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Introduction

Research Program

Tracing Sediment Sources in Eastern Iowa by Using Stable Carbon and Nitrogen Isotopes: An Exploratory Research

Basic Information

Title:	Tracing Sediment Sources in Eastern Iowa by Using Stable Carbon and Nitrogen Isotopes: An Exploratory Research
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Principal Investigators:	Thanos Nicholas Papanicolaou

Publication

Tracing Sediment Sources by Using Stable Carbon and Nitrogen Isotopes: An Exploratory Research

Thanos Papanicolaou

Summary

A fingerprinting technique that can help in elucidating the pathways of soil particles and temporal discontinuities in soil delivery is developed. Our composite fingerprint—due to its simplicity, low sampling requirements, and unique ability to distinguish soils produced from different land uses—can be effectively used in large-scale watersheds to quantify soil movement. The ability of our method to identify soil sources over large scales and to quantify soil movement at short time scales is inherent in the use of C-13, N-15, and C/N fingerprints. The stable isotope C-13 reflects the signature of the parent vegetation at different spatial scales; the mean elemental atomic ratio C/N ratio is reflective of plants and microorganisms that have inhabited the soil at different spatial scales; and the stable isotope N-15 reflects the temporal variations in the organic content of soils, primarily due to volatilization and organic decomposition. Our approach leads to the development of a composite tool that, for the first time, incorporates both statistically verified isotopic signatures of soils and a mixing optimization model to (1) identify the soil provenance originated from contrasting land uses; and (2) provide the proportion of soil particles produced by different land uses.

Introduction

Unwarranted soil erosion creates problems for land users, watershed ecosystems, and riverine habitats. While an urgent need exists to control soil erosion, mitigation is delayed as watershed managers and engineers are often unable to identify and measure erosion sources. Conceptually, soil erosion is easy to visualize. Soil stored in upland slopes erodes through fluvial erosion or mass failure (i.e., landslides, bank failure) to footslopes and floodplains, where it is either stored temporarily or continues movement into streams. While seemingly simple, measuring soil erosion has proven a complicated task; and existing monitoring techniques, such as erosion pins, erosion troughs, and scanning laser altimetry (LIDAR) are often expensive and face important spatial and temporal sampling constraints at the watershed scale. Spatial fractionalization of suspended soil serves as a practical approach to measure erosion from spatial regions within a watershed.

Spatial fractionalization refers to a field-based monitoring procedure that measures the relative soil eroded from spatially defined regions within a watershed. Spatial fractionalization involves a three-step process, including: (1) capturing eroded soil at the watershed outlet, (2) measuring the distributions of bio-chemical, geo-chemical, mineral magnetic, radionuclide, and/or physical soil properties, and (3) using the properties to partition or fraction the eroded soil into components derived from spatial regions within the watershed. The term fractionalization is derived from sediment transport literature. Fractionalization is traditionally used to define the distribution of particle sizes for stream sediments. Similarly, spatial fractionalization of soils defines the distribution of source-soils within the eroded sample.

Spatial fractionalization of eroded soils has been used previously, typically by researchers in the geologic, pedologic, and geographic fields under different taxonomy (e.g., suspended sediment fingerprinting, soil tracing). This past work has widely utilized mineral magnetic, geo-chemical, and radionuclide properties to connect suspended soils with their spatial provenance via a multivariate,

error-minimization model (i.e,. un-mixing model). At the watershed scale (>500 km^2), research has focused primarily upon fractionalizing eroded soils from pedologically contrasting sub-units (i.e., different soil associations) dependent upon geologic diversity.

The work herein aims to establish the use of nitrogen-15, $d^{15}N$, and carbon-13, $d^{13}C$, stable isotopes, and the C/N atomic ratio as bio-chemical soil properties that may be used to spatially fractionalize eroded soil from different source-soil land uses—herein from forest vs. agriculture land uses—at the watershed scale. Spatial fractionalization based on land use is important because forest vs. agriculture soils have very different erosion rates. For example, fields used for crop production are, typically, a significant source of fine soil particles because the soil surface is disturbed through several tillage operations over the growing season. On the other hand, forests are more stable, with lower amounts of soil delivery, unless disturbed by natural disaster or human activity. Quantifying these differences using spatial fractionalization will benefit erosion prediction model development and will aid watershed managers in decision strategies.

It is well documented that soil consists of inorganic and organic constituents within a composite aggregate matrix. The organic constituent is reflective of vegetative cover and land management (e.g., fertilizers, nutrient cycling due to tillage practices), and $d^{15}N, d^{13}C$ and C/N properties may be used to quantify these organics. $d^{15}N, d^{13}C$ and C/N are based on carbon and nitrogen atomic arrangement within soil and are dependent upon a number of processes, such as vegetation decomposition, fertilizer sorption, and denitrification. These processes are altered due to soil-environmental factors, such as vegetation type, soil moisture and temperature, concentration of soil gases, and land/crop management. Therefore, variations in $d^{15}N, d^{13}C$ and C/N exist when comparing forest vs. agriculture land uses but also may exist when moving across the landscape, deeper into the soil profile, to different plot locations within the same land use or during seasonal extreme conditions. Identifying the significant factors that induce variation of $d^{15}N, d^{13}C$ and C/N, and explaining that variation, is the thrust of the research presented herein. If the variation of $d^{15}N, d^{13}C$ and C/N may be accounted, then the soil properties may be adequately used for forest vs. agriculture land use fractionalization.

