in the sharing of the earth's limited resources and inter-human equality in the participation to decision-making – with compliant sharing of the responsibility – are seen as the leading objectives in the social science realm at large.

For society as a whole, the process of specialisation and spatial concentration has led to an enormous increase of production and consumption volumes, together with increased flows of goods (transport), services and finances. In the rich countries this led to overproduction and over-consumption, in particular of energy and animal proteins. It also went along with increasing power and wealth for less and less leading (upper class) people, and increasing dependency of increasing numbers of others: middle class people and poor.

In the biosphere realm it led to an increased waste of resources and an increased production of refuse. It also led to the removal of wildlife habitat, ecosystems degradation, decreasing species diversity and landscape features. All these changes led to a painfully perceived reduction in environmental quality and the loss of rural area's multiple values for other than industrial, urban or traffic uses.

To overcome the negative (side) effects of this development, a well-balanced coherence between society's vested and future interests seems an objective worth striving for. This statement holds for society's industry as a whole, as well as for agriculture (food production and landscape management) as one of industry's specific sectors. At the moment, agribusiness has a lot of power in the whole chain of food, feed and fibre production, ranging from farm (primary production) to household (final consumption). Global agribusiness at large still tends to demand for uniform standards: same units, same quality, and same product types (homogenisation and mono-cultures). Such a specialised economy tends to lose the benefits of diversity and so does a specialised agricultural sector wherein farmers get fully dependant on the demands of agribusiness (not necessarily consumers'). They restrict their farming to the production of large quantities of a limited number of products (raw materials for industrial upgrading). The contrast between fast food and slow food symbolically shows today's choice.

If private producers of valuable rural landscape do not receive any contribution to their costs of soil and landscape production, they will be reluctant or unwilling to invest in agro-landscape production or ecosystem's maintenance. The same holds for the challenge to make new wetlands or deserts productive for agriculture in an ecologically sustainable way.

Societies that tend to consume their soils, spoiling them, will sooner or later face bankruptcy as they will be unable to feed their people. Paying for their food with money earned from whatever mineral mining (oil, metals etc.) is on the long run not compliant with sustainable development's demands, as those minerals are fundamentally limited. Soil building societies will live as long as photosynthesis is cultivated in an ecologically sound and safe way. For economists and sociologists an crucial notion to apply their expertises in a responsible, ethically sound way.

Here another aspect of Russia's identity, its political history as well as its perspectives for the future generations, is at stake. The challenge as I see it is to elaborate a strategy to include ecology in the economy or, in other words, root the economy back into the regional, national and ultimately into the global ecology. Therefore, leadership explicitly dedicated to sustainable development is badly needed on all levels. This includes leadership with a proven capacity to formulate, coach and implement appropriate policies for all relevant ministries, to make them cooperate instead of compete. Cooperation with NGO's for fair sharing or the earth's limited resources seems efficient.

As for MOVIR et. al.in the context of sustainable development, the challenge as I see it is to find and establish new socio-economic partners along the food, feed and fibre chain as well as along the chain of landscape management and biosphere production. Thereby, tuning in into the various Russian regions' particular opportunities and demands, seems to open new doors that ad to those of centralised institutions. Breeding lines that perform excellent in specific regions may be much more promising than such that perform medium well in many. However, the latter are more interesting for centralised breeders, as they can sell larger volumes. The more environmental & biodiversity performance is included in the profit calculations, the more a shift to regional production will be recognised as a sustainable development.

3.1. Regarding the management of the agro-landscape's economic system, three main issues can be distinguished:

- Good farming should pay-off: 3.1.1.
- Greening the economy: 3.1.2.
- Regional autonomy: 3.1.3.

3.1.1. Good farming paying-off means that farmers' subsistence and thus farming systems' subsistence should be warranted. Therefore, good farming should pay-off, to make sure that the good farmers remain in or move to the rural areas in need of good agro-landscape management. As said before: farming is good for the food and landscape quality only when soil and landscape are included as such in farming's objectives. This obviously makes all farming multi-functional. Chapters 1. and 2. provide for the criteria of good farming as meant in this paper!

Some parameters to be used her are for example:

- Total net farm income;
- Total farm family income;
- Return on labour;
- Farm's market orientation;
- Financial autonomy.

For Russia's agricultural future, key issues are regional self sufficiency for food and fibres on a level of human basic needs for a healthy development. As energy prices boom, transport and processing together with exports and imports get increasingly expensive. When transport, storage and processing prices (waste product management included), are fully internalised in the food prices, a sustainable balance between exotic and rural food consumption will get established before too long. Moreover, regional production gives incentives to capacity building (appreciation, recognition), which again contribute to social welfare in the regions.

Accordingly, MOVIR et al would be encouraged to follow a regionalising policy.

Examples of on-farm multi-activities that increase the added value and farm income are:

Production of high quality products such as regional products, ecological and bio-dynamic products.

For Russia's wide range of particular regions - river basins, mountainous areas, plains – as well as historic ones, product diversification is definitely an option.

Creation of appropriate jobs and living conditions in the rural area.

For Russia's degraded lands this seems an option worth considering. Over urbanisation de-roots society. Land-flight leads to slums for jobless people with all the related problems such as increased criminality. Nowadays, with electronic connectivity spreading the country, (part-time) working in the urban fringe may well be an option for a reappraisal of the countryside life's Dascha culture.

Decentralising part of MOVIR as mentioned contributes to regional job creation.

Management and production of nature and landscape.

For Russia, in view of its enormous low populated areas, not so much the landscape's aesthetics as the landscape's environmental production seems an issue here. Fresh air and clean water for drinking, together with non-eroding soils (humus building) can be recognised as important for the urban population's food, fibre and climate production.

Agro-tourism, health farming, recreation.

Together with the before said, this will become increasingly important as a by-product of (agricultural and forestry) land management. Far away from urban stress and the presence of well cultivated biotopes (dry, semi wet and wet) can be cultivated compliant to each regions' particular character in various degrees of management intensity.

Farms facilitating care-tasks for people who need professional care (mentally handicapped, psychologically disturbed, burnt-outs, rehabilitation of criminals etc.).

For Russia this is a serious option for upgrading life on the land indeed. Training people to work with the mentioned client groups (agriculture and crafts) could be an additional goal for the people living on the land. In addition to the traditional 'Kurorts', Sanatoria and the like. Here good contacts with Russia's health & care organisations (governmental as well as non-governmental) is needed. In many European countries successful mergers between respectful (!) care for mentally disabled people and medium scale agricultural production have been *developed. Fair recognition for their reliable craftsmanship and serious motivation contributes to their social self acceptance which again stimulates their performances. This is especially the case in communities with mixed clientele (different diagnoses).*

3.1.2. 'Greening' the economy means an approach focusing on fysiocracy or in other words: including the ecosystem into the economy. This means that costs which today are largely not calculated, because they are regarded as external costs, are to be internalised in the calculation of the benefits. That is: they are incorporated in the production costs and thus in decision making of producers and consumers. This then is instrumental in adjusting society's behaviour away from today's 'eating the earth out' policy towards a sustainable mix of production and consumption. Thereto economic calculations at farm level and at regional level should include environmental costs and benefits. Although our society is nowadays perceived as highly economised, not all external benefits from multiple land use are yet included into the neo-classical, mercantilist, (semi-) capitalist economy prevalent in "western" society. As this approach to economy is oriented toward externalisation of production costs for the private entrepreneur as well as for each government, internalisation of environmental and social costs does not come easy. Studies like Herman Daly's 'Limits to growth' (Daly 1986) and 'Beyond growth' (Daly 1996) treated the issue already quite clearly in the '90's, focusing on the concepts of economy. Key notion is to distinguish physical growth from technological and cultural development. Growth relying on the earth's limited resources, development relying on the unlimited possibilities of intellectual and spiritual capacities.

More recently Nicky Chambers, Craig Simmons and Mathis Wackernagel (2000) published 'Sharing Nature's Interest: Ecological Footprints as an Indicator for Sustainability". And somewhat parallel William McDonough en Michael Braungart (2007) developed the so called 'cradle to cradle' policy (C2C) which fully complies to greening the economy. They phrased their policy for industrial development 'Design for Reincarnation', meaning that all materials used should be re-usable after their primary use (remnants ready for reuse). They argue that what we call 'waste' is basically a lack of thinking seriously.

Parameters to be used here are for example:

- Dependency on non-renewable inputs;
- Share of non-renewable inputs in total costs;
- Share of re-used on-farm production value in total costs;
- Costs-benefits ratio of investments in agro-landscape, environment and nature.

For Russia – large and resource-rich country as it is – there is a wide range of opportunities to contribute to a truly sustainable development of its agriculture for now and future generations. Re-cultivation of deserted lands will decrease future costs for society as a whole, though, in the non-sustainable way we calculate today, it means higher costs for agricultural production for the short time. As long as agriculture's costs of human health, nature's resources and environmental disasters are not calculated as costs, there will be no way to stop such abuses of the global ecosystem earth's life depends on.

3.1.3. Regional autonomy means that the region's own agriculture, fishery, and/or forestry provide for the rural region's subsistence. Obviously, wherever possible, the region's food and fibre surpluses can and should be used to serve neighbouring urban areas. Regional (rural) development policies can play an important role in favour of this regional autonomy. Today's on-farm specialisation and the excessive urbanisation (to macro and mega-poles) have led to considerable alienation between the rural area's farmers and the consumers. The latter expecting the farmers to produce fine landscapes and a clean & healthy environment, but only willing to pay for the cheapest foods as produced by agro-production systems that degrade precisely those much wanted 'by' products of their food's production. Re-linking consumer's awareness to the region's farming units they're eating from, which goes together with farmer's awareness of the consumers that appreciate their products, definitely contributes to an increase of product quality: directly and indirectly.

Parameters for regional autonomy are for example:

- Transport distance per food unit;
- Resource efficiency and regional labour possibilities;

- Swaps from single commodity support to management system's support;
- Translation of the good farming and greening of the economy to the regional level;
- Market access for regional speciality produce (Slow Food policy).

For Russia the challenge is finding a balance between the income production based on export to the urban area's and the world's wealthy consumers on the one hand, and the own regions' relatively poor people on the other. As you all know, Russia holds the world's largest natural gas reserves, the second largest coal reserves, and the eighth largest oil reserves. Russia is also the world's largest exporter of natural gas, the second largest oil exporter and the third largest energy consumer. Energy export makes about 60 % of Russia's export revenues. But its energy production accounts for over 98 % of its CO2 production is related to precisely that production because of outdated technology.

3.2. Regarding the socio-systems' management four main targets can be distinguished:
- Well-being in the area: 3.2.1.
- Permanent education of the rural community: 3.2.2.
- Access to participation: 3.2.3.
- Accessibility of the landscape: 3.2.4.

3.2.1. Well-being in the area focuses on the conditions allowing for ongoing acceptable life in the rural landscape: a pre-requisite for social reproduction of sustainable (agro-) landscape management. It is not only the farmer him/herself who is looking for sufficient well-being, but the farm family. Improvement of the well being counteracts rural degradation by increasing the social viability of the agro-landscapes. Serious perspectives for farmer's succession, warranting sufficient farm income and welfare services in the area are necessary social conditions. Here also land property and land-management structures from farm to regional level are crucial.

Some parameters to be used here are:
- Options for farmers' succession;
- Financial income for the rural community (tourism, local crafts, support services);
- Welfare services in the region (health, education, culture).

For Russia's non-urban regions both the regional people's nutrition and also their labour opportunity can be envisaged as an objective. The more people will be able to flourish in a region, the more such facilities as mentioned will pay off. Appropriate decentralisation is a wonderful tool to diminish the eco-terrorism of today's over-urbanised society. This is particularly the case as Russia still has more than half of its bio-capacity as yet as its eco-reserve (where The USA and the EU are more than 50 % in eco-deficit).

Short notice, history will look back at the kolkhoz and sovkhoz period as a failed opportunity to establish a sustainable and socially fair land-use policy. We would now perhaps prefer to call them sustainable, land-based food and landscape production societies (cultures). With good electronic communication and fair public transport connections countrywide, many people will prefer life on the land over the stressful urban life (as they prefer slow food over fast food). At least so for a considerable period of their lives.

3.2.2. Permanent education of the community is a must to warrant sustainable agro production, land use and landscape management. Farmers and other community members should have several possibilities to start and go on developing their knowledge about issues such as ecological and bio-dynamic agriculture, landscape management, etc. On farm processing of regional products, in village development of rural crafts (Slow Food etc) are parts of that development. Here the people's opportunities for self-realisation, as mentioned by Maslow's theory, is at stake (Maslow, 1943).

Some parameters for permanent education of the rural community are:
- Available levels of education in the area (up 'til pre-high school; then high school and university in subsequently wider circles);
- Participation in study circles, training and courses relevant for sustainable landscape development, forestry and all crafts related to food and wood production.

For Russia's degraded lands this complies with the plea for de-urbanisation.

3.2.3. Access to participation. The aforementioned global landscape degradation in general is often seen as a result of alienation between the various rural stakeholders and the values of rural landscape. Only an increased awareness of the mutual interests and interdependence of farmers, the rural community, urban people, and government will facilitate possibilities to co-operate (empathic coherence). At the moment there seems to be a lack of information, knowledge, understanding, and awareness among those actors regarding their common interest. That common interest of farmers and local community can merge in win-win solutions: farmers are paid for landscape production, which is appreciated by the local community, including consumers (empathic coherence). Here is a possibility of the before mentioned cross-compliance. Access to participate in local community and in farm activities will increase responsibility among farmers as well as among local community for sustainable landscape management. There are various ways how these two way participation and involvement can take place, e.g. through the membership of in regional councils, farmers' organisations, co-operation with NGOs and consumer groups, professional and lay excursions, etc.

This main criterion is subdivided into the following sub-criteria:

- Farmers' involvement in activities outside their farms: 3.2.3.1
- Outsiders' involvement in farm activities: 3.2.3.2.

3.2.3.1. Farmers' involvement in activities outside their farms can take place at various levels, from colleagues to governmental level, and will increase farmers' and other members of the rural communities' awareness of sustainable landscape management. The question here is where and how farmers and other rural people have access to participate in activities that will increase their awareness and willingness to contribute to the landscape development?

Some Parameters for farmers' involvement in activities outside their farms are:

- Membership to farmer organisations and farmer groups;
- Working part-time in the region;
- Involvement in organising outlets for their particular products;
- Co-operation with NGOs involved in landscape development;
- Membership of regional councils;
- Access to professional expertise and support programmes;
- Access to participate in dissemination programs.

For Russia's degraded lands this complies with the earlier plea for de-urbanisation.

3.2.3.2. Outsiders' involvement in farm activities can take place at various levels, from colleagues to municipal or regional level, and will increase farmers' awareness and willingness on sustainable landscape management.

Some parameters for outsiders' involvement in farm activities:

- Access to participate in landscape management in co-operation with the farmer;
- Professional and layman excursions to the farm;
- Community supported/shared agriculture (CSA);
- Financial commitment to landscape programmes;
- Access given to farmers to buy/rent and manage lands owned by landscape and nature institutions in a commonly agreed sustainable, ecologically and socially sound way.

