



ENVIRONMENTAL HEALTH FOUNDATION

**REDUCTION OF HAZARDOUS LEVELS
OF THE AGRICULTURAL APPLICATION
OF NITROGEN AND PHOSPHORUS RELATIVE
TO TOXIC GROUND WATER AND TOXIC LEVELS
IN THE SOIL**

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Reduction of hazardous levels of the agricultural application of nitrogen and phosphorus relative to toxic ground water and toxic levels in the soil

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Summary This paper proposes the hypothesis that microbial life chemically reduces levels of nitrogen (N_2) and phosphorus (P) that are toxic and threaten human health and safety. Bio-remediation uses microorganisms to decontaminate a polluted system, in situ, requiring a minimal amount of space and equipment. Data strongly suggest that bio-stimulation can assist one microbe to multiply up to one billion microorganisms in 24 hours. Biochemical literature postulates that microbial life chemically biodegrades nitrates by one of two methods: (1) assimilative reduction; or (2) dissimilative reduction, also known as denitrification. Assimilative reduction results in construction of microbial cell walls, cell membranes and various forms of amino acids. It is proposed that denitrification includes the venting-off of the excess amounts of N_2 not required by the soil or needed for additional microbial development. Nitrate reduction by way of denitrification is a functional part of anaerobic respiration. Alternatively, the denitrification process supports oxidative phosphorylation, a mechanism similar to aerobic respiration. Thus, denitrification and phosphorylation may be considered as forms of respiration. © 2000 Harcourt Publishers Ltd

INTRODUCTION AND JUSTIFICATION FOR THE STUDY

An increasing body of evidence suggests that controversy exists among scientists regarding the value, importance, and proper balance of nitrogen (N_2) and phosphorus (P) applications versus the effects of the applications on living organisms and resulting environmental health problems from the over-use of these inorganic chemical salts. It is proposed that the over-use of agricultural fertilizer salts contributes to hazardous levels of these inorganic salts in both ground water, animal manures and agricultural soils (1).

Consider the scenario that power from the sun, life in the oceans, and the living action within the soil, all combine and synergistically contribute to the accumulated reserve of power in carbon sources such as animal and poultry manure. Microorganisms can be considered one of the most successful representatives of life on our planet, and when and where allowed, microbial life continues to abound. It has been estimated that the combined weight of microbial cells on spaceship earth is about 25 times the weight of the earth's other animal life. While their individual size is incredibly small, one cubic inch of pure typical bacteria can contain over nine trillion microorganisms. It is reported by Dr Snyder of the Smithsonian publication that, even today, microorganisms continue to cleanse and balance this planet effectively (2).

It could be speculated that some bacteria oxidize inorganic compounds to obtain the necessary energy to produce their own food. The inorganic compounds oxidized

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by specific bacteria include ammonia, nitrites, phosphates, and sulfides and they use the small amount of energy released from these oxidations in the reductions that synthesize carbohydrates. For example, nitrifying bacteria reduce atmospheric nitrogen (N) to ammonia, and oxidize ammonia (NH) to nitrites (NO₂) and nitrites to nitrates (NO). The majority of bacteria break down dead organic substances by secreting digestive enzymes and then absorb the nutrient molecules. However, microbial life requires NH₄, nitrogen, PO₄³⁻, phosphorus, and SO₄, sulfur, along with other trace minerals for the creation of the microorganism cell wall, cell membrane, and for making amino acids and other nitrogen-phosphorus containing building blocks (3–5).

Does Nature hold the answers to balance our toxic problems?

This article's hypothesis proposes that the enzyme recruitment ecosystem, when biostimulated, offers the potential for the acquisition and evolution of new degradative pathways. This potential allows great flexibility in the capability of communities of microbes to degrade complex materials such as toxic levels of nitrogen and phosphorus representing out-of-balance substances that are unfriendly to a safe ecosystem. Inoculation of toxic waste products with various biostimulation generates many trillions of enzyme-secreting microorganisms, and indigenous molecules, active coenzymes, and exo-enzymes, that are able to hydrolyze and biodegrade efficiently the various forms of out-of-balance materials found in the remediation systems. It is reported by Dr James Dragun, soil chemist of Penn State University in Agronomy, that bio-remediation is powerful and demonstrates dynamic benefits. Up to 90% of the chemical substances on our earth considered toxic, including excessive nitrogen and phosphorus, can be biodegraded (6).