Recently, Papanicolaou et al. (2003) explored the feasibility of using $d^{15}N, d^{13}C$ and C/N for spatial fractionalization purposes in the Upper Palouse Watershed, Northwestern Idaho. However, $d^{15}N, d^{13}C$ and C/N variability warranted further experimental investigation within forest and agriculture land uses. The objective of the present report is to utilize 231 original soil data from the Upper Palouse Watershed to specifically address $d^{15}N, d^{13}C$ and C/N variation based on spatial and temporal factors including: land use, slope location, plot location within a given land use, sample depth in the soil profile, and sampling season. It is expected that the results presented herein will be utilized to further establish spatial fractionalization of forest vs. agriculture land uses as a soil erosion measurement technique at the watershed scale.

Background Regarding $d^{15}N, d^{13}C$ and C/N

As previously mentioned, $d^{15}N, d^{13}C$ and C/N are soil properties based on carbon and nitrogen atomic arrangement within soil organics. In the following subsections, a brief introduction of the three

bio-chemical properties is presented including a discussion of the processes influencing variation. Realization of $\delta^{15}N, \delta^{13}C$ and C/N spatial and temporal variation sets the framework for the experimental investigation detailed in the methods.

$\delta^{15}N$. $\delta^{15}N$ is a signature proportional to the *15N:14N* isotopic ratio and has been used most prominently by environmental scientists to study nitrogen pollution in the hydrosphere and atmosphere; but $\delta^{15}N$ has also been used significantly for studying issues such as nitrogen cycling in plants and nitrogen cycling in lakes and coastal regions. While $\delta^{15}N$ fractionation—defined as the partitioning of isotopes (i.e., 15N and 14N) and thus the altering of $\delta^{15}N$ —within soil environments has been difficult to implicitly quantify and is still under investigation, it is accepted that the variation of $\delta^{15}N$ values for inorganic and organic soil constituents is induced by processes including assimilation, mineralization, volatilization, denitrification, and decomposition. These processes are accompanied by fractionations, whereby plants or soil discriminate between nitrogen isotopes, tending to favor the incorporation of *14N* over *15N* or visa versa, and, thus, the soil $\delta^{15}N$ value is depleted or enriched. $\delta^{15}N$ values are typically higher for agriculture soils as compared to forest soils under similar environments.

$\delta^{13}C$. $\delta^{13}C$ is a soil property proportional to the *13C:12C* isotopic ratio which highly retains the signature of the parent vegetation. That is, $\delta^{13}C$ of soil reflects that of vegetation with only a small enrichment during decomposition. Due to this fact, $\delta^{13}C$ has been extensively used in the soil research for paleo-environmental studies of changes in vegetation and climate and to investigate soil carbon dynamics.

The well documented difference in $\delta^{13}C$ values for C3 and C4 plants induced by the unique photosynthetic pathway of each plant type results in the average plant tissue $\delta^{13}C$ values of –12‰ for C4 plants and –26‰ for C3 plants. Within the C3 or C4 plant types, $\delta^{13}C$ values of individual plant species can additionally vary by several parts per mil and are dependent upon a number of factors including soil water, humidity, genetic response to water or salinity stress, drought stress, irradience levels, plant nutrition, altitude, and the assimilation of soil repired CO_2 in closed canopies. Thus, forest vs. agriculture soils attain distinct $\delta^{13}C$ fingerprints due to contrasting vegetation and distinct environmental regimes.

C/N. The *C/N* atomic ratio—defined as the ratio of total atomic carbon to total atomic nitrogen—exists as a proxy typically used for ecosystem health and processes by soil fertility experts. The soil *C/N* ratio is reflective of plants and microorganisms which have inhabited the soil. Terrestrial plants have a wide *C/N* ratio range primarily between 10 to 40 (e.g., gymnosperms=16.4; pteridophytes-ferns, plants with spores=25.6); however, values as high as 90 are not uncommon. Most microorganicsms have *C/N* ratios between 4 and 9. As plants decompose, the trend is the loss of carbon due to microbial oxidation and respiration of CO_2 and the sequestering of nitrogen, resulting in a decrease of the soil organic matter *C/N* ratio relative to plants. A number of reasons account for differences in *C/N* when comparing forest vs. agriculture soils, including vegetation type, decomposing organic complexes, carbon and nitrogen losses during cultivation, and chemical fertilizers.

Study Watershed

Figure 1 illustrates the study area located within the Upper Palouse Watershed in Northwestern Idaho. Figure 1 details the designated watershed outlet within the city limits of Princeton, Idaho, located upstream of river mile 140 at Hattercreek Creek Road Bridge 1, approximately ½ mile south of the junction of Idaho SR 6 with Hatter Creek Road. This reach drains an area of approximately 600 km^2. The Palouse River, a gravel bed-cohesive bank river, and its tributaries occupy the drainage of the watershed. The majority of the study area is either public land controlled by the USDA Palouse Conservation Field Station, and is a target watershed designated by the Natural Resources Conservation Service (NRCS) for water quality and soil erosion research, or is part of the St. Joe (Clearwater) National Forest.