For Russia's degraded lands this complies with the before said plea for de-urbanisation. All relevant activities can be developed in dialogue with the regional workers, their family's and local leadership. Here a renaissance of dacha's and dacha farming could be at stake. For dacha's surrounding large farms, situated in their fringes, would allow a closer contact between both: farmers, villagers and urban people.

3.2.4. Public accessibility of the (agro-) landscape, this is crucial to let the rural community experience and appreciate the landscape as such. If farmers do participate in the decision making process and responsibility of the local community, then this local community wants to 'consume' what they are paying for. Accessibility of the landscape facilitates commitment of consumers to farming practices and landscape management. Farmers, like other professionals of any job, want and need appreciation for their products. But farmers are not always enthusiastic about public accessibility, because they feel as disturbance of their privacy or damage to fields and animals.

In former days, public access to “church-paths” (School paths) and “rights of way” were general accepted by farmers and the local community. Farmers that become initiative in participating trough excursions of consumers, NGO, etc. to their farms may reduce the threshold of resistance towards accessibility and will facilitate commitment of consumers to their farm practices and land use and landscape management.

Some parameters for the accessibility of the landscape may be:

- Excursions to and on the farm;
- Rights of way;
- Tracking roads crossing the countryside.

For Russia’s degraded lands this complies with my earlier plea for de-urbanisation. All is to be considered in the balance between over – exclusive fencing-out of all ‘others’ (not part of the party) and loss of identity by failing limitation of the socio-ecosystem’s use.

4. Human sciences and the (agro-) landscape: landscape psychology, physiognomy and identity

Here we come to the agro-landscape’s cultural sphere of history, architecture, anthropology, aesthetics and ethics. Therein we meet the land in its least material phenomena, in its most non-excludable and non-depleting use. Here it is the perceivable appearances of the earth’s surface, which result from the interaction between man and nature. At large, its colours, smells, structures and its picturesque qualities are open for public consumption with little threat of being worn down from that sensorial consumption.

All peoples’ histories are embedded in (combinations of) landscapes: the sea, the mountains, the desert, the steppe, the forest, the delta, the riverside, the small or the large towns cities and mega-poles. Landscape as seen as characteristic spatial arrangements of land-units. Therein the *characteristic arrangement* of the units (species, bio-topes, infrastructure, buildings) refer to an artistic, aesthetic notion, addressing the vision of the whole. It is that perception of the pictorial quality that makes the landscape into a landscape. The landscape as a whole delivers the context wherein the mentioned elements of the nature, which can be analysed by natural sciences and used for design by technical sciences, do figure. Those landscape elements of nature and culture must be discerned within and reduced from the landscape as a whole, before they can be analysed in one way or another. Therefore, it makes no sense to consider landscape as a static phenomenon that should definitely be conserved in a certain fixed state, and thus landscape and sustainable development fit nicely together.

In broad circles of European society and authority, restoration of the ecological quality of the landscape is seen as a step further in the cultural development. Organic, Biodynamic and Permaculture types of agriculture do play an important role in this restoration process, as they represent a feasible style of farming based in a concept of respect for and an attitude of co-operation with nature and the environment. Thus quite contrary to a large trend of modern agro-production’s eco-terrorism indeed. In general, the three before mentioned styles already provide a clearly richer type of landscape with more naturalness than non-organic (conventional) farming.

Amongst the landscape problems at stake, the following major categories of detrimental effects can be indicated:

1. Desertification or standardisation of the landscape, as its regional diversity decreases together with the vanishing of many natural landscape elements from the rural area (bio-topes of various sizes);
2. Fragmentation of the landscapes, especially in the neighbourhood of urban areas, took place as new infrastructure lines were recklessly forced upon the existing ones;
3. Simplification of the landscape, as too many extensive and abrupt changes have too often been taken place, disturbing the appropriate development of landscape elements, making mature landscapes that show the full potential of their features and qualities rather rare;
4. Ongoing and alarming decrease of bio-diversity, along with the before mentioned trends.

For Russia these trends are quite recognisable, and need appropriate policy to change toward sustainable development.

For MOVIR et al, such policies as recommended before, do fully fit here as well.

In addition, here the issue of species’ collection and conservation is at stake (Gene banks). This holds for edible and pharmaceutical species as well as for aesthetic ones, odorous and *painting pigment producing ones. Thereby it has become clear that the localisation of such banks influences the genes stored therein, particularly through the need for reseeding to keep the seeds alive. The rationalisation of plant breeding as argued before, nicely fits the demand for rationalised gene banking.*

4.1. The (agro-) landscape's psychological features, as spontaneously experienced and appreciation by all participants, are important for the landscape's sustainable development. If the land is experienced as worthless no-ones-land, people tend to contribute to its degradation. On the other hand, the more people appreciate its features the more they will effectuate social control to warrant its quality.

For all the various demands of its farmers, inhabitants, tourists and people who need to recover from stress, mental or physical diseases, the landscape should offer appropriately fitting niches. Places and possibilities in the landscape to feel comfortable, at home, secure, inspired and empowered to recreate, relax, recover, or, on the other hand, be efficiently engaged in professional production activities. Therefore, landscape's multitude and ranges of qualities should provide sufficient interesting information and artistic pleasure.

Features to check are for example:

- Compliance to the natural environment, understood as the clear presence and cultivation (conservation) of the region's special natural features like water-bodies of all sorts;

- slopes, peaks, marshes, forests, river basins, dunes and cliffs;

- Good use of the landscape's potential utility, understood as:

• Rationality of the sustainable land-use and the way it looks like. The good used of the land should be visible;

• Percentage of sustainable areas in proportion to the whole landscape and those managed in unsustainable ways;

• Possibilities for activities other than food and fibre production, their feasible locations and their appropriate intensity of actual use;

Presence of naturalness:

• Presence of natural, non-productive sites/areas;

• Balance of natural elements, lines, patterns, materials, as compared to artificial ones;

- A rich and fair offer of regional specific sensory qualities, such as forms, colours, smells and sounds;

- Experiences of unity as for example in: completeness, wholeness and spaciousness;

- Experienced historicity, in elements of art and crafts and in historic landscapes patterns;

- Presence of cyclical developments, for example growth cycles and seasons.

Nearly half of urban Russian households grow food on their dacha plots. In her study, the American anthropologist Jane R. Zavisca (2003) investigates the meaning of this activity for both those who embrace it and those who reject it. Existing scholarship frames the post-Soviet dacha as a survival strategy and debates its efficiency. Ethnographic evidence reveals that the dacha provides not simply a source of food but a discursive arena for debating the rationality and morality of the transition to a market economy. Due to their rich history, dachas may be interpreted as sites of production or of consumption, as economic necessities or status signifiers. This ambiguity makes dachas particularly salient in disputes over the proper relationship between economic power and social esteem in the shifting stratification order.

This obviously is far from today's dacha's of several Russia's oligarchs and successful entrepreneurs, athletes, pop musicians and mafia bosses now choose dacha as their primary residence. Their estates, often surrounded by solid fences equipped with barbed wire, surveillance cameras, and/or motion detectors, and are sometimes even protected by heavily armed guards, are quite contrary to the culturally, socially and ecologically integrated land-use advocated here.

4.2. The (agro-) landscape's physiognomy and cultural geography

They are objective features showing the regional landscape identity and can be studied in:

- Diversity of landscape components:

• Diversity of landscape types per country;

• Diversity of landscape units (bio-topes) per landscape type;
• Diversity of elements (crops and planting) per landscape unit;
• Diversity of species per bio-tope.

- Coherence among landscape elements:
• Hydrology;
• Infrastructure;
• Farming;
• Ecology.

- Continuity of land-use and spatial arrangement:
• Cultural history;
• Duration and continuity of land use and spatial arrangement;
• Presumed future sustainability.

For Russia, Christopher Ely wrote the study 'This Meager Nature. Landscape and National Identity in Imperial Russia '(2002). Two poems cited therein:

1. *Fedor Tiutchev's poem of 1859, 'These Poor Villages':*

These poor villages,
This meager nature:
Long-suffering native land,
Land of the Russian people!
Proud foreign eyes
Will not notice nor grasp
The light that shines through
Your humble barrenness.
Worn by the weight of the cross,
The Heavenly King in the guise of a slave
Has passed through all of you,
Native land, blessing you.

2. *Nekrasov's, 1867, 'Who can be Happy ?*

You are wretched
You are abundant
You are downtrodden
You are all-powerful
Mother Russia

5. On agro landscape planning an management

From the paper as presented it will be clear that in the authors' view agro-landscape planning and management are tasks for interdisciplinary teams. The term interdisciplinary meaning here that the team members are aware of the way in which their expertise's strong and weak points can be beneficial *to* as well as compensated *by* those of the team members and their disciplines. Therein the awareness of the mutual dependence of holism and reductionism, as equally important steps in research, helps to see facts in context and define concrete steps in sustainable developments over long periods of time.

Therewith we presume to contribute some tools to Russia's agro-landscape planning debate.

5.1. Integration of the diversity

Now, after proposing such a long range of themes and parameters for (agro-) landscape research, it seems wise to propose as well a scheme on the interrelationships of the mentioned issues.

In the scheme below, referring to the scheme on links between People and Landscape organisms earlier in this paper, I elaborate on the Maslow triangle, placing to Scheme 4 the various scientific disciplines mentioned above in a consistent context (Fig. 4) (Maslow, 1943).

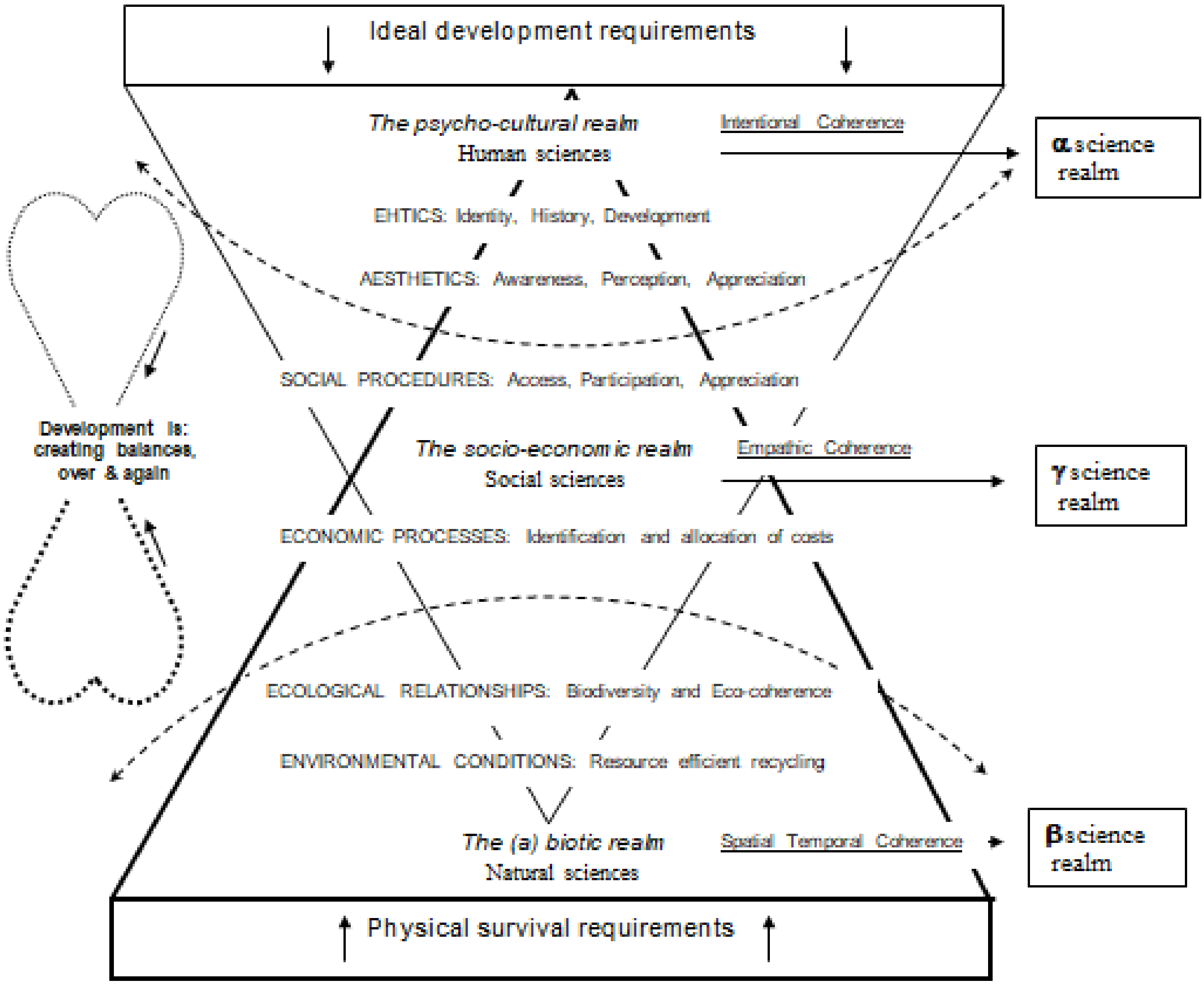

Fig. 4. Maslow's triangle adapted to the agro landscape management by the author

Human, social and natural sciences are mentioned again, but each in their own realm, and each realm now including its particular type of coherence: intentional (goal oriented), empathic (appreciation oriented) and spatial-temporal (ecologically oriented) successively.

It also shows how all three together they are instrumental to balance the fundamental opposition between ideal developments on the one hand and physical survival on the other, or in other words 'the world or agro landscape as it is' versus 'the world or agro landscape as it should be'.

Supportive ideas for MOVIR and the Vavilov Institute of Plant Industry

We all know that plant breeding is meant to serve agricultural crop production (forestry included), which again is to serve people's use of plants for human and animal nutrition, fibres and flowers, as well as pharmacy. We're also aware that, at the same time, agriculture is to warrant the agro-ecosystems autonomous production capacity, landscape quality and environmental health.

Thus I here list some demands from society on plant breeding for the future, which are to make sure there are lines available that suit for more then only quantitative food, feed and fibre production. But subsequently, I add a list of compliant demands from breeders on society, which must be fulfilled by society in order to empower plant breeders appropriately.

Demand on breeders

- regional adaptation
- food (etc.) quality

- roughage production for livestock
- deep rooting for soil upgrading
- economic use of water in dry areas
- optimal nitrogen fixation
- weed suppression
- pest tolerance
- salt tolerance
- drought tolerance

Demand from breeders:
- integration in the food (industry) chain
- integration in the regional society
- participation in farmers / agronomists education
- participation from farmers / agronomists education
- cooperation with plant and animal production research
- cooperation with human nutrition research
- cooperation with soil research

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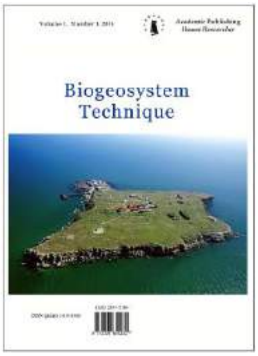
Appendix. Herman Daly's (1996) requirements for sustainable development:

- Reduce the physical demands on our global ecosystems. They are all essentially sensitive and limited. The earth is round!

- Distinguish between growth (quantity) and development (quality), starting with GNP. Whereas growth should be limited in face of the earth's limited resources and ecosystems (the earth is round), development is as unlimited as mankind's ever creative mind. This holds as well for sub national units like regions, micro projects and individual households. They can not, however, be left to the latter alone.