Livestock manure, phosphorus, nitrogen, water quality and soil toxicity

To narrow the proposed subject matter and thus become more specific, consider the example of bio-treatment of animal and poultry manure. One of the problems with animal and poultry manure (and with excess levels of chemical fertilizers), is the amount of time it takes to compost these materials. Animal and poultry manure, and excess chemical fertilizers, can be toxic with an abundance of ammonium nitrate, which is a threat to stream life, and can percolate down into the ground water, thereby contaminating it (7).

Phosphorus is an essential element in the diet for every living organism. For plant functions, phosphorus serves as a constituent of sugar phosphates, nucleotides, nucleic acids, phospho-lipids and co-enzymes. In the form of phosphates, it is a major contributor to the minerals,

which are essential for bone development (8). It also is involved in all basic metabolic processes, including its important role in cell metabolism. Phosphorus is essential for the metabolism of protein, calcium, and glucose. Phosphates are extremely important, particularly in the storage and use of cell energy and the transmission of genetic information within the cell and from one cell to another.

Phosphorus in excess is extremely poisonous. The inhalation of its vapors may cause narcosis. Free phosphorus causes fatty degeneration of the liver and other internal organs of the body, including the heart, lungs, and intestines.

The clear, colorless, odorless liquid known as phosphoric acid is irritating to the skin and eyes and moderately toxic if ingested. Phosphoric acid is used in the production of fertilizers, soaps, detergents, animal feeds, and certain drugs (9).

Nitrogen is essential to the synthesis of proteins that the body must have, particularly nitrogen-containing compounds or amino acids derived directly or indirectly from plant food. It also is essential as an element for plant nutrition because it supplies additional co-enzymes, nucleic acids, chlorophyll, and other components necessary for plant growth.

Nitrogen follows a cycle from atmospheric gas into nitrogen-fixing bacteria, into green vascular plants, into humans and animals, by decay, or in excreted nitrogenous wastes, and then back into the soil. Denitrifying bacteria in the soil breaks down nitrogenous compounds and releases gaseous nitrogen (10).

While nitrogen and phosphorus are so vital to the total life cycle, the United States Environmental Protection Agency (EPA) has been required to distinguish between a number of water treatment toxic problems, especially those problems that cause immediate threats to human health. These two substances for which standards have been set include excess nitrates and phosphates (11). For example, nitrates in drinking water, above the national standard of 10 mg/liter (as N) pose an immediate threat as the substance in harmful excess, reacts with the hemoglobin in the blood. This reaction reduces the oxygen carrying ability of the blood and may produce an anemic condition.

Mechanical depiction of a bio-chemistry schematic

Figure 1 is offered as a schematic of a bacteria microorganism (12). Observe its function and consider the suggested design: topsoil is developed from macroorganisms (earthworms, as an example) and microorganisms ~ their castings and their spent bodies.

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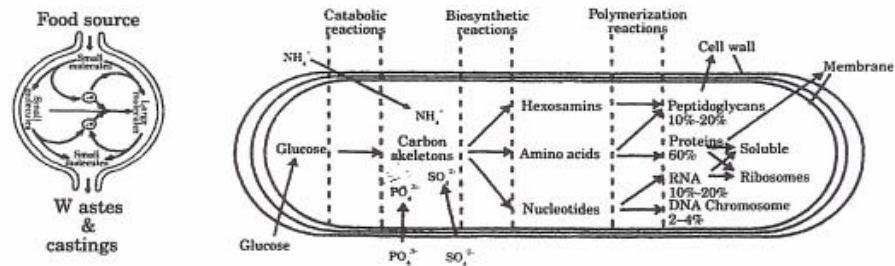


Fig. 1 Mechanical depiction of a bio-chemistry schematic.