The Palouse River watershed was chosen due to its variable land uses. Deep intermountain valleys with relatively small floodplains characterize the upper portion of the Palouse River watershed. This topography prevails above Laird Park, located at elevation 809.4 m above mean sea level (Palouse Subbasin Summary, NWPPC 2001). In these upper portions of the watershed, the land is predominantly conifer forest. This region comprises the forest sampling area, and locations are illustrated in Figure 1. Rolling hills used for agriculture (primarily wheat and hay) dominate downstream of Laird Park. The hills have 15.6 % to 27.9 % steepness at approximately 750 m above mean sea level. About half or less of this cropland is in annual production. Roughly, there is a two- to three-year rotation of winter wheat/spring lentils or peas, or winter wheat/spring barley/spring lentils or peas. The remainder of the cropland is permanent grass used for hay or grazing, or is committed to the Conservation Reserve Program. This lower portion of the watershed comprises the agriculture sampling areas of the study and sites are illustrated in Figure1.

The climate in the Palouse Watershed consists of generally mild winters and summers punctuated by occasional high or low temperatures. The soil of the basin can freeze to 20–30 inches of depth during the more extreme winters. The average annual precipitation (1961–1990) is 536 mm, falling mostly between October and May. The main erosion season is late winter to early spring, at which time approximately 249 mm of the annual precipitation occurs. As much as 90% of the soil loss is caused by surface thaws and snowmelt, primarily during February and March. The watershed soils are formed in a combination of parent materials including loess derived from south central Washington, underlying geologic rock, and volcanic ash from the eruption of Mt. Mazama approximately 8,000 years ago.

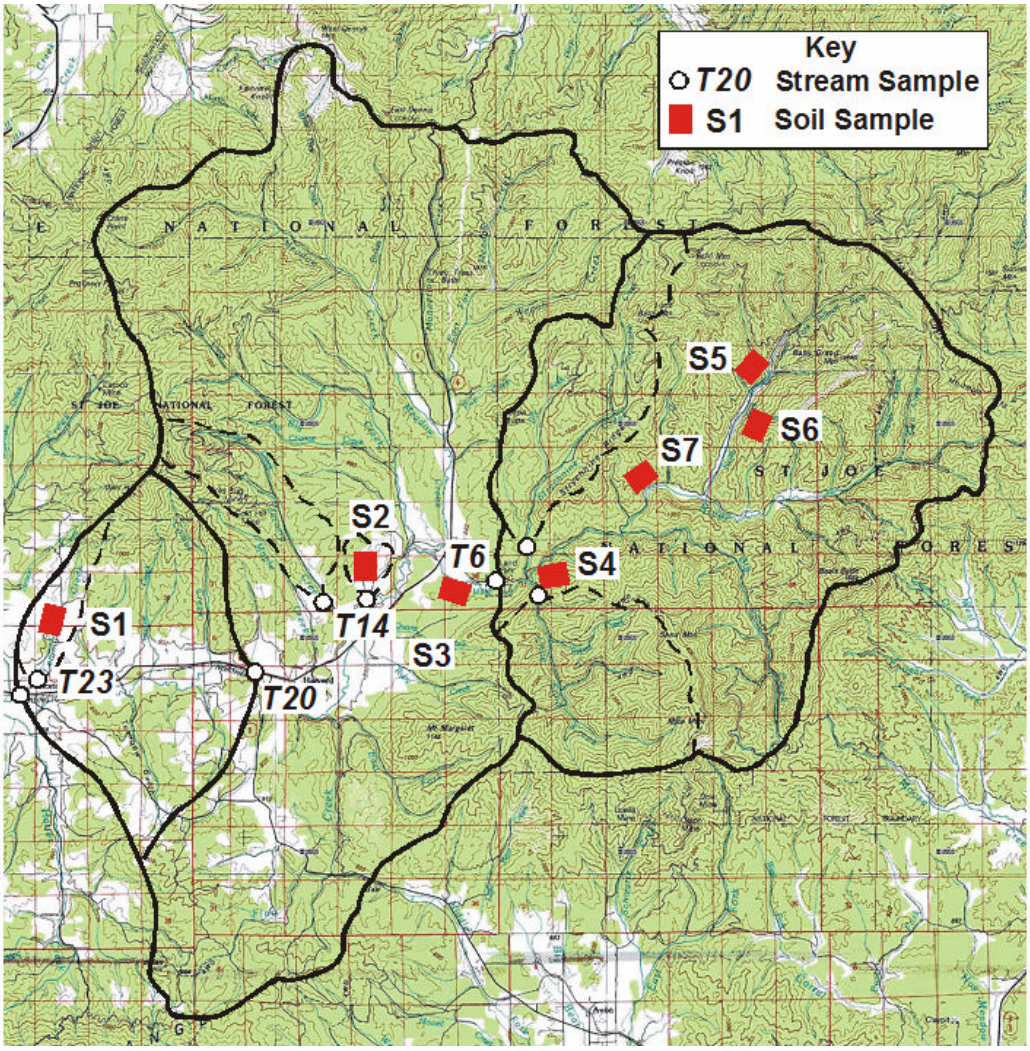


Figure 1. Upper Palouse Watershed and designated sampling sub-watersheds. Stream sampling and soil sampling locations are depicted with white circles and red rectangles, respectively.