- Settle fair prices on depletion and pollution of the earth's limited resources as they are no free assets.
- Set socio-political limits to resource throughput on a sustainable scale.
- Total consumption (population x per capita consumption) should be stabilised to warrant sustainable development worldwide.
- Reduced levels of consumption are badly needed; reducing throughput (growth) is a tool to induce progress (development).
- Greater sharing, more population control (education!) and true development are badly needed.
- Costs of depletion and pollution to be internalised in the product's prices.
- Tax throughput instead of income & tax high incomes instead of lowest incomes.
- More growth for all without any sacrifice is a misleading illusion: definitely unsustainable!
- After scale and distribution have thus been settled: let the individualistic market rule allocation.
- The world's nations are key instruments to start implementing this policy.
- Irreducible uncertainty about new technologies' environmental effects are real costs, to be included in the price of the commodity that imposed the costs. To be paid as assurance bond and returned over time as experience reduces the uncertainty.
- Thus liability of industry should be strengthened and the general public not be burdened by industrial development efforts.
- The fair demand for free institutions regards market's freedom from monopoly as well as collective social freedom to democratically enact rules for the common good.
- GATT, WTO and Codex Alimentarius do not at all comply to this fair requirement. They are by and large tools for trans-national industry to increase their monopolising position.
- Free trade among regimes of different degrees of cost internalisation will result in a spiral of standards lowering competition worldwide. Trans-national corporations will take over standard setting from democratic governments; see GATT. WTO and Codex Alimentarius.
- Self sufficient countries are less likely to go to war then countries depending on other countries for their welfare.
- Advances in science and technology are beneficial, increasing both our understanding and range of choices about how humanity and the environment relate. This holds when technologies for new sustainable developments are meant. It does not hold when only unlimited up-scaling and growth of existing technology are concerned.



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Conceptual and Mathematical Statement of the Process of Heavy Metals Migration in the System «Soil – Agricultural Plant»

Tatyana V. Perevolotskaya [a, *], Vyacheslav S. Anisimov [a]

[a] Russian Institute of Radiology and Agroecology, Obninsk, Russian Federation

Abstract

The mathematical modeling was used to describe the functioning and organization of natural ecosystems as well as various approaches to the classification of environmental models to describe both the individual processes and their interactions on the base of the real system patterns.

Using the system analysis, the components of the system «soil – agricultural plant» are identified and analyzed separately interconnected by bilateral cause – effect relationships. It was shown that the origin of the studied system is artificial, while specific of the components is material. By the nature of interaction with the environment it is an open system, the sequence of events in which is not deterministic and is probabilistic. In developing the conceptual model of heavy metal migration in the system «soil – agricultural plant», a number of assumptions were made: the main mechanism of heavy metals input into crops is the root one; the process of chemical element transfer between the components of the system can occur in forward and reverse directions; in the processes of migration between the components of ecosystems, the transit of heavy metals can take place; the foliar flow of heavy metals is taken into account, mediated, in the transfer constants from the soil to the above-ground phytomass of crops; it is assumed that the investigated chemical element is isolated from the parts of plants into the soil.

The accepted limitation in the development of the conceptual model of the TM migration process in the system «soil – agricultural plant» is the formalization of the initial information to quantifiable indicators. Based on the results of the system analysis, adopted approaches and assumptions, a conceptual model of TM migration in the system of «soil – agricultural plants» is developed, with the allocation of the main blocks (compartment): arable soil layer (0-30 cm), aerial phytomass of plants, the root system of plants (root and tuber fields), the subsurface layer of the soil. Mathematical description of the dynamics of migration of chemical elements between the individual components of the studied system «soil – agricultural plant» is presented by a system of linear differential equations of 1st order. The process of migration between the compartments are determined by the constants of transfer of a chemical elements, based on literature data and average values of coefficients of accumulation of Zn, cu, Pb, Cd in the most common crops.

Keywords*:* heavy metals, chemical elements, accumulation coefficient, conceptual model, algorithm, factor.

1. Введение

Использование методов и подходов системного анализа с применением методов математического моделирования представляет собой концептуальный инструмент,

[*] Corresponding author
E-mail addresses: forest_rad@mail.ru (T.V. Perevolotskaya)

ориентированный на анализ и прогнозирование процессов функционирования и организации природных экосистем, что позволяет значительно расширить экспериментальные исследования (Ляпунов, Багриновская, 1975; Краснощеков, Петров, 1983; Пеннинг де Фриз, Ван Лаар (ред.), 1986; Карпачевский (ред.), 1987; Марчук, 1982; Кремер, Морозов, 1988; Dube et al., 2001; Сурмин, 2003; Новосельцев (ред.), 2006; Guala et al. 2010; Guala et al., 2013; Franklin et al., 2014; Campbell, Paustian, 2015; Lurgi et al., 2015; Endovitsky et al., 2015; Endovitsky, Kalinichenko, Minkina, 2015; Batukaev et al., 2016; Field et al., 2016; Naskova, 2017; Antle et al., 2017; Chuine, Régniére, 2017; Donatelli et al., 2017; Bourhis et al., 2017; Janssen et al., 2017; Okamato et al., 2018; Amuti et al., 2018). В зависимости от формы реализации математические модели можно разделить на имитационные и аналитические (Сельскохозяйственная радиоэкология, 1992; Новожилов, Семенова, Петрова, 1999; Семенова и др., 2003; Boulangeat et al., 2014; Jones et al., 2017; Sándor et al., 2017; Ehrhardt et al., 2017; Cammarano et al., 2017; Donatelli et al., 2017; Van Oijen et al., 2018). Под имитационными моделями обычно понимают «формализованное описание с помощью современных компьютерных технологий изучаемого явления во всей его полноте на грани нашего понимания» (Нейлор, 1975, Шеннон, 1978, Джефферс, 1981). Значение аналитических моделей определяется тем, что они дают достаточно простые формулы для приближенных практических расчетов и часто служат методической основой для создания больших имитационных моделей. В зависимости от степени определенности получаемых результатов как имитационные, так и аналитические модели делят на детерминированные и стохастические (Сельскохозяйственная радиоэкология, 1992; Bai1 et al., 2007; Porporato et al., 2015). При этом, если в детерминированных моделях значения переменных определяются точно, то стохастические модели оперируют с распределениями параметров. В этом случае они рассматриваются как случайные величины и характеризуются вероятностными показателями, такими, как математические ожидания, дисперсия и т.п. В зависимости от того, описывают ли модели временную динамику процессов или нет, их можно классифицировать на статические и динамические (Фрид, 1987; Рыжова, 1987; Иванова, 1989; Полуэктов, 1991, Прохорова, Фрид, 1993; Хомяков, 1994; Полуэктов и др., 2002; Кошелева, 2009; Полуэктов и др., 2012; Snell et al., 2014; Yu et al., 2014; Guillem et al., 2015; Баденко и др., 2015; Medvedev et. al., 2015; Harding, Twine, 2015: Sun et al., 2016; Tan et al., 2016; Ozturk et al., 2017; Jones et al., 2017; Winter et al., 2017; Renzi et al., 2018).

Широкий диапазон моделируемых процессов (от глобальной экологии до отдельных компонентов агроэкосистем) обусловил различные подходы классификации экологических моделей (Джефферс, 1981; Бондаренко, Жуковский, Мушкин, 1982; Дмитриев, 1995; Франс, Торнли, 1987; Робертс, 1986; Poluektov, Topaj, 2001; Tipping et ai., 2012; Duru et al., 2015; Bellocchi et al., 2015; Campbell, Paustian, 2015; C Rowe et al., 2015; Antle et al., 2017; Holzkämper, 2017). Наиболее часто, для описания сложных процессов, происходящих в агроэкосистемах, применяют статистические модели, балансовые, вероятностные модели (Рыжова, 1987; Бровкин, 1988; Прохорова, 1993; Кошелева, 2002, 2004; Keller et al., 2001; Москвин, 2011; Hou et al., 2014; Porporato et al., 2015; Demková et al., 2017; Lázaro et al., 2017; Hu et al., 2018). Построение статистических моделей агроэкосистем основывается на допущении о случайности исследуемого процесса и может быть изучено с помощью методов математической статистики, что отражено в целом ряде работ (Иванова, 1989; Рыжова, 1987; Образцов, 1990; Литвак, 1990; Уланова, Забелин, 1990; Laflen, Jane, 1991; Прохорова, Фрид, 1993; Porporato et al., 2015; Demková et al., 2017; Lázaro et al., 2017; Hu et al., 2018). Моделированию потоков вещества и энергии в относительно однородных средах посвящены работы (Дмитриев, 1991; Икконен, Толстогузов, 1996; Фельдман, 1999; Tipping et al., 2012 и др.), балансовые модели на основе дифференциальных и интегро-дифференциальных уравнений, описывающих динамику развития систем как совокупность процессов переноса вещества и энергии, применены в работах Пачепский, 1992; Богатырев, Рыжова, 1994; Кошелева, 2009; Bergez et al., 2013. Оптимизационные модельные разработки ведения сельского хозяйства в регламентированных условиях нашли отражение в работах (Сельскохозяйственная радиоэкология, 1992; Фесенко, Яцало, Спиридонов, 1994; Porporato et al., 2015; Amuti et al., 2018 и др.).

Вне зависимости от типа моделей математическое моделирование представляет собой сложный процесс и включает в себя постановку задачи исследования, создание концептуальной модели, формальное описание объекта моделирования, алгоритмическое описание модели, верификацию, оценку адекватности модели.

Основу для создания концептуальной модели миграции тяжелых металлов (ТМ) в системе «почва – сельскохозяйственное растение», определяющей переход от реальной системы к логической схеме ее функционирования, посредством логико-математического описания объекта составляет цель исследования с учетом всех допущений, необходимых для построения данной модели.

2. Объекты и методы

Методическую основу концептуальной и математической постановки процесса миграции тяжелых металлов в системе «почва – сельскохозяйственное растение» составляют методы теории систем и системного анализа, системного подхода, аналитически-численный.

Методика проведения исследования включает в себя сбор, обработку и обобщение информации о процессе миграции ТМ в системе «почва – сельскохозяйственное растение». В качестве информационной базы исследования были применены накопленные и систематизированные литературные данные (Свидетельство о государственной регистрации…, 2016), монографии и публикации отечественных и зарубежных ученых, отражающие результаты исследований согласно целям и задачам настоящего исследования.

Принятые гипотезы, ограничения и допущения концептуальной и математической постановки процесса миграции тяжелых металлов в системе «почва – сельскохозяйственное растение»:

Исходные гипотезы:

– объектом для разработки концептуальной схемы модели является процесс миграции ТМ в системе «почва – сельскохозяйственное растение»;

– процесс миграции ТМ в системе «почва – сельскохозяйственное растение» может быть описан системой дифференциальных уравнений I порядка.

Принято ограничение:

– разработанная концептуальная схема модели процесса миграции ТМ в системе «почва – сельскохозяйственное растение» должна позволять формализовать исходную информацию до количественно измеряемых показателей.

Приняты допущения:

– ТМ Cu, Zn, Pb, Cd относятся к числу приоритетных как для фонового мониторинга окружающей среды, так и экологических оценок территории;

– рассмотрены коэффициенты накопления (КН) Cu, Zn, Pb, Cd в наиболее распространенных сельскохозяйственных культурах (пшеница (зерно), ячмень (зерно), соя (зерно), капуста, свекла (корнеплоды), салат, картофель (клубнеплоды), морковь (корнеплоды));

– основной механизм поступления ТМ в сельскохозяйственные культуры – корневой;

– в процессе взаимодействия компонентов системы друг с другом происходит перенос материи и энергии, как в прямом, так и в обратном направлении (в частности, учитывается вынос ТМ с продуктами ассимиляции в почву с корневыми выделениями);

– между компонентами системы существует обмен химическими элементами различной интенсивности, что определяется физико-химическими свойствами и биологической ролью последних;

– допускается транзитный перенос ТМ между компонентами системы (например, из почвы в ассимилирующие или генеративные органы без учета содержания ТМ в проводящей (стебли) системе);

– внекорневое поступление ТМ учтено, опосредовано, в константах переноса от почвы к надземной фитомассе сельскохозяйственных культур;

– предполагалось выделение исследуемого химического элемента из надземной фитомассы в почву (за счет потерь с надземной фитомассой в виде опада, с корневыми выделениями и другими процессами);

– в течение вегетационного периода происходит постепенное снижение корневого поступления химического элемента за счет миграции последнего за пределы корнеобитаемого слоя;

– в начальный момент времени (первые сутки вегетационного периода, t=0) начинается поступление исследуемого химического элемента из почвы в сельскохозяйственное растение. Предполагалось, что во всех компартментах экосистемы, на данный момент времени, содержание элемента равно 0;

– дополнительные агротехнические и агромелиоративные мероприятия на кормовых угодьях не проводятся;

– содержание ТМ во всех компартментах системы принято в мг на 1 м² поверхности почвы для слоя почвы 0–30 см;

– при расчете концентрации ТМ в имеющих хозяйственную ценность частях сельскохозяйственных растений их фитомасса принята максимальной, что соответствует периоду сбора урожая (зерно 0,3 кг/м² (30 ц/га), картофель 2,50 кг/м² (250 ц/га), свекла 5,0 кг/м² (500 ц/га), капуста 7,5 кг/м² (700 ц/га), салат 1,8 кг/м² (200 ц/га), морковь 6 кг/м² (700 ц/га), соя (зерно) 1 кг/м² (18,3 ц/га) (Романов, 1993).

3. Результаты и их обсуждение

Концептуальная постановка задачи – один из важнейших шагов операционного исследования, результатом выполнения которого является концептуальная модель исследуемого объекта с содержательным описанием состава системы, ее компонентов и взаимосвязей между ними на основе принятых подходов и допущений.

На предварительном этапе применение методов системного анализа позволило выделить отдельные, взаимосвязанные двусторонними причинно-следственными связями, компоненты системы «почва – сельскохозяйственное растение», существенные по отношению к цели исследования: пахотный слой почвы (0–30 см), подпахотный слой почвы, надземная продуктивная (1) и вегетативная (2) фитомасса сельскохозяйственных растений, корневая система (корне- и клубнеплоды) растений (рис. 1).

По происхождению анализируемая система искусственная, по специфике составляющих компонентов – материальная. По характеру взаимодействия со средой представляет собой открытую систему, последовательность событий в которой, не детерминирована и носит вероятностный характер.