What may be deduced from this illustration? Where does all of the extra ammonium nitrate go? Where does the excess phosphorus go? For example, denitrification is the process by which nitrates or nitrites in the soil or organic deposits are reduced to lower oxides of nitrogen by the action of denitrifying microorganisms (various bacteria) in both aquatic and terrestrial environments. **The process results in the escape of nitrogen back into the atmosphere.** So, what happens if too much nitrogen is converted to nitrates? As designed by nature, **denitrifying bacteria counter-balance the process of nitrogen fixation to assure balance.** However, a problem can develop when there is an excess of nitrogen fixation due, for example, to the overuse of artificial chemical fertilizers. **The soil could become chemically toxic, reducing or eliminating microorganism life; then nature's balancing system would be destroyed.**

After plants have absorbed nitrogen in a usable form, they can synthesize or manufacture proteins and nucleic acids. **Some of the newly courted organic nitrogen quickly returns to the soil as fallen foliage or as animal waste, known as humus.** The remaining **organic nitrogen reserve returns only when plants and animals die.** Decomposing mechanisms then break down these organic nitrogen compounds through decay and release ammonia. Under this scenario, **the ammonia often is tamed into oxidized nitrate by nitrifying bacteria; this process is referred to as nitrification.** Thus, there is a sub-cycle among members of food chains **that does not involve atmospheric nitrogen (N₃) at all.**

This courtship of nitrogen and phosphorus sometimes makes nitrogen and phosphorus, among other minerals, available to crops from organic matter through two additional reactions. Protein and allied compounds are broken down into amino acids through a reaction called **arinization.** **Soil microorganisms acquire energy from this digestion.** They also utilize some of the available amino-nitrogen in their own cell structure. For example, ammonia-nitrogen is formed by a second reaction that converts amino compounds into ammonia (NH₃) and ammonium (NH₄) compounds. **Microorganisms in this**

process decompose the remains of all living tissues and their waste products. This reaction is called **ammonification.** The ammonia that was just yielded can now leave the soil or be converted into other nitrogen compounds, depending to some degree on the soil conditions. The dual reaction of **aminization** (proteins to amino acids) and **ammonification** (amino compounds to ammonia, NH₃, and ammonium, NH₄) is referred to as **mineralization (13).**

Recorded in US Bureau of Mines Report 7203 and US Bureau of Mines Circular 8471 is the indication that this sub-cycle within the nitrogen cycle **allows nitrogen compounds to be released naturally over the entire growing season rather than a shocking dose of a limited duration as experienced with an artificial substance applied periodically (14).** A serious concern surfaces relative to **chemically abused and toxic, crop-worn soils that have been stripped of basic huniic substances and left sterile of microbial life.**

In the example of animal and poultry manure, even after remediation, the toxic amounts of nitrogen and phosphorus, have actually made available more useable nitrogen and phosphorus for the soil in organic form. **Microorganism activity in one acre of soil** expends about the same amount of energy in soil preparation, as 10 000 people would burn for the same work, for the same period of time (15).

US Government Involvement

The agricultural community would benefit if they established the following plan of action. On October 28, 1997, a Bill — United States Senate Bill 1323 — was introduced

and read twice to the 105th Congress, 1st Session. This Bill, introduced by Senator Harkin, was to consider the regulation of animal feeding operations for the protection of the environment and public health. It is now with the Agriculture, Nutrition, and Forestry committee.

Pages 12 through 14 of United States Senate Bill 1323 include the following concerns:

A description of the methods, structures, or practices to be used by the concentrated animal feeding operation to prevent or minimize the following issues:

- A. soil loss;
 - B. surface water pollution;
 - C. ground water pollution; and
 - D. odors caused by animal waste during collection, storage, and application.
- Technical specifications for the design and construction of containment systems to be used by each concentrated animal feeding operation.
 - A description of methods, procedures, and practices to be used by each concentrated animal feeding operation for the following processes:
 - A. operation, monitoring, maintenance, and inspection of animal waste storage facilities; and
 - B. Handling, transportation, application, and treatment of animal waste, including storage volume, schedules for emptying storage facilities, and application schedules, rates, and locations.

A description of contingency measures to be used by the concentrated animal feeding operation to minimize environmental pollution resulting from any unexpected waste leak or discharge.

- Maximum levels for nitrogen and phosphorus – the application of animal waste to land by any person is prohibited if (taking into account all sources of nutrients, including commercial fertilizer) the application of animal waste would result in the application of nitrogen or phosphorus in a quantity that results in either one, or both, of the following problems:
 - A. exceeds the quantity necessary to meet crop nutrient requirements; and
 - B. significantly increases the risk of increased soil toxicity or the pollution of surface or ground water.