Methods

Herein, a sampling experimental design is established and completed with the intent of capturing spatial and temporal variation of $d^{15}N, d^{13}C$ and C/N. The following sub-sections present details regarding soil sampling, lab procedures, and isotopic analyses.

Soil Experimental Design and Field Sampling. The experimental source-soil sampling design is established with the intent to explain $d^{15}N, d^{13}C$ and C/N variation via statistical analysis of variance (ANOVA). $d^{15}N, d^{13}C$ and C/N are established as three independent response variables, and a set of factors are varied during sampling to assess their effect on each response variable. Factors are chosen to represent pedologic variation within the watershed soils; thus a range of soil processes is indirectly accounted. Factors considered most relevant to induce $d^{15}N, d^{13}C$ and C/N alterations include: (1) *land use* (i.e., agriculture vs. forest), (2) *plot location* of sample within the land use, (3) *slope topography* of sampling, (4) *season*, and (5) *profile depth.*

Land use is the focus of this study and is expected to produce $d^{15}N, d^{13}C$ and C/N differences due to vegetative cover and land management. Over 100 samples are excavated within each land-use to provide 200+ samples for source-soil distributions. Plot location induces elevation and, thus, climatic gradients and has been shown to modify carbon isotopic composition of plants. In addition, various plot locations may introduce soil variation due to differing land management and pedologic history; therefore, numerous plots are sampled within both the forest and agriculture land uses. Table 1 compiles the plot location names, and Figure 1 illustrates plot locations with a red box for sampling.

Table 1. Names of soil sampling plot locations.

Number	Name	Description
1	Pienesta Grove	AG - 2650'
2	Private Land	AG - 2750'
4	Flooplain near Laird	AG - 2550'
5	Laird Park	FOR - 2600'
6	Eldorado Gulch	FOR - 3200'
7	N.F. of Palouse R.	FOR - 3000'
8	Student Sample location	FOR - 2700'

Slope topography may create microclimates and affect soil moisture conditions—important for denitrification and decomposition processes. To assess these possible effects on $d^{15}N, d^{13}C$ and C/N values, sampling is completed high on the slopes, referred to here as "slope samples," and at the toe of the slope near waterways, referred to here as "floodplain samples." Typically, sampling includes three repetitions at the slope and floodplain locations to address random variability among plots.

Seasonal sampling is deemed important because extreme experimental drought treatments have modified stable carbon isotope ratios by as much as 2‰ for plant species. This past work introduces uncertainty for soil isotopic values; therefore, sampling is completed in eight seasons to assess seasonal variability. In March 2003, sampling was restricted to one forest location due to inaccessible forest roads.

When excavating deeper into the soil profile, an older, more highly decomposed organic constituent is attained; therefore, sampling of the surface topsoil (0–5 cm) and subsurface soil (5–20 cm) is completed to assess $d^{15}N, d^{13}C$ and C/N variation among profile depth. Soil deeper than 20 cm is not sampled due to the fluvial-erosion mechanism and low probability of very deep soils experiencing erosion.

Sampling techniques for soils rely on accepted methods of pedologic and environmental scientists. For each sample, soil pits are excavated and one sample is removed from the 0–5 cm level, omitting the root mat and litter layers from the agriculture and forest soils, respectively. A second sample is then removed from deeper in the profile, generally 5–20 cm. The samples are individually wrapped in aluminum foil, labeled, and placed into plastic Zip-lock bags. Aluminum foil is chosen due to its

absence of carbon or nitrogen components and, therefore, a decreased chance of contaminating soil during transport and storage. Packaged samples are placed into a cooler in order to keep samples close to field conditions (i.e., cool and free of light) until return to the laboratory.

Lab Methods. In the lab, sub-samples for $d^{15}N, d^{13}C$ and *C/N* analysis are removed from each field soil and placed in an aluminum drying dish, labeled with a lab control number, weighed, and dried at 55°C until a constant weight is reached. Roots and plant material representing coarse particulate organic matter, coarse POM, and detritus (i.e., diameter, $d > 250$ µm) are removed from soils, and the samples are ground. Soil particles with diameter, $d < 250$ µm are targeted due to their fate as eroded soil. Soil particulates with $d < 250$ µm, are termed mineralized-SOM ($d < 52$ µm) and fine-POM (52 µm $< d <$ 250 µm) by environmental scientists. By indicating the $d < 250$ µm size range, a highly recalcitrant (i.e., non-labile) organic matter fraction is targeted and, thus, conservative soil properties are attained. Soil samples are checked for carbonates (i.e., inorganic sedimentary carbon), and all soils passed the effervescence test.

Isotope Analysis. Following preparations, sub-samples are transported to the University of Idaho Natural Resources Stable Isotope Laboratory. For isotopic analysis, the material is packed into tin cups, sealed and flash-combusted in the presence of oxygen and a series of catalysts and chemical scrubbers in the Carlo Erba CHN-2500. CO_2 and N_2 produced during combustion are separated with a GC column and delivered by a continuous flow inlet system to a Finnigan MAT Delta Plus isotope ratio mass spectrometer. The mass spectrometer ran in "jump" mode to direct first the CO_2 and then the N_2 beams to the Faraday cups. Precision of this method is typically better than 0.2‰ for nitrogen and 0.1‰ for carbon. Reference gas peaks are placed immediately before and after the sample peaks to correct for instrument drift. Samples of dried egg albumen calibrated against an NIST standard are placed in every tenth position in the runs to provide a means of correcting the data to a known standard. The mass spectrometer analysis ultimately provides the C/N ratios and the isotope ratios for $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