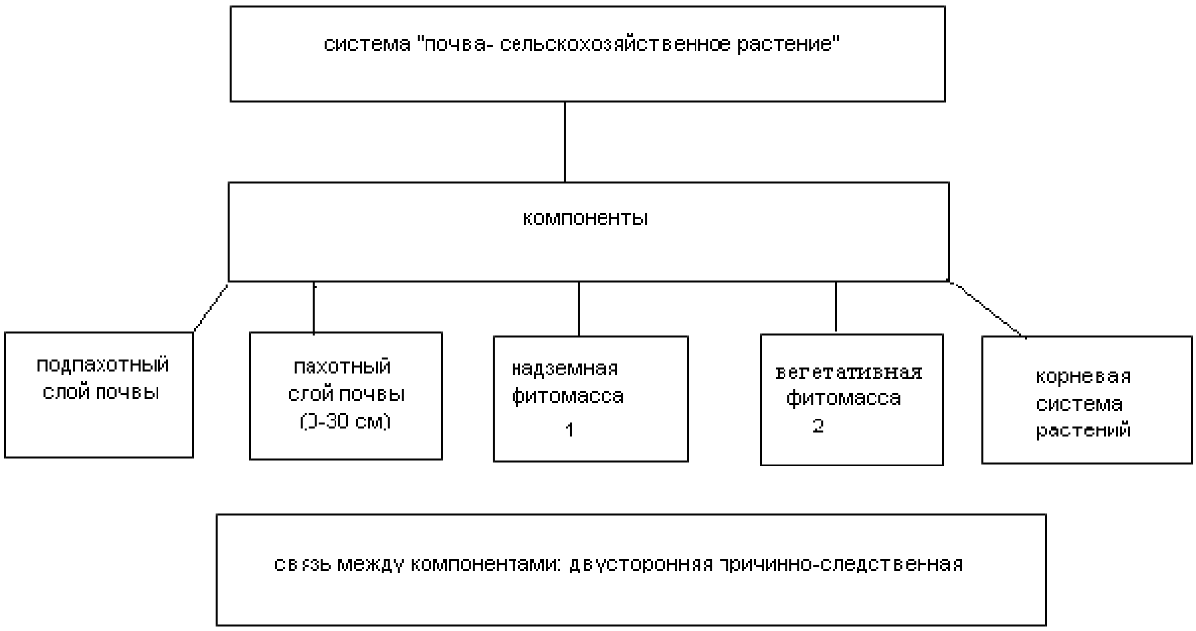


Рис. 1. Структура системы «почва – сельскохозяйственное растение»

Результаты системного анализа объекта исследований были приняты в качестве основы концептуальной модели миграции ТМ в системе «почва –сельскохозяйственное растение». Разработанная модель включает в себя 5 основных блоков: A_1 – пахотный слой почвы (0–30 см), A_2 – надземная фитомасса растений (1 – продуктивная), A_3 – надземная фитомасса растений (2 – вегетативная), A_4 – корневая система растений (корне- и клубнеплоды), A_5 – подпахотный слой почвы (рис. 2).

При этом следует отметить, что концептуальная модель миграции ТМ в системе «почва – сельскохозяйственное растение», основу которой составляет систематизация информационных данных исследуемого процесса миграции, не отражает, происходящие в нем, серьезные изменения. Для того, чтобы решить данную задачу, необходима формализация концептуальной (содержательной) модели с учетом принятых гипотез и установленных ограничений на основе выбора адекватного математического отображения.

Для исследования процессов миграции элементов в системе «почва – сельскохозяйственное растение» наиболее широко распространен компартментный подход, в основу которого положен метод системного анализа (Сельскохозяйственная радиоэкология, 1992). Моделируемая экосистема разбивается на блоки, которые называются компартментами, а сама модель – компартментальной. Сложность данных моделей связана главным образом с неоднозначностью выделения компартментов, преимущество заключается в их относительной точности и простоте, описании процессов переноса веществ в потоковой форме в виде констант переноса k_{ij}.

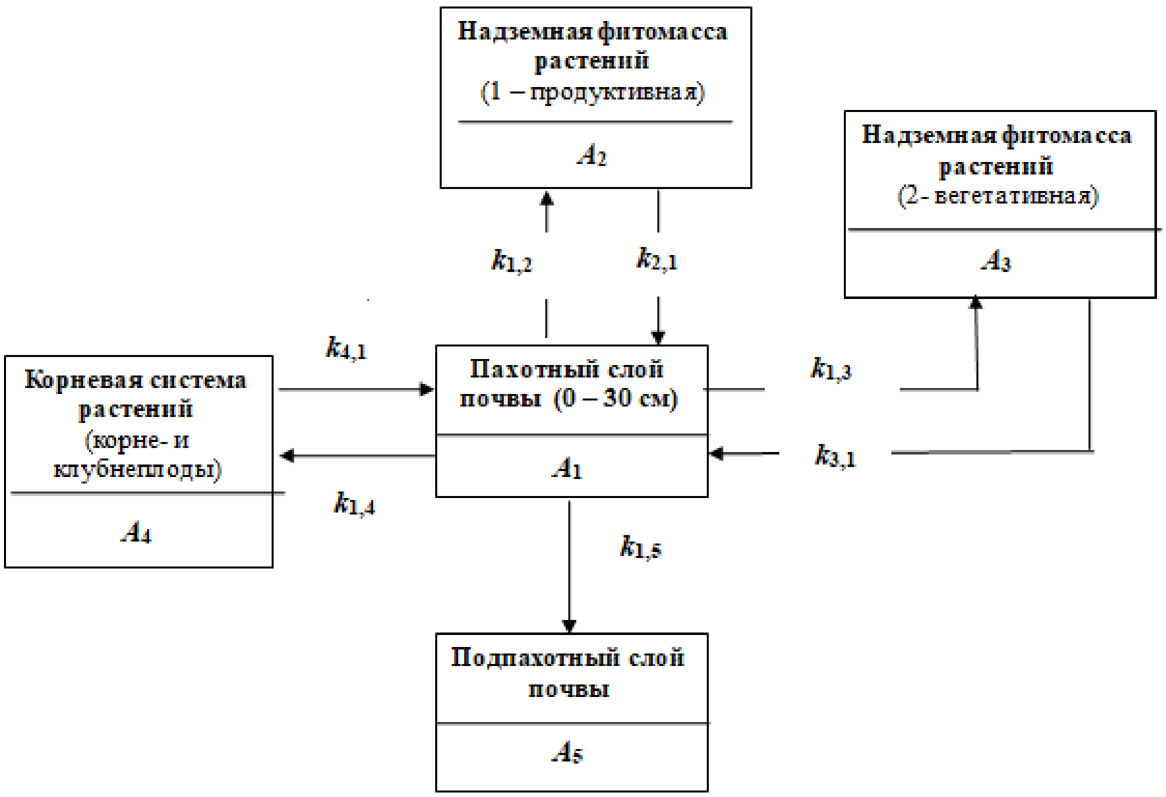


Рис. 2. Концептуальная модель миграции ТМ в системе «почва – сельскохозяйственное растение»

Константы k_{ij} (1/с) связаны с переносом химического элемента между компартментами системы вследствие следующих процессов: $k_{1,2}$ и $k_{1,3}$ – поступления за счет корневого усвоения (транзитный перенос); $k_{2,1}$ и $k_{3,1}$ – транзитного переноса из надземной фитомассы растений с продуктами ассимиляции в почву, $k_{1,4}$ – поступления в корневую систему растений, $k_{4,1}$ – формальная константа переноса, обеспечивающая пропорциональность содержания элемента в компартнентах 1 и 4, $k_{1,5}$ – сток за пределы корнеобитаемого слоя почвы (рис. 2).

В общем виде динамика обмена химических элементов между отдельными компартментами исследуемой системы «почва – сельскохозяйственное растение» (рис. 2) может быть описана системой дифференциальных уравнений I порядка:

$$\frac{dA_i}{dt} = A_i^0 + \sum_{i=1}^{n} k_{ij} \cdot A_n - \sum_{j:=1}^{m} k_{ji} \cdot A_m \ , \qquad (1)$$

где A_i, A_m, A_n – содержание химического элемента в компартментах системы, мг на 1 м² поверхности почвы; A_i^0 – поступление химического элемента извне, мг/с; k_{ij} – константы переноса химического элемента из компартменты i в компартменту j, характеризующие перенос химического элемента в соответствующие компартменты из почвы 1/с; k_{ji} – константы переноса химического элемента из компартменты j в компартменту i, характеризующие перенос химического вещества в почву из соответствующих компартмент, 1/с.

Система дифференциальных уравнений I порядка, описывающая миграцию исследуемого химического элемента между отдельными компартментами системы (рис. 2), имеет следующий вид:

$$\begin{cases} \frac{dA_1}{dt} = k_{21} \cdot A_2 + k_{31} \cdot A_3 + k_{41} \cdot A_4 - (k_{12} + k_{13} + k_{14} + k_{15}) \cdot A_1 \\ \frac{dA_2}{dt} = k_{12} \cdot A_1 - k_{21} \cdot A_1 \\ \frac{dA_3}{dt} = k_{13} \cdot A_1 - k_{31} \cdot A_3 \\ \frac{dA_4}{dt} = k_{14} \cdot A_1 - k_{41} \cdot A_4 \\ \frac{dA_5}{dt} = k_{15} \cdot A_1 \end{cases} \qquad (2)$$

Первое уравнение определяет динамику содержания химического элемента в пахотном слое почвы (0–30 см), а пятое уравнение – в подпахотном слое почвы. Второе и третье уравнение характеризуют интенсивность изменения содержания элемента в надземной продуктивной (1) и вегетативной (2) фитомассе растений, соответственно. Четвертое уравнение описывает изменение содержания элемента в корневой системе (корне- и клубнеплоды) растений. Положительные члены уравнения определяют собой интенсивность входящего потока элемента в компартменту системы, отрицательные – исходящий поток из данной компартменты.

Для решения системы дифференциальных уравнений использовался метод конечных разностей:

$$\frac{dA_1}{dt} = \lim_{\Delta t \to 0} \frac{\Delta A_1}{\Delta t} \approx \frac{A_{1,t+\Delta t} - A_{1,t}}{\Delta t}\Big|_{при \Delta t=1} = A_{1,t+1} - A_{1,t} \qquad (3)$$

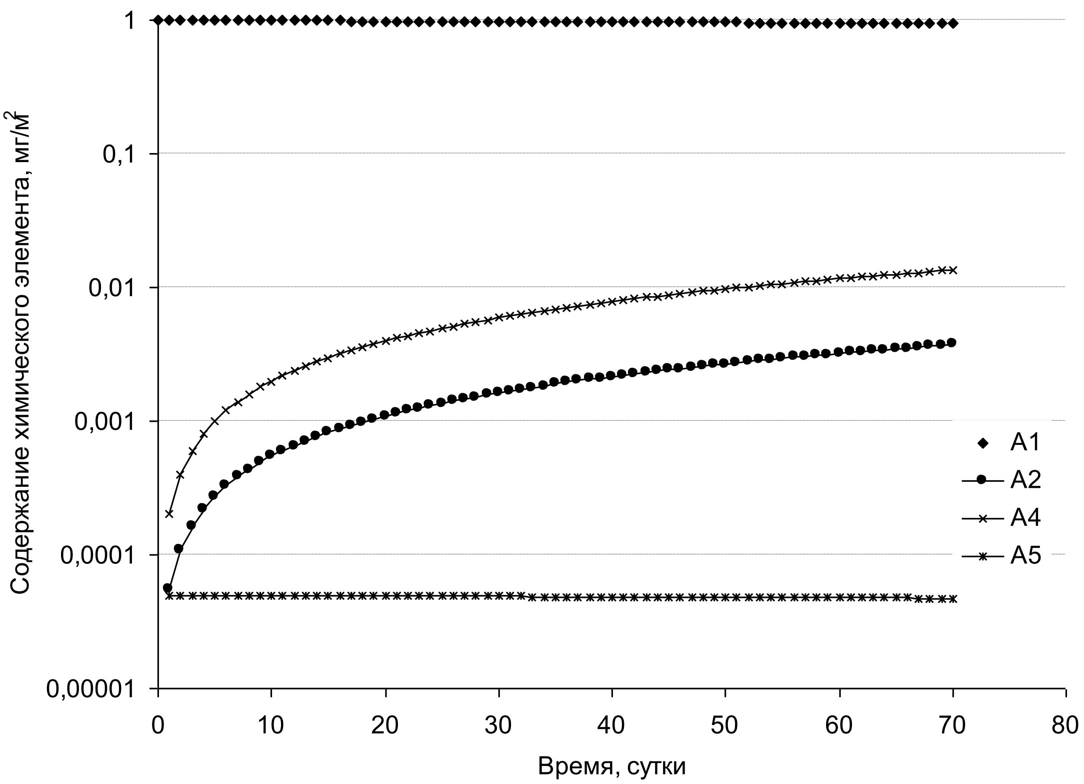
После подстановки конечно-разностного аналога (3) в первое уравнение (2) и соответствующих преобразований система конечно-разностных уравнений имеет вид:

$$\begin{cases} A_{1,t+1} = k_{21} \cdot A_{2,t} + k_{31} \cdot A_{3,t} + k_{41} \cdot A_{4,t} + A_{1,t} \cdot (1 - k_{12} - k_{13} - k_{14} - k_{15}) \\ A_{2,t+1} = k_{12} \cdot A_{1,t} + (1 - k_{21}) \cdot A_{2,t} \\ A_{3,t+1} = k_{13} \cdot A_{1,t} + (1 - k_{31}) \cdot A_{3,t} \\ A_{4,t+1} = k_{14} \cdot A_{1,t} + (1 - k_{41}) \cdot A_{4,t} \\ A_{5,t+1} = k_{15} \cdot A_{1,t} \end{cases} \quad (4)$$

Для расчета параметров модели миграции ТМ в системе «почва – сельскохозяйственные растения» применены литературные данные о константах переноса химических элементов (Гусев, Беляев, 1991) и средние значения величин коэффициентов накопления Zn, Cu, Pb, Cd в наиболее распространенных сельскохозяйственных культурах (Свидетельство о государственной регистрации..., 2016). Уточнение констант переноса для каждого вида сельскохозяйственной культуры и типа почвы могут быть выполнены путем решения системы линейных уравнений при известных величинах содержания ТМ в соответствующих компартментах системы.

Расчет концентрации ТМ в хозяйственно ценных частях растений выполняется путем деления содержания химического элемента в соответствующей компартменте (мг/м²) на величину ее массы (кг/м²), соотнесенной к единице площади поверхности почвы.

Пример результатов расчета содержания Zn в моделируемой системе «почва – сельскохозяйственное растение» для капусты, произрастающей на дерново-подзолистой почве (П^Д^), приведен на рис. 3.



A_1 – пахотный слой почвы, A_2 – надземная (продуктивная) фитомасса растений,
A_4 – корневая система растений, A_5 – подпахотный слой почвы

Рис. 3. Содержание Zn в компартментах исследуемой системы на примере капусты, произрастающей на дерново-подзолистой почве, 1/сут

4. Заключение

Для описания процессов, происходящих, в частности, в агроэкосистемах, применяют статистические модели, балансовые, вероятностные модели. Выполнен обзор проблем, возникающих при математическом моделировании процессов функционирования и организации природных экосистем, отражены различные подходы к классификации экологических моделей. Показано, что математическое моделирование, основным инструментом которого выступает системный анализ, позволяет адекватно описывать как отдельные процессы системы, так и взаимодействие процессов на основе установленных закономерностей поведения реальной системы. Это определяет перспективу использования рассмотренных моделей для изучения и предсказания сложных агроэкосистем.

Применение методов системного анализа позволило провести анализ и выделить отдельные взаимосвязанные двусторонними причинно-следственными связями компоненты системы «почва – сельскохозяйственные растения», существенные по отношению к цели исследований. Показано, что по происхождению анализируемая система искусственная, по специфике составляющих компонентов – материальная, по характеру взаимодействия со средой представляет собой открытую систему, последовательность событий в которой не детерминирована и носит вероятностный характер.