HYPOTHESIS

The hypothesis of this article is that various bio-stimulants may be used to increase the indigenous microorganisms rapidly, thereby digesting and processing hazardous levels of nitrogen and phosphorus, and reducing the detrimental effects of toxic levels of nitrates by: (1) an accelerated action of denitrification thus venting NO₂ to the ambient air; (2) the implementation of the nitrification subcycle; and (3) the incorporation of nitrates into microbial cell development. By the same token, the stimulation of microorganisms, including the work of enzymes, may reduce the detrimental effects of toxic levels of phosphates.

Phosphatase, including phytase, are enzymes that act as a catalyst in chemical reactions involving phosphorus. This action in the presence of inorganic phosphate, catalyzes the conversion of glycogen into glucose-1-phosphate.

Nitrogen constitutes approximately 78% of the atmosphere and is a component of all proteins and a major component of most organic substances. Thus, compounds of nitrogen are essential constituents of all living organisms, as included in the proteins and the nucleic acids that are basic elements of all life forms. Non-toxic balance can and must be maintained for human health and safety.

SAMPLE DELIMITATION STUDIES

The following three locations were selected, as sample studies: (1) a swine and agriculture field study in the State of Kansas; (2) a swine and agriculture field in the State of Iowa; and (3) a poultry study by the University of Delaware, in the State of Delaware. The various studies were selected on the basis of different circumstances relative to: (1) standard chemical fertilization application; and (2) manure lagoon management and field application, accounting for the hazardous levels of agricultural nitrogen and phosphorus accumulation.

The **methodology** employed in each study was a modified single organism, multiple baseline design that provided inter- and intra-subject replication. The pretest, post-test measures, as well as control versus treatment samples were used to compare reduction and performance changes regarding nitrogen and phosphorus levels.

FIELD CASE STUDIES (16)

In light of the issues of this article, several examples are submitted by way of illustrations that corroborate the article's hypothesis.

I. Dale Love project, as reported to the Kansas Department of Natural Resources

This project involved an accidental spill that covered approximately 1 acres with 336 000 gallons of swine manure from the lagoon. The facts indicate that there were approximately five gallons of swine manure applied to each square foot of a 1 z> acre area.

A bio-stimulant was used to increase the rapid reproduction of indigenous microorganisms. This study reports that the microorganisms dramatically digest and process toxic levels of nitrogen, thereby reducing the harmful levels of nitrogen and the detrimental effects of the excess nitrogen. More specifically, through bio-degradation, the harmful levels of nitrogen in the field

were reduced by the following process: (1) accelerating the denitrification action, venting nitrogen oxide (NO₂) to ambient air; (2) incorporating nitrogen (N) into microbial cell development; and (3) implementing the nitrification sub-cycle. According to the report, field test results indicate that this remediation reduced the harmful levels of phosphorus that were excreted in the manure of swine, poultry, and other animals.

II. ALCECO Cooperation project, as reported to the Iowa Department of Natural Resources

The following list describes the purposes of this project:

- A. Determine the approximate amount of hog manure, liquified by treatment with EC&S Bio-Stimulant, that can be applied via top-spray on a corn crop without destroying the crop. This approximate amount is referred to throughout this report as the threshold amount.
- B. Assess the negative and positive effects of applying the threshold amount of liquified hog manure on the yields of the corn crop.
- C. Assess the negative and positive effects on the soil of the corn field after the crop sprayed with the threshold amount of liquified hog manure is harvested.
- D. Determine whether the odor from the treated hog manure is less offensive than the odor from untreated hog manure.

Fields of corn were foliar sprayed with hog manure, pre-treated with a bio-stimulant: field #1, @ 4000 gallons of hog manure per acre; field #2 @ 8000 gallons of hog manure per acre; field #3 @ 4000 gallons of hog manure per acre, and field #4, the control field, received no pre-treated hog manure. The bio-stimulant, pre-treated hog manure, experienced a major reduction of offensive odor compared to the untreated hog manure.

The average yield per acre from the three test plots of the corn fields foliar sprayed with bio-stimulant treated hog manure was 3.19 bushels per acre greater than the yield per acre from the corn field control plot. Applying current corn prices, about \$8.93 more per acre was derived from the corn test plots, when compared to the amount per acre derived from the control corn plot.