All *C/N* values reported in this paper are expressed in the form of atomic ratios and are dimensionless numbers. Isotope data for carbon and nitrogen are expressed in "*delta*" (d) notation indicating depletion (-) or enrichment (+) of the heavy (higher-mass) stable isotopes ($^{13}C, ^{15}N$) compared to the lighter-mass stable isotopes ($^{12}C, ^{14}N$) relative to standard materials. Because of the small differences in isotopic ratios, the delta values are commonly multiplied by 1,000 and termed per mil (‰) notation, so that the resulting numbers are greater than 1 or -1, depending on the sign. The *delta* notation (d) refers to differences between the isotopic ratio of the sample and accepted standard materials expressed as:

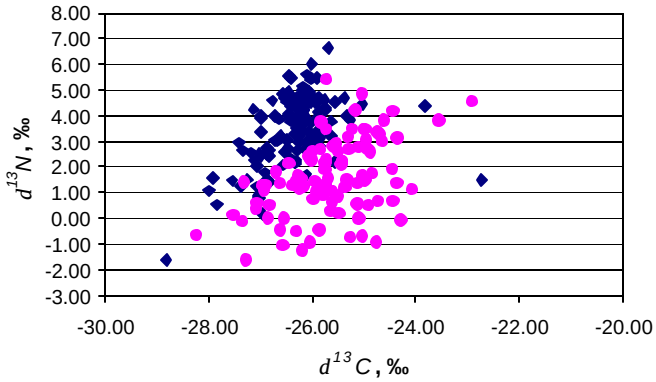
$$dX\ (in\ ‰) = \left(\frac{R_{sample}}{R_{std}} - 1 \right) \bullet 10^3 \qquad (1)$$

where X in our case ($^{13}C, ^{15}N$), R_{sample} is the isotope ratio ($^{13}C/^{12}C$, or $^{15}N/^{14}N$) of the sample and R_{std} is the isotope ratio of the standard (Vienna Pee Dee Belemnite, VPDB, and atmospheric nitrogen, respectively). The dX is measured by mass spectrometry in the laboratory (for more details see the methods/under tasks section).

Results

Figure 2 illustrates scatter plots of a) $d^{15}N$ vs. $d^{13}C$, b) C/N vs. $d^{13}C$, and c) $d^{15}N$ vs. C/N for the 231 soil data. In the figure, pink circles represent forest data and blue diamonds represent agriculture data. Values are presented separately on *x*- and *y*-axis with $d^{15}N$ and $d^{13}C$ in units of ‰ and C/N as dimensionless.

a)



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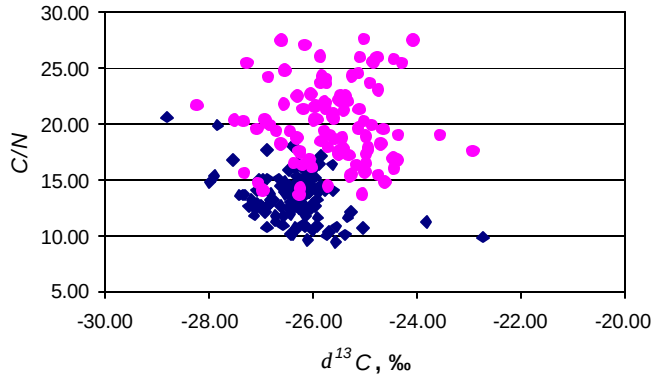

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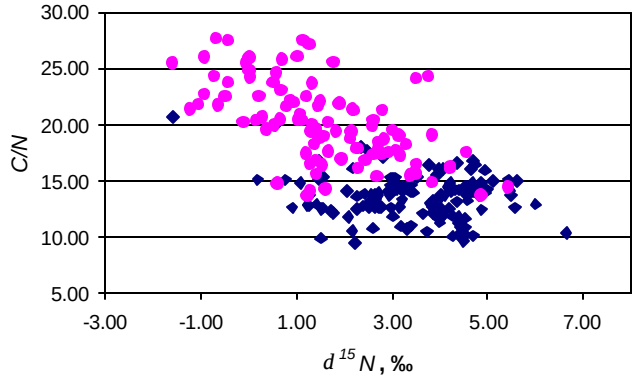

Figure 2. Scatter plots of a) $d^{15}N$ vs. $d^{13}C$, b) C/N vs. $d^{13}C$, and c) $d^{15}N$ vs. C/N for the 231 soil data. In the plots, pink circles represent forest data and blue diamonds represent agriculture data. Fingerprint values are presented separately on *x*- and *y*-axis with $d^{15}N$ and $d^{13}C$ in units of ‰ and C/N as dimensionless.