На основе результатов системного анализа, принятых подходов и допущений разработана концептуальная модель миграции ТМ в системе «почва – сельскохозяйственные растения», с выделением основных блоков (компартмент): пахотный слой почвы (0–30 см), надземная фитомасса растений, корневая система растений (корне- и клубнеплоды), подпахотный слой почвы. Математическое описание динамики миграции химических элементов между отдельными компартментами исследуемой системы «почва – сельскохозяйственное растение» представлено системой линейных дифференциальных уравнений I порядка. Процессы миграции между компартментами определяются константами переноса химического элемента, основанными на литературных данных и средних значениях величин коэффициентов накопления Zn, Cu, Pb, Cd в наиболее распространенных сельскохозяйственных культурах.

Представленная модель миграции ТМ в системе «почва – сельскохозяйственное растение» позволяет оценить содержание тяжелых металлов в имеющих хозяйственную ценность частях сельскохозяйственных растений с учетом почвенно-экологических условий. Она может быть использована в системах поддержки принятия решений при возделывании сельскохозяйственных культур на почвах, загрязненных ТМ.

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Концептуальная и математическая постановка процесса миграции тяжелых металлов в системе «почва – сельскохозяйственное растение»

Татьяна Витальевна Переволоцкая [a, *], Вячеслав Сергеевич Анисимов [a]

[a] Всероссийский научно-исследовательский институт радиологии
и агроэкологии, Российская Федерация

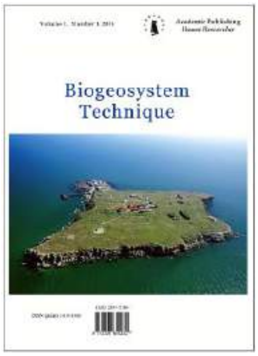
Аннотация. Выполнен обзор проблем, возникающих при математическом моделировании процессов функционирования и организации природных экосистем, отражены различные подходы к классификации экологических моделей. Показано, что математическое моделирование, основным инструментом которого выступает системный анализ, позволяет адекватно описывать как отдельные процессы системы, так и взаимодействие процессов на основе установленных закономерностей поведения реальной системы.

С применением системного анализа выделены и проанализированы отдельные взаимосвязанные двусторонними причинно-следственными связями компоненты системы «почва – сельскохозяйственное растение». Показано, что по происхождению исследуемая система искусственная, по специфике составляющих компонентов – материальная. По характеру взаимодействия со средой представляет собой открытую систему, последовательность событий в которой, не детерминирована и носит вероятностный

[*] Корреспондирующий автор
Адрес электронной почты: forest_rad@mail.ru (Т.В. Переволоцкая)

характер. При разработке концептуальной модели миграции тяжелых металлов в системе «почва – сельскохозяйственное растение» принят ряд допущений: основным механизмом поступления тяжелых металлов в сельскохозяйственные культуры является корневой; процесс переноса химического элемента между компонентами системы может происходить в прямом и в обратном направлениях; в процессах миграции между компонентами экосистем может происходить транзитный перенос тяжелых металлов; внекорневое поступление тяжелых металлов учтено, опосредовано, в константах переноса от почвы к надземной фитомассе сельскохозяйственных культур; предполагается выделение исследуемого химического элемента из частей растений в почву. Принятым ограничением при разработке концептуальной модели процесса миграции ТМ в системе «почва – сельскохозяйственное растение» является формализация исходной информации до количественно измеряемых показателей. На основе результатов системного анализа, принятых подходов и допущений разработана концептуальная модель миграции ТМ в системе «почва – сельскохозяйственные растения», с выделением основных блоков (компартмент): пахотный слой почвы (0–30 см), надземная фитомасса растений, корневая система растений (корне- и клубнеплоды), подпахотный слой почвы. Математическое описание динамики миграции химических элементов между отдельными компартментами исследуемой системы «почва – сельскохозяйственное растение» представлено системой линейных дифференциальных уравнений I порядка. Процессы миграции между компартментами определяются константами переноса химического элемента, основанными на литературных данных и средних значениях величин коэффициентов накопления Zn, Cu, Pb, Cd в наиболее распространенных сельскохозяйственных культурах

Ключевые слова: тяжелые металлы, элементы, коэффициент накопления, концептуальная модель, алгоритм, фактор.



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Soils Pollution with Oil Products (Ulaanbaatar, Mongolia)

Gantumur Sambuu [a, *], Haliun Gantumur [a, b], Galina V. Kharitonova [c]

[a] Mongolian State University of Science and Technology, Mongolia
[b] China University of Petroleum, People's Republic of China
[c] Institute of Water and Ecological Problems, Far East Branch of the Russian Academy of Sciences, Khabarovsk, Russian Federation

Abstract

As for most megacities, the problem of soil pollution with oil products is acute for the capital of Mongolia. In Ulaanbaatar, it is provided (aggravated) with dust pollution from the burning of brown coal, containing heavy metals and As, used in fuel and industrial complex and yurt part of the city. In this paper soil contamination of Mongolian capital Ulaanbaatar with oil products was investigated. In addition, the state of soils in the zone of influence of gas stations was studied to reveal the patterns of accumulation of oil products and heavy metals (and arsenic), which fall into the soil due to atmospheric dust. Such surveys for Ulaanbaatar have not been conducted previously. Based on soil-geochemical survey, a map of soil contamination of the city with oil products was constructed. According to the obtained data, the content of oil products in the presence of local anomalies with medium and heavy soil contamination of Ulaanbaatar are classified as slightly contaminated. Elevated (above the maximum allowable concentration of 100 mg/kg) content of oil products in soils was found along the city's main highway, industrial zone, CHPP and yurt zone, high value were found in oil tank farms and filling stations (up to 1000 mg/kg soil and more). The visualization of the obtained data (the content of oil, heavy metals and As in soils) by GIS methods showed cumulative effect of As accumulation with petroleum products most likely due to atmospheric dust from the burning of brown coal.

Keywords: soil, pollution, oil products, coal.

1. Introduction

At present, due to the rapid growth of the urban population and the formation of large agglomerations, the assessment of pollution of urban soils acquires a special relevance (Davies, Wixson, 1987; Li et al., 2001; Lu et al., 2003; Lado et al., 2008; Andersson et al., 2010; Chen et al., 2010; Dao et al., 2010; Wei, Yang, 2010; Kasimov et al., 2011; Xia et al., 2011; Tóth et al., 2016). The growth of the urban population occurs around the world and Mongolia is no exception. Almost half of the country's population lives in the capital of Mongolia, Ulaanbaatar. Over the past 25 years, the population of Ulaanbaatar has increased from 600 thousand to 1.4 million people. In Mongolia, migration processes have a historical tradition and are closely related to economic management and living conditions. Securing on the legislative level the rights of citizens to free movement and choice of residence led to uncontrolled settlement of the capital by nomads, chaotic placement of yurts and cottages in the territory of Ulaanbaatar. The active construction of yurts

* Corresponding author
E–mail addresses: gntm2000@mail.ru (Gantumur Sambuu)

began after 2003, when each family was allowed to occupy 0.07 hectares of land. This led to a negative impact on urban areas, and primarily on soils and atmospheric air. The process of migration is intensifying, and its consequences acquire a social character. The influx of people and the growth of the capital's territory causes an exacerbation of environmental problems related not only to large–scale construction, development of the industrial sector and the yurt sector of the city, but also to the fleet and network of filling stations (Cogbadrahyn, 2003; Bajarsajhan , 2009a, 2009b; Sambuu, 2016).

The most common contaminants in urban soils are oil products and heavy metals (and As). According to recent studies, the content of heavy metals and As in the presence of local anomalies with moderate and severe soil contamination, Ulaanbaatar are classified as slightly contaminated (Bathishig, 1999; Dorzhgotov, Bathishig, 2008; Batjargal et al., 2010; Kosheleva et al., 2010; Kasimov et al., 2011). Regarding the regional background, urban soils are enriched with Pb, Zn, Cu, As, Cr and Mo. Excess of MPC is revealed for As, Pb and Zn. The strongest tendency to the accumulation of pollutants is manifested in the central part of the city, which includes blocks of multi-story buildings, industrial zone and the largest thoroughfares.

In the urban environment, pollution of soils with oil and oil products is the most dangerous, as it leads to prolonged multicomponent pollution with toxic substances (Galitskaya, Pozdnyakova, 2011; Zektser, 2001; Banwart, Thornton, 2010; Bauer et al., 2009; Vela et al., 2012). The composition of oil and oil products includes a large number of various toxic substances, differing in both the degree of solubility and the degree of resistance to biodegradation. The hydrophobic component of oil products remains in soils for a long time, in the aeration zone, and in aquifers, is gradually microbiologically transformed and is a source of secondary pollution of the components of the natural environment. At the same time, water–soluble constituents of oil products migrate at different rates in the soil system – groundwater (Luckner, Schestakow, 1991; Widdel et al., 2010; Stempvoort Van, Biggar, 2008). It should be noted that for Ulaanbaatar studies of soil contamination with oil products are few, and the level of study of polycomponent pollution of soils (oil products and heavy metals) remains extremely weak.

The purpose of this work is to assess the pollution of the city's soil with oil products, its degree and spatial structure. The work is a continuation of geoecological studies of the authors of the Ulaanbaatar soils (Sambuu, 2016). A feature of this work is the visualization of polycomponent pollution of the most representative sites using GIS technologies.

Environmental conditions

The area of Ulaanbaatar at present is 4704.4 km^2, the population according to data for 2017 is 1405 thousand people. The city includes areas with urban buildings (multi-story apartment houses, buildings of various institutions and organizations, industrial, transport enterprises, etc.) and areas of private yurt housing, most of which were formed on the outskirts of the city after 2003 (Boldyn, 2004; Bajarsajhan , 2009a, 2009b).

The city is located in an intermountain basin, drained by the Tuul River, stretching from west to east for 30–35 km and from north to south for 6–10 km. The slopes of the surface in the bottoms of the intermontane depressions make the first degrees, towards the mountain framing they increase to 20–25°, sometimes steeper slopes are noted (Zaitsev et al., 1982). The valley of the Tuul River has a width of 5–10 km with absolute elevations of 1230–1240 m. Within the city the river accepts a number of small tributaries (Salbe, Uliastai, Gachuurt, etc.).

The Urginian Basin refers to the Khangai soil-bioclimatic provincia, the Prehentei district with chestnut and dark chestnut soils under eluvial and trans-eluvial conditions and alluvial stony-pebble soils in the accumulative landscapes of the Tuul River valley and its tributaries (Nogina, 1984). The main soil-forming rocks in the city are quaternary deposits of light granulometric composition with a low content of most trace elements. In intermontane depressions they are represented by alluvial-proluvial deposits of loamy and sandy loam composition with inclusions of crushed stone, gravel and pebbles. In some places there are outcrops of Paleogene–Neogene sediments enriched with Fe, Mn and Co. The indigenous rocks are represented by Archean granites, coal metamorphic clay shales and Neogene variegated clays. Slates and clays are enriched in Fe, Mn, Cr, Co, Pb, Ni, Ti, while granites, sandy sediments and river alluvium are impoverished by them (Bathishig, 1999).

The region is characterized by a sharply continental climate with large annual and daily fluctuations in air temperature. Winter is long and severe, summer is cool. Precipitation is low all in the summer, in winter there is almost no precipitation. The high altitude also greatly reduces the temperature in the city, the average annual temperature is –0.4 ° C. Among all the capitals of states, Ulaanbaatar has the lowest average annual temperature and the coldest winters (colder than in Moscow, Astana and Helsinki). The average annual rainfall in Ulaanbaatar is 240–260 mm, of which 60–90 % falls on July and August, and they have a stormy character (Beresneva, 2006). In winter, due to the prevalence of a windless anticyclone weather regime, stable temperature inversions are formed in the city.

Soil formation in the region under study proceeds in extracontinental conditions characterized by a slowed down rate of chemical weathering, biological cycling, short–term periods of biologically active temperatures and the activity of living matter, the duration of the cold period with strong supercooling of the upper horizons of the soil and their deep freezing (Nogina, 1989). Soils of sandy loamy and light loamy granulometric composition with high water permeability, low humus content (0.5–1.5 %), medium to neutral reaction (pH 7.5–9.0) and $CaCO_3$ content from 0.7 to 5.0 % predominate. On the slopes of the Bogdo-Ula Mountains, soils (mountain sod–taiga and mountain meadow–forest soils) are formed with humus content higher than 7.0 %.

Within Ulaanbaatar, soils are subject to a strong anthropogenic transformation, the extent of which is decreasing in the direction from the central part of the city to the areas of yurt housing. In the central part of the city, in connection with the construction of modern multi–stored buildings, the paving of asphalt roads, the soil cover undergoes a radical transformation – from mixing and "sealing" until its complete destruction and formation of man–made surface formations. In the areas of yurt housing there are no profound changes in the soil cover. The negative impact of urbanization in these areas is manifested in the elimination of natural vegetation and the reduction of the sludge of soils, which accelerate the processes of water and wind erosion and increase the dustiness of the atmosphere.

Sources of pollution

Ulaanbaatar is a megapolis with a diversified industry (machinery construction, metal-working, woodworking, homebuilding, food and other industries). The main part of industrial enterprises and all the CHPPs are located in the western part of the city. The districts of the yurt housing surround the central districts of the city, rising to the south, south-west and south-east spurs of the Baga-Hentay Range. The predominance of winds of western and north-western bearings creates increased pollution in the eastern and south-eastern part of the city.

The fuel and energy complex is one of the main sources of pollution of the environment of Ulaanbaatar. It includes three operating CHP plants, inter-district boiler houses and boiler houses of industrial enterprises. The main type of fuel is the brown coals of the deposits of Baga-Nura, Nalaiha and Chulut, which are enriched with Pb, As and Mo by a factor of ten, compared with clark values. Elevated values are also noted for Cu, Sr, Cd, and Ni (Kosheleva et al., 2010). The maximum emissions fall on the heating season, which lasts in Ulaanbaatar from October to May (Gunin et al., 2003).

Half of the volume of pollutants entering the city's atmosphere is emissions from the combustion of brown coal in the yurt part of the city. In Ulaanbaatar there are about 134 thousand yurts and they occupy more than half of the territory of the capital (Fig. 1A). In the yurts there are more than 600 thousand inhabitants. For heating and household needs in the yurt zone 443 thousand tons of coal are used per year (Report, 2010). With the heating of yurts is associated with the emergence of smog, which affects half the city of Ulaanbaatar. In winter, the windless anticyclone weather regime and stable temperature inversions create an ecologically unfavourable environment in the city, associated with an increased content of many toxic substances in the air, which are gradually deposited in the snow cover and in the upper horizons of the soils. Due to winter smog, Ulaanbaatar has turned into one of the most environmentally unfriendly cities in the world (Arguchintsev et al., 2009; Sambuu, 2016).