III. Poultry manure study, conducted by the University of Delaware (17)

(University of Delaware CI^T 3-4 Poultry Water Additive to Reduce Nutrients in Broiler Manure)

CI 3-4 poultry water additive contains multiple sources of enzymes that reportedly enhance the utilization of ingredients in poultry feed. With increased digestibility of feed and/or more effective absorption of nutrients, broiler performance, particularly feed conversion, should be improved. Because there is a potential for increased uptake of nutrients by the birds, fewer nutrients should be excreted. Due to increased environmental concerns

with potential excess of poultry manure (particularly nitrogen and phosphorus in the excreta) being generated in some concentrated poultry producing areas, solutions to this problem are being sought. This study was conducted to make a *preliminary* assessment of the effect of CI 3-4 water additive on potentially reducing the nutrients excreted in broiler manure. Nutrient composition of manure from male broilers provided CI 3-4 water additive from 34 to 39 days of age. During this five-day assessment period, composition of the manure did not differ significantly between treatments. Compared to the control, there was a trend for less total N (12%), P₂O₅ (3%) and K₂O (12%) in the manure from birds provided the CI 3-4 water additive.

Discussion of poultry study

The duration of this study would typically represent only 10% of the broiler life. Considering the effects of this additive on performance may be cumulative with age, the minor improvements observed in this study could be more pronounced when broilers are subject to the additive for the full production period. Furthermore, response to this additive should be evaluated for both sexes, over a wider range of feeding regimes and under commercial-like growing conditions. The manure composition results, particularly N and K₂O data, suggest there may be a reduction in some major nutrients in manure from broilers provided the CI 3-4 water additive. Considering the possible reduction of some nutrients in manure and the slight improvement in body weight gain observed in this limited assessment, a more comprehensive evaluation appears warranted.

DISCUSSION AND SUMMARY

Whatever pathway of modification prevails, the results include data that metabolic competence of various bacteria can be used by man to perform services ranging from the bioremediation or digestion of animal waste, sewage and oil spills, to the generation of such products including vitamins and even antibiotics. Included in this group are also the complex conversions of such inorganics as nitrogen, phosphorus and sulfur. Nature pivots on natural balance. As reported in the literature, decomposer bacteria, as they are often referred to, can digest such a large variety of molecules that there seems to be no organic molecule that cannot be digested by at least one species of bacteria. Thereby, the decomposing bacteria serve a critical function in recycling matter in eco-systems and in making inorganic molecules available to

photosynthesizers, thus assisting human health and safety.

Thus, it seems we can formulate that microbial life chemically biodegrades nitrates by one of two methods: (1) assimilative reduction; or (2) dissimilative reduction, also known as denitrification. With the assimilative method of nitrate reduction, microbial life reduces nitrites (NO_2) to nitrates (NO) which developed from oxidized ammonia (NH_3). For a specific definition, this is the follow-up of nitrification, which included the formation of nitrates and nitrites from ammonia (or ammonium compounds) as in soils by microorganisms.

Interestingly, it is the reduction of the inorganic form of nitrogen, which includes the assimilative process of microbial oxidation of ammonia, where the nitrogen forms specifically NH_4 (as well as phosphorous and sulfur) that becomes part of the microbial construction of cell walls, cell membrane, and various forms of amino acids. On the other hand, denitrification includes the venting off of the excess amounts of nitrogen (N) not required by the soil or needed for additional microbial development. Denitrification includes the process by which nitrates or nitrites in the soil or organic deposits are reduced to lower oxides of nitrogen by bacterial action. Again, the process results in the escape of excess nitrogen into the air.

It would therefore seem likely that nitrate reduction by way of denitrification is a functional part of a metabolic process referred to as anaerobic respiration. Included in this process, the facultative anaerobic microbes incorporate nitrate as the terminal electron acceptor at the end of their electron-transport chain when two events occur: (1) when they run out of oxygen; and (2) when nitrate is present. The above mechanism's description also supports oxidative phosphorylation, which would be a mechanism similar to aerobic respiration. Thus, denitrification and phosphorylation may be considered as forms of respiration.

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