The plots qualitatively display the separation of the forest and agriculture data using the soil properties. The plots illustrate the variability associated with the soil properties for the spatial and temporal sampling throughout the watershed but offer promise for the soil properties as a spatial fractionalization tool. In the following sub-sections, results of soil $d^{15}N, d^{13}C$ and C/N isotopic analysis are presented, and the statistical analyses to assess variability are established. Soil sample results are evaluated with the ANOVA analysis to address variation of $d^{15}N, d^{13}C$ and C/N, focusing upon variation based on spatial and temporal factors. Within ANOVA, $d^{15}N$ or $d^{13}C$ or C/N are used independently as response variables, and land use, plot location, season, profile depth, and slope location are specified as factors. ANOVA models are performed for each response variable (i.e., $d^{15}N$ or $d^{13}C$ or C/N) with the inclusion and exclusion of factors and factor interactions. Results of the analysis are included herein for only the models which highlight the prominent role of the factors and best explain data variation. Analysis of means (ANOM) is also administered in order to quantify values for the trends deciphered from ANOVA. *Minitab Statistical Software 13.0* is utilized for the ANOVA and ANOM analyses.

$d^{15}N$ **Variation.** Table 2 illustrates the results of ANOVA "Model 1" with $d^{15}N$ as the response variable and land use, profile depth, slope location, the interaction of land use and slope location, plot location, and season as factors. In Model 1, plot location is specified as nested within land use (i.e., PlotLoc(Land-use)), that is, plot locations 1–4 in Table 2 are only associated with agriculture land use, and plot locations 5–8 are only associated with forested land use. Table 2 compiles F and p-values indicating the factor's importance and the probability for the factor's effect to be insignificant; therefore, a low p-value indicates high significance. In Model 1, land use, profile depth, and plot location exhibit significance as factors, indicated by their low p-values, < 0.0001.

Table 2. Results of ANOVA Model 1 for $d^{15}N$ variation.

Factor	F	P
Land Use	43.370	0.000
Profile Depth	20.410	0.000
Plot_Loc(LandUse)	17.550	0.000
Slope Location	1.820	0.165
LandUse*SlopeLocation	2.010	0.137
Season	3.240	0.023

Table 3 presents the ANOM for the land use factor and includes all 231 soil data. $d^{15}N$ values are 3.46 and 1.59‰ for agriculture and forest land uses, respectively. The values differ as compared to preliminary data for the Palouse Watershed (i.e., forest mean $d^{15}N$ = 0.78‰ vs. agriculture mean $d^{15}N$ = 4.74‰), primarily attributed to the inclusion of multiple plot locations in the new data (Papanicolaou et al., 2003); however, the trend of an increase in $d^{15}N$ value of agriculture soil relative to forest soil is maintained in the more extensive dataset. This trend agrees well with forest vs. agriculture $d^{15}N$ values in other physiogeographic regions.

Table 3. ANOM for agriculture and forest land use soils.

Land Use	Samples	$\delta^{15}N_{AIR}$	$\delta^{15}N_{AIR}$	$\delta^{13}C_{PDB}$	$\delta^{13}C_{PDB}$	C/N	C/N
		Mean	StDev	Mean	StDev	Mean	StDev
		‰	‰	‰	‰	-	-
Agriculture	134	3.46	1.33	-26.35	0.68	13.48	1.97
Forest	97	1.59	1.47	-25.65	0.90	20.07	3.56

Conclusions

Our findings from the research can be summarized as follows:

1) $d^{13}C$ reflects the signature of the parent vegetation effectively and can clearly distinguish soils produced by land uses with contrasting parent vegetation (C3 vs. C4).
2) $d^{13}C$ can distinguish soils produced from different plot locations, even in monoculture environments, if the factors related to C biogeochemical cycle introduce variations that are greater than the variation (error) introduced by the isotopic analysis performed via the mass spectrometer. Mass spectrometer errors do not typically exceed 0.1 ‰ .
3) C/N is an effective indicator of different land uses.
4) $d^{15}N$ has the highest variation in comparison to the other two fingerprints (see figure 1(b)).
5) The factors considered most relevant to induce $d^{15}N, d^{13}C$ and C/N alterations include: (a) land use, (b) plot location of sample within the land use, (c) slope topography of sampling, (d) season, and (e) profile depth. These factors depict pedologic variation within the watershed soils; thus, a range of biogeochemical soil processes is indirectly accounted.
6) Well established statistical tools can be used to adequately describe the factors considered most relevant to induce $d^{15}N, d^{13}C$ and C/N alterations.

Based on the Palouse Watershed study, it was concluded that some research issues must be further investigated, including:

1) The fact that $d^{15}N$ presents a high variability can be advantageous for conducting short-term erosion studies as long as the prominent factors introducing such variability are understood and their variability can be statistically verifiable.
2) In order to develop a reliable composite tool, the spatial variability of the signatures as function of the above factors needs to be considered and modeled via statistical tools.
3) The Palouse study is limited to the fingerprinting of agriculture and forest land uses. The contributions of roads, gullies, and other sources need to be explored in the future research if the development of a general fingerprint tool is the ultimate goal.
4) Soil samples used for isotopic analysis and soil fingerprinting should offer adequate spatial and temporal representation of the different signatures.

References

Papanicolaou, A., Fox, J., and Marshall, J. (2003). Sediment Sources Fingerprinting in the Palouse River Watershed, USA. *International Journal of Sediment Research*. 120:23–29.