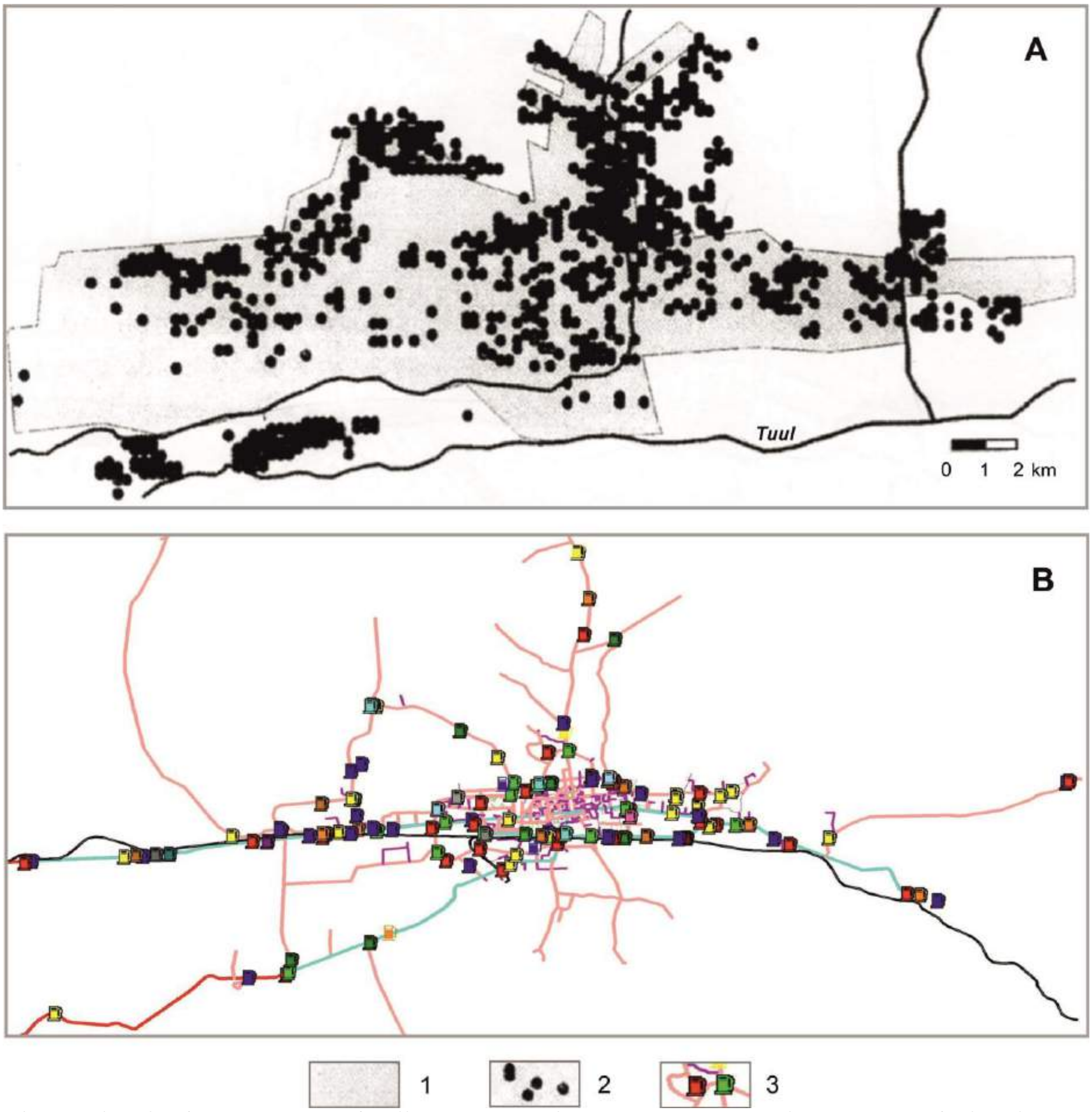

Fig. 1. Sketch of yurt sources of pollution (A) and gas stations (B) in the territory of Ulaanbaatar: 1 – the main part of the city, 2 – yurts (Arguchintsev et al., 2009), 3 – gas stations

The influx of people and the growth of the capital's territory caused an exacerbation of environmental problems related not only to the development of the industrial sector and the yurt part of the city, but also to the vehicle fleet and, accordingly, the gas station network. According to the information agency REGNUM for 2016, more than 458 thousand cars were registered in the capital. The sphere of oil products supply is developing rapidly and chaotically, which contributes to contamination and degradation of soil ecosystems (Sarapulova et al., 2007). In Mongolia, there are more than 130 fuel depots and oil depots, about 1200 gas stations, of which 186 are in Ulaanbaatar. The location and density of the gas station network in the territory of Ulaanbaatar are presented in Fig. 1B. At modern gas stations with sealed equipment, the probability of fuel leaks is minimized. However, an analysis of the operation of the gas station network by a company alone revealed that the number of straits in the fuel dispensers and on the fuel discharge site remains high: up to 100 g per 1 ton of gasoline and 50 g per 1 ton of diesel fuel. Surface water run–up contains oil products up to 28.7; lead to 0.005, copper to 0.05, zinc to 0.08 mg /L (Sambuu et al., 2010). The concentration of the gas stations network of increases the negative impact of oil products on the city's soils and can lead to the formation of geochemical abnormal pollution fields.

The absence of a system of environmental control of soils creates a risk of both primary and secondary pollution of the environment by oil products. In conditions of multifactorial impact on urbanized soils, the ecological danger of pollution of the territory increases.

2. Materials and methods

To identify areas and assess the pollution with oil products in the city's soil, the sites and districts of the city that are subject to negative impact of man–made facilities and oil products facilities were chosen as research objects. Such areas were the districts in the center of the city, the territory along the main highway, the areas of CHPP–3 and CHPP–4, the central market area and others. According to disparate surveys (Bajarsajhan, 2009a, в; Boldyn, 2004; Gonchigzhav, 2005; Sarapulova et al., 2007, 2009, 2010; Sambuu et al., 2010; Sorokina, Enkh–Amgalan, 2012), soils of these areas are polluted with oil products.

On the selected sections of the city with a single topographic grid samples of soils were taken with account the "packing" of the territory. The coordinates of the points were determined with GPS. Sampling was carried out from a depth of 0–30 cm (the boundary of the root–habitat zone) during 2009–2014 in spring and autumn. In total, more than 200 samples were selected (in each region from 10 to 30 samples). To assess the content and identification of soil contamination with oil products, an express method of IR spectroscopy and a standard gravimetric method for determining the mass fraction (GOST, 2011) were used. In addition, the content of organic carbon in soils was determined (GOST, 2008). To build a map–scheme for the distribution of oil products in the city soils, the GIS MapInfo Professional (version 5.0) was used. The map-scheme is a continuous raster surface obtained by interpolating point data.

Of particular interest was the study of the state of soils in the zone of influence of gas stations in order to reveal the patterns of distribution of oil products and heavy metals (and arsenic) falling into the soil due to atmospheric dust. These territories are a kind of polygon for studying geochemical features of polycomponent soil pollution. These studies are carried out for the first time for Ulaanbaatar. According to the environmental standards of Mongolia, only the content of oil products in the ambient air is monitored at the gas station. The monitoring of the state of the territory adjacent to the gas station is not carried out. Analysis of soils in the city is carried out singly during engineering and geological surveys. The main tasks of the study were (1) to identify zones of accumulation and areas of soil contamination with pollutants (oil products and heavy metals), (2) to obtain thematic landscape maps of their (pollutants) distribution.

The studies were carried out at the gas stations №14 and №90 OOO MT (Magnai Trade). Both are located in the eastern part of the city, which, due to the prevalence of winds in the west and north–west of the city, is experiencing an increased impact of dust pollution from the emissions of thermal power plants and stoves in the yurt part of the city. The first of them was built in 1992, the total number of oil products (gas and diesel fuel) released on it is 23,000 L / day. The second more modern one was launched in 2011; the quantity of oil products released is significantly less – 3,500 L / day. Sampling for dust pollution was taken from a depth of 0–5 cm in the spring and autumn of 2015. In total, more than 200 samples were taken, determination of the content of oil products was performed (GOST, 2011). The Pb, Zn, Hg, Cu, and As contents were determined by atomic absorption spectroscopy using a Perkin Elmer–5000 instrument in accordance with the ISO / TC190 / SC3 / WG1 method. The choice and list of identified chemical elements is determined by the specificity of contamination of the territory under study: lead, mercury, zinc, arsenic and copper. The measurements were carried out at the Institute of Physics and Technology Mongolian Academy of Sciences.

3. Results and discussion

According to the obtained data, the content of oil products in the presence of local anomalies with medium and severe soil contamination of Ulaanbaatar are classified as slightly contaminated (Fig 2). Increased content of oil products in soils (above the MPC of 100 mg/kg) was found along the main highway of the city, industrial zone, CHPPs and yurt zone, high – in the territories of oil depots and gas stations (up to 1000 mg/kg soil and more). So, in the territory of the gas station № 8 "Zhast-Oil" (the city center, from the Burd market to the northwest), where oil spills often occur, their content is more than 1000–1200 mg/kg. The content of oil products on the territory and in the area of the Tolgoit tank farm, where leakages (filling equipment) are also often fixed, is

800 to 1200–1500 mg/kg (multiples of MPC). The size of the distribution zone of oil products is about 200x1200 m^2 with the thickness of a highly contaminated layer of 10–15 cm. There are practically no plants on this area. And the pollution spreads up to 3 km from the source.

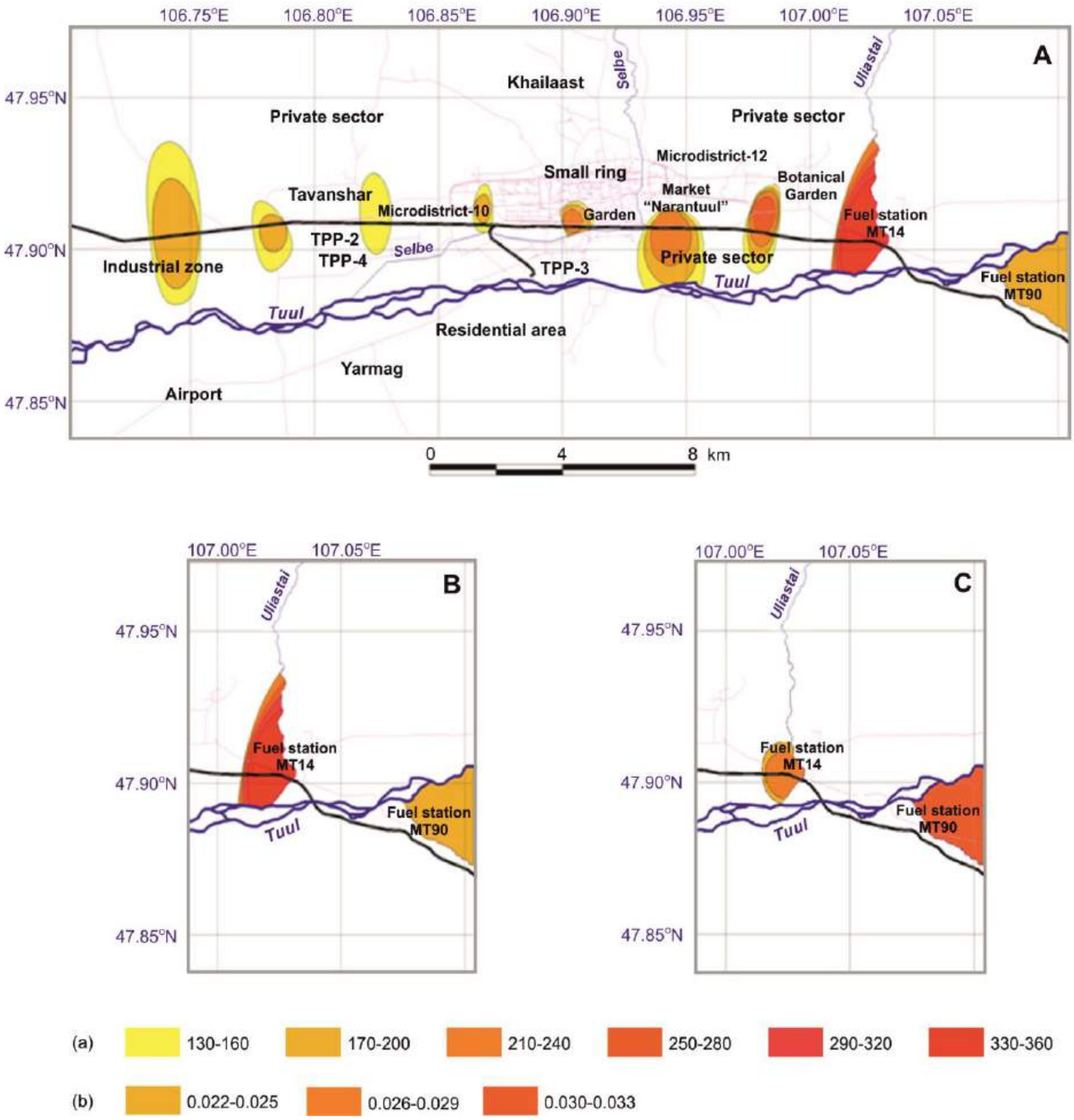

Fig. 2. Distribution of oil products (A, B) and C_{org} (C) in the territory of Ulaanbaatar: a – scale for oil products (mg/kg), b – scale for C_{org} (g / g). The remaining explanations see in the text

The quantitative approaches adopted in various countries to standardize the content of oil products in soils are determined by the nature of regional pollution, the degree of its industrialization, environmental policy, and the physic geographical conditions on which self–purification of the environment depends. In many countries, the oil content level of about 1000 mg/kg is increased pollution, which requires observation, analysis and elimination of the cause of pollution (VROM, 1988; Pikovskii et al., 2003).

A comparative analysis of soil contamination with oil products for the gas station AZS–14 MT and the adjacent territory indicates their significant accumulation in the station itself and lateral removal along the slope towards the Tuul River (Fig. 3a). The direction of their migration is subject to the landscape slope of the surface (the catenary principle for geochemical components). At a distance of about 100 m from the source of pollution, the content of oil products is 800 mg/kg. And at a distance of 250 m from the source, the content of oil products in the soil at 500 mg/kg does not comply with generally accepted standards (minimum content is 100 to 300 mg/kg soil). This creates a risk of contamination of the territory in general and surface water: the gas station is 1.6 km away from the Tuul River.