Veterinary antibiotics: Transport to and degradation in surface water

Basic Information

Title:	Veterinary antibiotics: Transport to and degradation in surface water
Project Number:	2003IA36B
Start Date:	3/1/2003
End Date:	2/29/2004
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Research Category:	Not Applicable
Focus Category:	Toxic Substances, Non Point Pollution, Water Quality
Descriptors:	
Principal Investigators:	Joel Robert Coats

Publication

Veterinary Antibiotics: Transport to and Degradation in Surface Water

Joel R. Coats

Summary

Tylosin: mobility in a manure-soil matrix and dissipation in surface water. Tylosin is a veterinary antibiotic commonly used as a livestock feed additive for growth promotion and disease prevention. Tylosin enters the environment via application of manure to soil and has recently been detected in many surface water bodies. Little is known about the fate of tylosin in a manure-soil matrix. In this study, the mobility of tylosin was investigated using intact soil columns treated with swine manure spiked to 5 mg/kg tylosin. Following a single rain event, leachate was examined for tylosin. Using ELISA and LC/MS/MS, 0.8 ng/mL total tylosin was detected in leachate, with tylosin isomers A and D comprising 22% and 65%. Over 80% of total tylosin applied to soil columns was tylosin A, thus indicating differential mobility and/or persistence of tylosin D. Our data indicate that tylosin can move in an agronomic soil. Our second objective was to determine the fate of tylosin in surface water and the potential for phytoremediation by an aquatic plant (*Ceratophyllum demersum*). Dilute pond water fortified to 10 ng/mL tylosin received treatments including 0.1% manure solution and vegetation, alone and in combination. Dissipation of tylosin was monitored over 24 days. Solutions receiving manure treatments had a significant decrease in tylosin concentration compared to manure-free treatments beginning at day 4; this decrease could be due to increased microbial degradation or binding of tylosin to organic matter. Vegetation did not have a significant effect on dissipation during the 24-day test; however, trends indicate a possible effect beyond day 24. This study provides data and methods that may be useful in risk assessment of antibiotics and manure management in the environment.

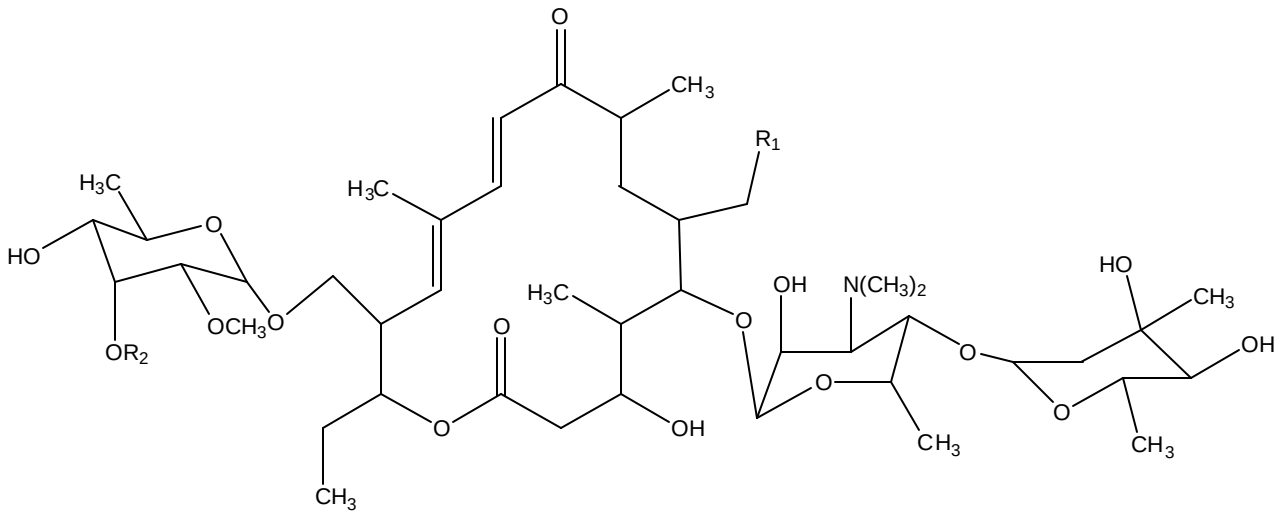
Introduction

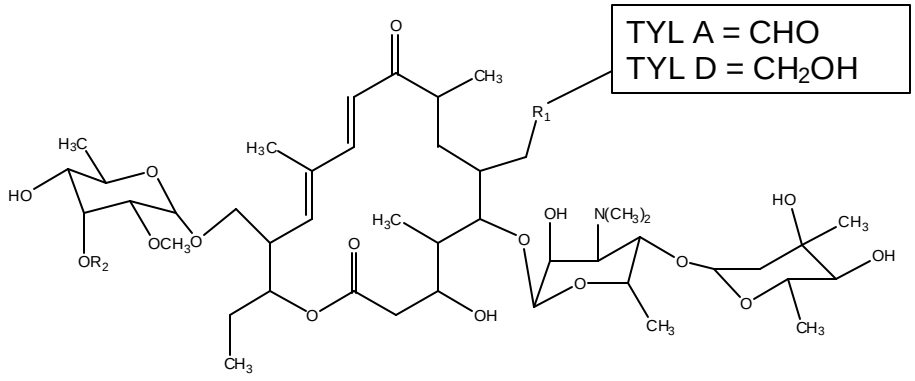
Tylosin

- Macrolide class, related to erythromycin.
- Used only in veterinary medicine.
- Mostly active against gram-positive, but also some gram-negative bacteria.
- Mode of action: Inhibition of transcription at 50S ribosomal subunit.