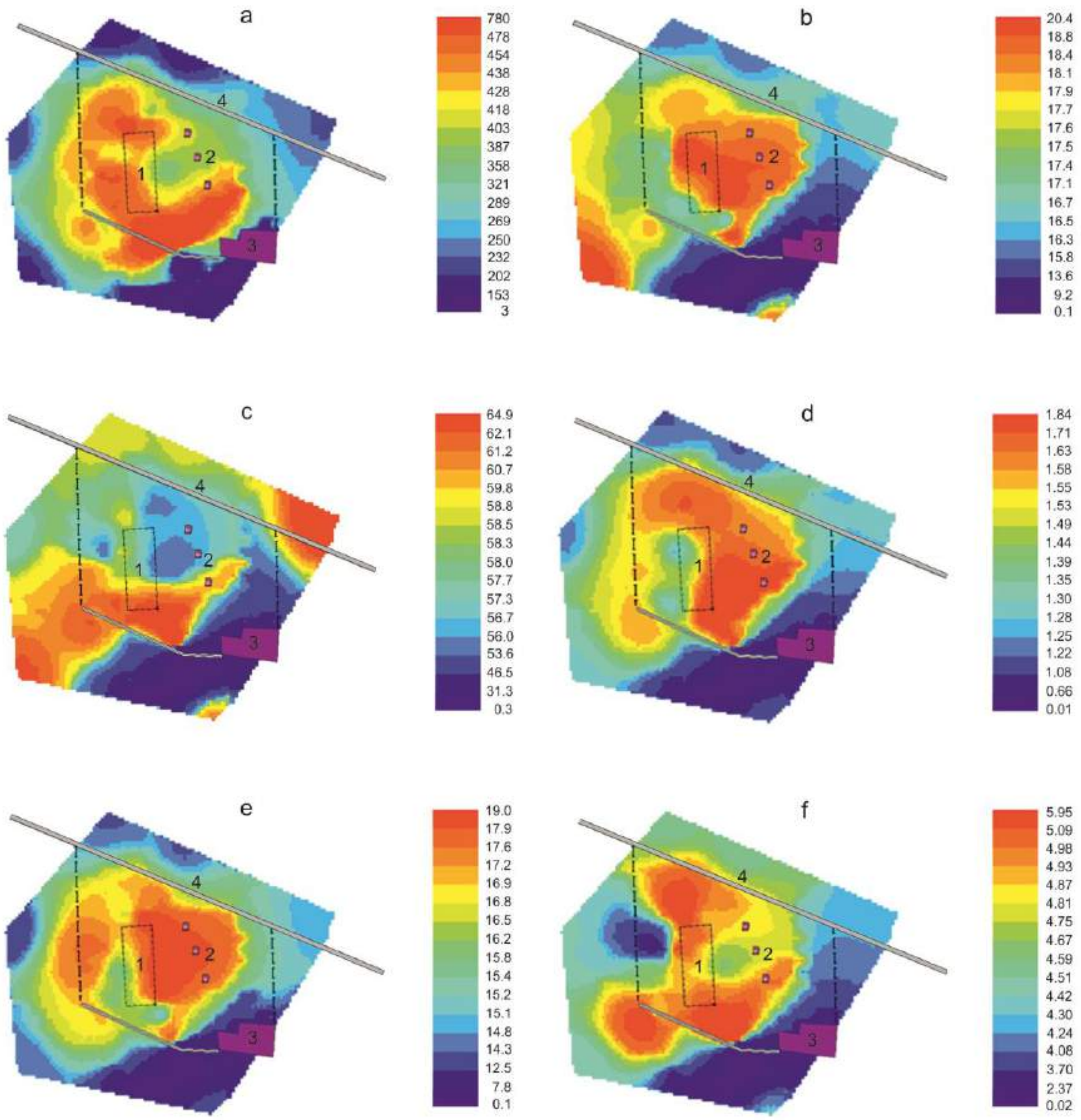

Fig. 3. Distribution of pollutants at gas station №14 MT and the surrounding area: a – oil products, b – Pb, c – Zn, d – Hg, e – Cu and f – As (mg/kg). Other explanations see in the text

An analysis of the spatial distribution of Pb, Hg, Cu and Zn in the territory under consideration indicates their higher concentrations in the oil leakage zone compared to the surrounding area (Fig. 3 b–e). For Zn, there is an increased migration activity compared to Pb, Hg and Cu, the solubility of compounds of which is much less. However, the content of Pb, Hg, Cu and Zn in the territory under consideration, with the exception of the latter, does not exceed the maximum permissible concentration – 32, 2.1, 33 and 55 mg/kg, respectively. For Zn excess is less than 20 %. But it is noteworthy that soil contamination with As exceeds the MPC by more than 2.5 times (Figure 3f). Excess of MPC for As content was also noted in the city soils before (Kosheleva et al., 2010; Kasimov et al., 2011), but in our case it is important that the area of its accumulation practically coincides with that of oil products. The latter allows assuming the affinity of As to oil products and the joint accumulation of pollutants with a cumulative effect as a result.

For the new gas station №90 MT, lower pollution levels were detected as oil products (90–190 mg/kg), Pb, Hg, Cu and Zn content does not exceed the MPC (Fig. 4). With a low variance (difference) of the obtained analytical data, the tendency of migration of these pollutants beyond the territory of the gas station is clearly recorded. As for As, the joint accumulation of it with oil products both at gas station №14 MT is not even observed as a trend. Its (As) content on the entire gas station and the adjacent territory is high (4.2–4.4 mg/kg). The result is somewhat unexpected, even taking into account the fact that the gas station №90 MT is relatively new, launched in 2011. But if we turn to the data of organic carbon content in soils (Fig. 1C), the picture becomes clear. Soils of the region under consideration are much more humus than in the area of gas station №14 MT. The latter is located in the mouth of the valley of the mountain river Uliastai, alluvial and proluvial deposits of which are mainly represented by cobble and gravel. Accordingly, the organic matter in the soils of this region is less and the absolute values of sorption by them (organic matter) of arsenic are also smaller (Vodyanitskii, 2009).

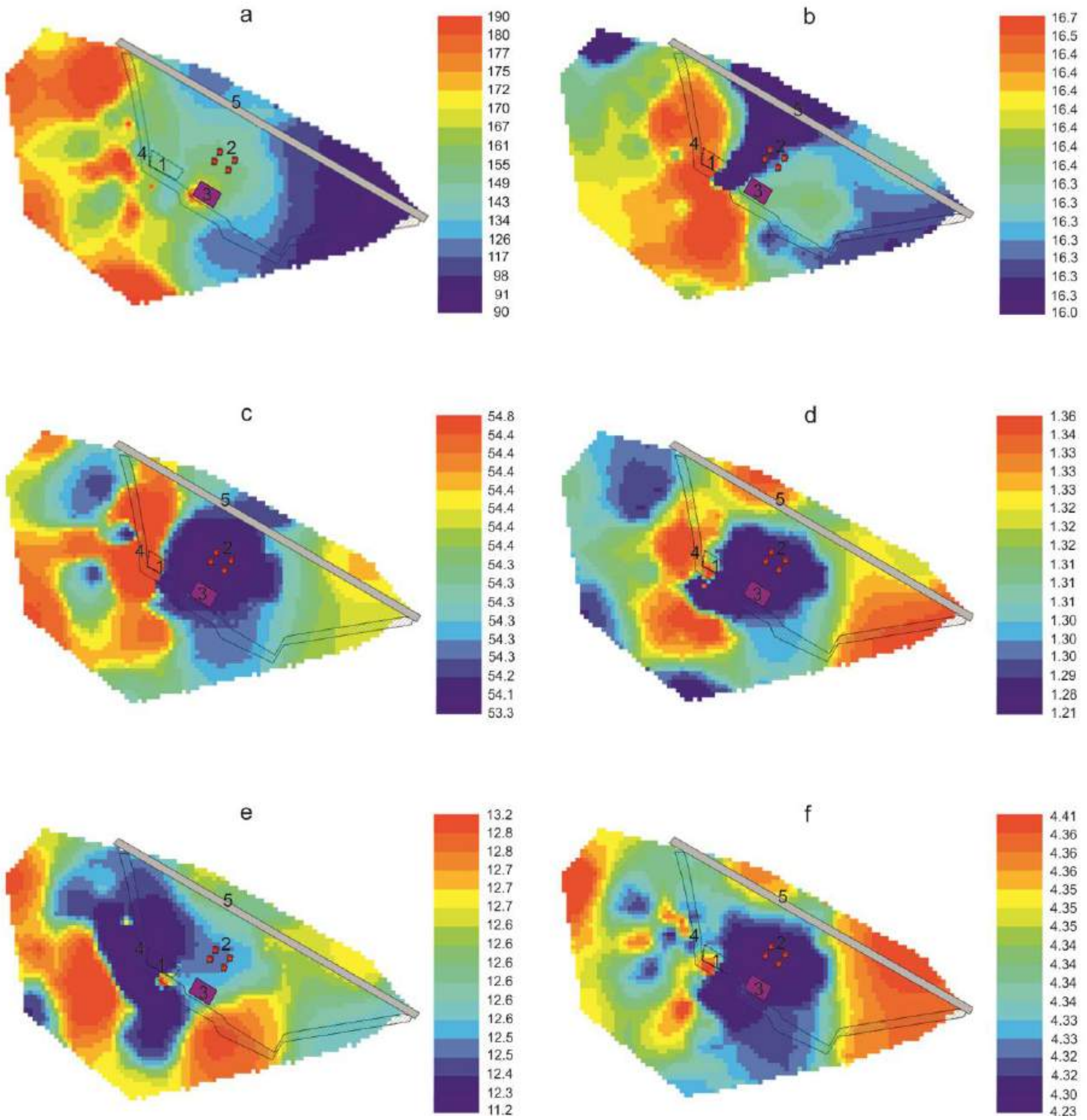

Fig. 4. Distribution of pollutants at gas station №90 MT and the surrounding area: a – oil products, b – Pb, c – Zn, d – Hg, e – Cu and f – As (mg/kg). Other explanations see in the text

The main source of arsenic in the city's soils is the dust from the combustion of used brown coal (fuel and industrial complex and yurt). The problem of soil contamination with arsenic is a problem not only for Ulaanbaatar, where emissions from burning solid fuels in the yurt part are half of the volume of pollutants entering the atmosphere of the city. Arsenic is found widely in nature. Annual global emissions from natural sources have been estimated to be approximately 8,000 tons per year (Becher, Wahrendorf, 1992; WHO, 1987) whilst emissions from anthropogenic sources have been estimated to be approximately three times higher, in the region of 23,600 tons per year (WHO, 1987). The main source of arsenic emissions has been the combustion of fossil fuel (notably coal), other sources being small in comparison. The arsenic contamination exists in UK, Slovakia, Turkey, and the Guizhou Province of China. This occurs primarily as a result of the concentration of arsenic in coal which is known to vary from trace to 1–2 % (Yudovich, Ketris, 2005; Garelick et al., 2008).

Consider, for example, England. In the 70s of the last century a big problem of air and soil pollution with arsenic arose. The UK National Air Emissions Inventory (NAEI) indicate that combustion of fossil fuels such as lignite (brown coal), hard coal and heating oil account for the largest proportion of total UK emissions of arsenic (Maggs, 2000). At the government level, the solution to the problem was initiated with the organization of pollution monitoring, followed by a decrease in the use of fossil fuel and the introduction of new technologies. The reduction of arsenic emissions in United Kingdom over the past decades has resulted as a consequence of a decline in coal use, in favour of natural gas use as a cheaper alternative. NAEI results show that emissions have declined by 78 % since 1970.

Currently, the Government of Mongolia is extremely concerned about the problems of environmental pollution and is doing a great job to improve life in the city. So in the yurt part of the city, to replace the dust emissions, a significant part of the furnaces was replaced by new ones, regular measures were taken for landscape gardening. But the problem of soil and air pollution in the capital is still far from the solution. At this stage in Mongolia, it is important and realistic to organize the monitoring of pollution. As our studies have shown, the main attention should be paid to monitoring soil contamination with oil products (areas of the city with levels of about 1000 mg/kg) and arsenic, analysis and eliminating the causes of pollution.

4. Conclusions

The soil contamination of Mongolia's capital Ulaanbaatar with oil products was investigated. To identify areas and assess the pollution of the city's soil with oil products, the sites and districts of the city that are subject to negative impact of man-made facilities and oil products facilities were chosen as research objects. Based on soil-geochemical survey, a map of city pollution was built. According to the obtained data, soils of Ulaanbaatar on the content of the oil products are classified as slightly contaminated. However, there are local anomalies with medium and strong pollution. Elevated (above the maximum allowable concentration of 100 mg/kg) values for oil products were found along the city's main highway, CHPPs, industrial and yurt zones, high in oil tank farms and gas stations (up to 1000 mg/kg soil and more).

Of particular interest was the study of the soils state in the zone of influence of gas stations in order to reveal the patterns of distribution of oil products and heavy metals (and arsenic) falling into the soil with atmospheric dust from burning brown coal (fuel and industrial complex and yurt part of the city).

These territories are a kind of polygon for studying geochemical features of polycomponent (oil products, heavy metals and As) soil contamination. Such studies for Ulaanbaatar have not been carried out previously. Visualization of obtained data by GIS methods made it possible to establish that at high oil content (of the order of 1000 mg/kg), there is a symbate accumulation of As, which exceeds the MPC at times. Areas of accumulation of arsenic practically coincide with those of oil products. The latter allows assuming the affinity of As for oil products and the joint accumulation of pollutants with a cumulative effect as a result. With less contamination of soils with oil products (up to 200 mg/kg), As content corresponds to the content of humus (organic matter) in them – more humus soils contain more As.

Currently, the Government of Mongolia is extremely concerned about the problems of environmental pollution and is doing a great job to improve life in the city. So in the yurt part of the city, to replace the dust emissions, a significant part of the furnaces was replaced by new ones, regular measures were taken for landscape gardening. But the problem of soil and air pollution in the capital is still far from the solution. At this stage in Mongolia, it is important and realistic to organize the monitoring of pollution. As our studies have shown, the main attention should be paid to monitoring soil contamination with oil products (areas and areas of the city with levels of about 1000 mg/kg) and arsenic, analysis and eliminating the causes of pollution.

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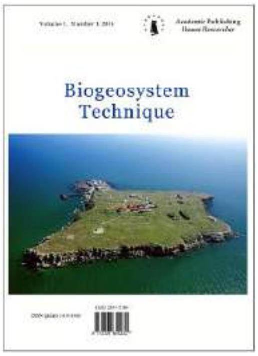

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Organic Carbon and Total Nitrogen in Soils of Kon Ka Kinh National Park (Central Vietnam)

Nguyen Van Thinh [a,*], Do Phong Luu [a], A. A. Okolelova [b], Ngo Trung Dung [a]

[a] Joint Russian–Vietnamese Tropical Scientific Research and Technological Center, Southern Branch, Ho Chi Minh City, Vietnam
[b] Volgograd State Technical University, Volgograd, Russian Federation

Abstract

The article presents the results of analysis of organic carbon (OC) and total nitrogen (TN) in the soils at the 6 different types of tropical forest: Mixed forest between broad-leaved trees and conifers (*Lagerstroemia calyculata* + *Pinus kesiya*); Bamboo forest (*Bambusa*); Three-needled pine (*Pinus kesiya*); Primary broad-leaf evergreen forest has dominated by Fagaceae; Primary broad-leaf evergreen forest, dominated by Fagaceae, near the streams, can be flooded in the rainy season and Mixed forest between broad-leaved trees and conifers (*Fagaceae* + *Dacrycarpus imbricatus*, *Dacrydium elatum*) of Kon Ka Kinh National Park in Central Vietnam. OC concentrations ranged from 1.1 % in the Primary broad-leaf evergreen forest to 3.5 % in the Mixed forest between broad-leaved trees and conifers (*Fagaceae* + *Dacrycarpus imbricatus*, *Dacrydium elatum*). TN concentrations varied from 0.33 % in the Three-needled pine to 0.04 % in the Bamboo forest. In the Primary broad-leaf evergreen forest has dominated by Fagaceae, OC decreases while TN increases according to the depth of soil. In the Primary broad-leaf evergreen forest, dominated by Fagaceae, near the streams, OC does not change, and TN decreases with soil horizon.

Keywords: organic carbon, total nitrogen, soils, tropical forest, Kon Ka Kinh National Park, Vietnam.

1. Introduction

Organic compounds are very important for the formation of the tropical forest soils (Fridland, 1964; Thai, Nguyen, 2002). The number and the nature of their strong impact to the process of land formation are decided in many branches of Natural Sciences: Physics, Chemistry, Biology and Fertility of soil. Currently, scientists are convinced that one of the main causes of forest degradation, reducing fertility and overall produce of soil biological product is reduced organic matter and decreased humus reserves in soil (Nguyen, 1996; Do, Nguyen, 2001). The soil OC and TN determining is urgent to individualize the location of ecological regions because OC and TN concentrations have a significant influence on the chemical reactions in the soil or sediment (Schumacher, 2002; Okolelova, 2006).

In natural forest, the primary source of organic matter contained in soil includes dead plants, animals and micro-organisms. This supply capacity depends on each status of different forest and species featured plants, domination plants distributed in habitat. This is also not the exception to the Vietnam natural forest ecosystems. In a survey by Vietnam-Russia tropical center held in Gia

* Corresponding author
E–mail addresses: thinh39b@gmail.com (Nguyen Van Thinh)

Lai province (Central Vietnam), we have conducted research, identified the OC content and TN in the soils to find out the relationship between them and the habitats which is characterized by different plant species of the Kon Ka Kinh National Park.

2. Study Area

Kon Ka Kinh National Park is located on the Central Highlands of province Gia Lai (Vietnam). Kon Ka Kinh National Park covers an area 41,780 ha. This is the only place in Vietnam that owns mixed forest between broad-leaved trees and conifers (2,000 ha) (Nguyen, Bui, 2018).

In terms of our expedition, conducted research on pedology and some ecological characteristics at the 6 different types of tropical forest in the southwestern part of the Park. Location of research points in Fig. 1.

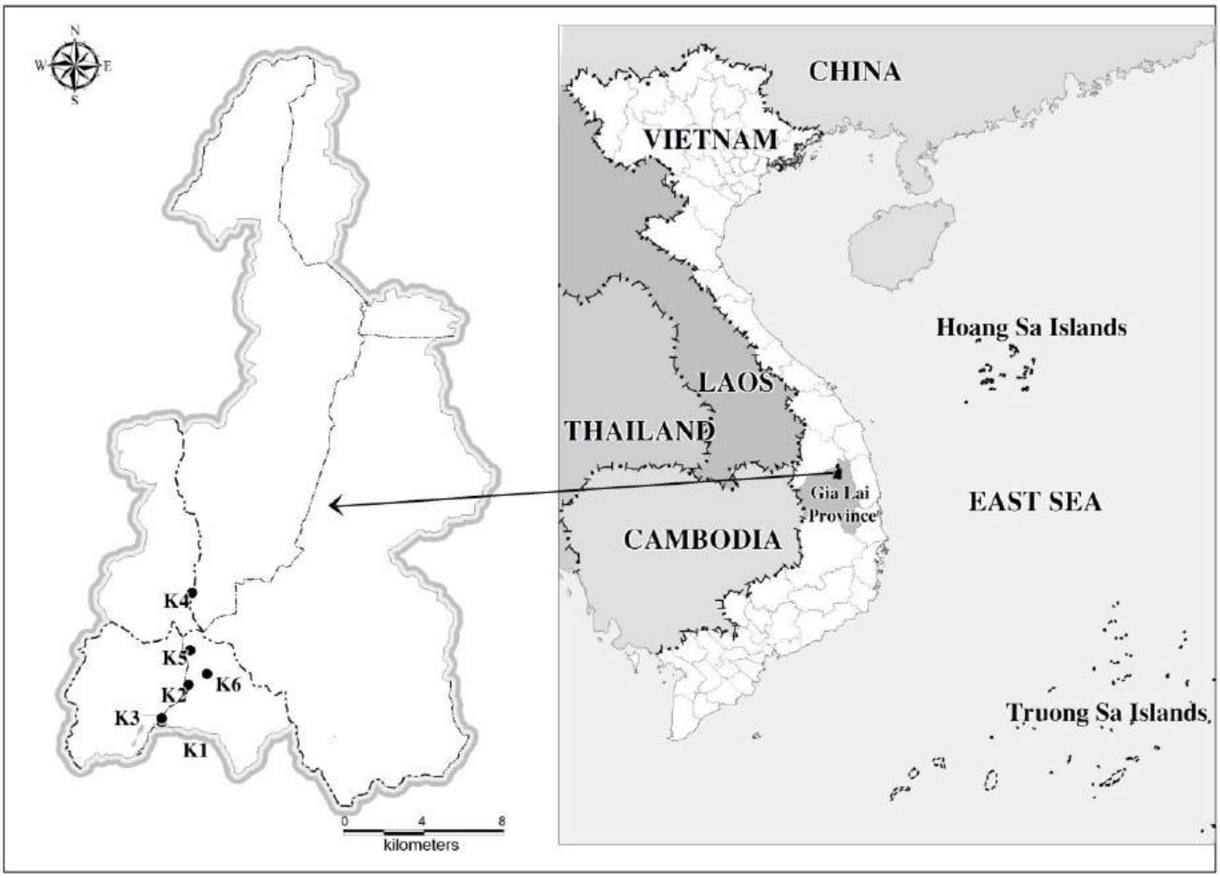

Fig. 1. Location of research points

K1-Mixed forest between broad-leaved trees and conifers (*Lagerstroemia calyculata* + *Pinus kesiya*);

K2-Bamboo forest (*Bambusa*);

K3-Three-needled pine (*Pinus kesiya*);

K4-Primary broad-leaf evergreen forest has dominated by *Fagaceae*;

K5-Primary broad-leaf evergreen forest, dominated by *Fagaceae*, near the streams, can be flooded in the rainy season;

K6-Mixed forest between broad-leaved trees and conifers (*Fagaceae* + *Dacrycarpus imbricatus, Dacrydium elatum*).

3. Materials and methods

The sampling areas: based on the distribution characteristics of plant in the habitat (primary forest, mixed forest, regrowing forest) according to the classification of Thai Van Trung (1978); Anichkin (2011); Kuznetsov, Kuznetsova (2011); Nguyen, Okolelova (2014).

Soil sampling: sample collection, profiles in the ground floor according to Vietnam standard TCVN 7538-2:2005, process templates according to Vietnam standard TCVN 6647:2007. In some cases, does not determine the ground floor then sampling under the depths of 0–20, 20–40 and 40–60 cm.

The determination of OC to Vietnam standard TCVN 4050-85 is based on the method of Walkley-Black (1934). Oxidisable matter in the soil is oxidised by $K_2Cr_2O_7$ solution. The reaction is assisted by the heat generated when two volumes of H_2SO_4 are mixed with one volume of the dichromate. The remaining dichromate is titrated with ferrous sulphate. The titre is inversely related to the amount of C present in the soil sample (Walkley, Black, 1934; Carter, Gregorich, 2007).

The determination of TN to Vietnam standard TCVN 6498: 1999 is based on the Kjeldahl method: Denitrification of the sample by Dewarda in sulphuric acid is converted to ammonia. Distillation of ammonia released from ammonium sulphate with sulphuric acid and catalyst. The end of the condenser is dipped into a solution of boric acid. The ammonia reacts with the acid and the remainder of the acid is then titrated with a sodium carbonate solution by way of a methyl orange pH indicator (Carter, Gregorich, 2007).

Data processing on software Microsoft Excel 2010.

Preparation of soil samples and analysis were performed in the laboratory of Vietnam–Russia tropical Centre, Ho Chi Minh City, Vietnam.

4. Results and discussion

Characteristics of study areas

K1: Mixed forest between broad-leaved trees and conifers (*Lagerstroemia calyculata* + *Pinus kesiya*). The brown soil, soft, mixing with gravel from 1–3 cm. Stratification not visibly. Litter layer of pine leaf. Position 14°11'12" N, 108°18'10" E, at elevations of 880 m a.s.l.

K2: Bamboo forest (*Bambusa*). The brown land, many bamboo roots. Difficult to distinguish the soil layers. Plants mostly bamboo (*Bambusa*). Litter layer of bamboo leaves. Position 14°12'42" N, 108°18'55" E, at elevations of 902 m a.s.l.

K3: Three-needled pine forest (*Pinus kesiya*), 5–7 m high, steep hills, ferralsol. Layer A1 (0–30 cm) of humus soil, the soil is black and soft. Layer A2 (30–54 cm) the color of soil is back and brown then change to red- yellow in layer B1 (55–70 cm).In A2 and B1, the attributes of soil are flexible and wet. Litter layer of pine leaf. Position 14°11'47" N, 108°18'10" E, at elevations of 897 m a.s.l.

K4: Primary broad-leaf evergreen forest has dominated by Fagaceae. The soil mix with rocks, 3–5 cm diameter. The first layer is humus covers the sandy soil layer below which has pale yellow color. The layer A1 (0–18 cm): humus, black and soft. The layer A2 (18–32 cm), B1 (32–54 cm): sandy soil mixes with a huge gravel and stone, less roots. Position 14°15'13" N, 108°19'01" E, at elevations of 965 m a.s.l.

K5: Primary broad-leaf evergreen forest, dominated by Fagaceae, near the streams, can be flooded in the rainy season. The sandy soil, possible inundated in the rainy season, litter layer of leaves. The layer A1 (0–20 cm), sandy soil, soft. The layer A2 (20–40 cm), B1 (40–60 cm) are golden sand. At elevations of 965 m a.s.l, position 14°13'39" N, 108°18'58" E.

K6: Mixed forest between broad-leaved trees and conifers (*Fagaceae* + *Dacrycarpus imbricatus, Dacrydium elatum*). The layer A1 (0–40 cm), the soil is wet, the color of soil is brown-red. Field texture: medium clay. The layer A2 (40–63 cm), the soil is reddish brown, slightly moist, medium clay, mixes with large tree roots. The layer B1 (63–120 cm) the soil is red, coarse clay, moisture, mixes with large tree roots. Position 14°13'03" N, 108°19'26" E, at elevations of 1,172 m a.s.l.

Content of OC and TN at study areas

The results analyzed at Table 1 indicate the OC content in study areas varies from 1.1 % in the Primary broad-leaf evergreen forest (K5) to 3.5 % in the mixed-leaved forests (K6). The result can be explained as: Although in the K5 is the primary forest, but stay in coastal position which has sandy soils, gravel, litter layer often gets washed by rain and streams so the land should not be sufficiently for biodegradable, additional organic substances for soils. The K6 with high humidity by 1,127 m height, increases decomposition of leaves and plant-body which makes clay in field texture so the ability to accumulate organic matter is better than sandy soils. Consequently, this is the highest level of OC in comparison to other areas, including the depth of the soil horizons.

Table 1. Organic carbon and total nitrogen in soils research

Habitats – Soil types	Soil layer (cm)	OC (%)	TN (%)
K1 Ferralic Acrisols	0–20	3.0	0.26
	20–40	2.8	0.18
	40–60	2.7	0.17
K2 Ferralic Acrisols	0–20	3.2	0.22
	20–40	1.3	0.17
	40–60	1.2	0.04
K3 Xanthic Ferralsols	0–30	2.9	0.33
	30–54	1.7	0.23
	54–70	1.8	0.17
K4 Fluvisols	0–18	3.0	0.06
	18–32	1.6	0.06
	32–54	1.3	0.10
K5 Fluvisols	0–20	1.1	0.24
	20–40	1.1	0.18
	40–60	1.1	0.14
6 Rhodic Ferralsols	0–40	3.5	0.27
	40–63	3.4	0.18
	63–120	3.3	0.12

As shown in Table 1, OC content tended to decrease in depth in 5 habitats (K1, K2, K3, K4, K6) and fluctuated from 3.5 % (K6 habitat) to 2.9 % (K3 habitat). In deeper soil layers, OC change from 3.4 % (K6) to 1.2 % (K2). This is a common feature, due to the surface layer, under the influence of micro-organisms, temperature and humidity, the resolution of organic matter is stronger, the resolution speed of organic matter occurred more powerful, while the lower soil layers, humus formation is quite stable so the resolution speed is slower, resulting in lower OC content. In habitat K5, OC content virtually does not depend on the depth of horizon. This may be due to the place of sampling, the soil component is sand and loamy sand, along with the flow effects of the streams, the organic matter has been washed away, with the lowest content determined, including on the upper ground and forward layers.

Besides the nature of the soil, the types and composition of vegetation in that habitat will be a determining factor for the OC content of the soil. On ferralsols land, the studies of several authors suggest that OC content in the habitats dominated by *Afzelia xylocarpa* reached 2.9 % (Nguyen, 1996); in the rubber forest of Dak Nong Province (Central Vietnam) is about 1.75–3.81 % (Nguyen et al., 2009). While in the Pleiku area, 50 km to the east of study areas, Nguyen V.D. (2013) analyzed the OC content in the ground surface of low-shrub land that is about 2.8%, and in grasslands is 3.2 %.

In Cat Tien National Park and Dong Nai Nature Reserve, OC in the topsoil achieve from 1.71 % in the habitats dominated by *Dipterocarpus alatus* to 5.34 % in the habitats dominated by *Lagerstroemia calyculata*, *Afzelia xylocarpa*, *Tetrameles nudiflora*, while in the habitats dominated by *Ficus*, the OC ranges from 2.15 to 4.75 %, while the bamboo forest (*Bambusa*) is 1.14 to 1.93 % (Okolelova et al., 2014). Thus, as in our analysis, in organic bamboo habitats, OC content is generally lower than in other habitats. In the Evergreen Broad-Leaved forest (Cat Tien National Park, Dong Nai Nature Reserve, South Vietnam) the OC content was higher than our analysis. As for the remaining habitat, the OC content in the soil is equivalent to our analysis.

Analysis of TN content shows that, basically in the topsoil, this value is highest compared to the next layers of the soil profile. This was recorded in 5/6 study areas (except areas K4). In these areas, the TN content was from average or higher than variation in surface layer from 0.22 % (K2 habitat) to 0.33 % (K3) and from 0.04 % (K2) to 0.23 % (K3) in subsequent layers. For the K4, in the upper soil layer, the TN content was 0.06 % but in the down soil layer this number increased 0.1 %.

An analysis of soil properties in some habitats at Cat Tien National Park shows that TN has a large variation, from 0.09 % in *Dipterocarpus alatus. D. turbinatus; Bambusa* and *Dipterocarpus alatus* to 4.8 % in *Lagerstroemia calyculata*, *Bambusa* sp., *Calamus* sp. (Okolelova et al., 2014).

In the *Bambusa* (K2) habitat of the study, our analysis also determined that TN was the smallest (0.04 %) compared to all study habitats.

5. Conclusion

The OC content of the soils in the study areas is highest in the topsoil (3.5 % in K6 – Mixed forest (*Fagaceae* + *Dacrycarpus imbricatus, Dacrydium elatum*)) and tends to decrease in depth of the soil layer (1.1 % in K5 – Primary forest, Evergreen Broad-Leaved forest near the stream). Except for K5 – there is almost no change in depth of profile (1.1 %).

TN content was highest in the topsoil (0.33 % in K3 – *Pinus kesiya* forest) and gradually decreased in the soil profile (0.04 % in K2 – Bambusa forest). In Bambusa areas (K2), TN determination was lowest.

Although research land have the same nature as sandy soil and gravel in K4 and K5 areas - Fagaceae) and the same type of evergreen forest, but the OC and TN content recorded are not similar. The OC content of the soil in K4 is higher than K5, while the TN content is opposite.

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