(FAO/WHO, 1991)

Tylosin





- Tylosin A (TYL A) is most prevalent in formulation at >80%.
- Tylosin D (TYL D) is 2nd at ~10%.
- Tylosins B & C are less prevalent forms.
- Koc = 500 to 8000, pKa = 7.1, water solubility = 5 g/L.

- Tylosin is ranked first among antibiotics used in swine production (31.3% of sites
- surveyed) (Bush & Biehl, 2001).
- It has been well documented that antibiotics, including tylosin, are excreted in urine and feces and may be converted from metabolized forms back into parent compound.

Purpose

Need for investigation of environmental fate of veterinary antibiotics because of high usage rates and concern over microbial resistance.

Objectives

- To evaluate the mobility of tylosin and *E. coli* in a manure-soil matrix.
- To determine the fate of tylosin in surface water and the potential for phytoremediation by a submerged aquatic plant species.

Results

Manure treatment appears to have an effect:

- Possible binding to organic matter.
    - Extraction.
- Possible microbial degradation related to manure.
    - LC/MS/MS at end of study to determine metabolites.

Immunoassay

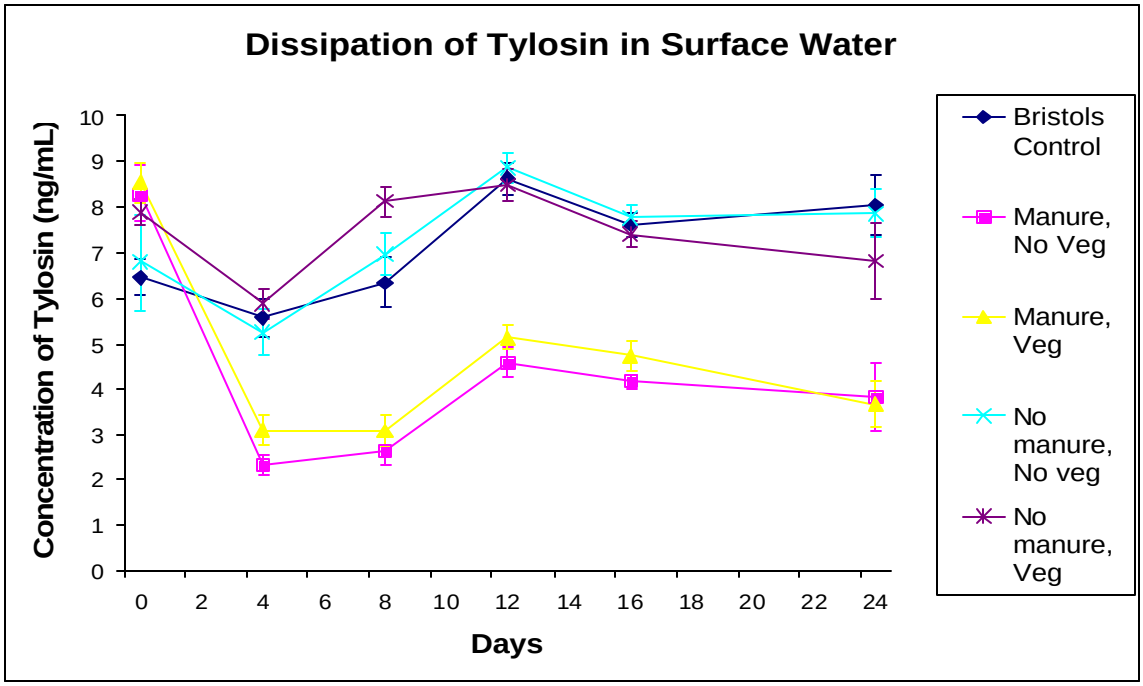
- Treatment leachate mean value of tylosin 0.60 ng/mL (se=0.08).
- Background interaction of control leachate.
- Mean=0.35 ng/mL; se=0.07.
- Detection limit = 0.5 ng/mL.

No significant difference between treatment and control values (two-sided p-value = 0.0577).

Methods—Dissipation in Surface Water

- No significant differences at initial time point.
- Significant differences at days 4, 8, 12, 16, and 24 between treatments with manure and those without manure (0.05 level; p-value <0.001).
- Coontail did not appear to have an effect on tylosin dissipation (two-sided p-value = 0.0809).
- Manure treatment appears to have an effect.
- Possible binding to organic matter.
- Possible microbial degradation related to manure.

Results


Dissipation of Tylosin in Surface Water
Concentration of Tylosin (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
14
16
18
20
22
24
Days
Bristols Control
Manure, No Veg
Manure, Veg
No manure, No veg
No manure, Veg

Conclusions

- Tylosin D vs. Tylosin A
  - D may be more stable or more mobile.
  - TYL A may be more easily degraded or less mobile.
- Interaction with immunoassay needs to be explored.
  - Actual background tylosin highly unlikely, possible cross reactivity with native microorganisms producing very low levels of tylosin.
- Preliminary results indicate that tylosin can move in an agronomic soil.
- Previous work by Rabølle & Spliid (2000) in sandy soil indicated no tylosin in leachate.
  - Limit of detection 7 µg/L.

Future Directions

- HPLC-UV and LC/MS/MS methods correlated with enzyme immunoassay.
- Repeat study with varied manure application rates and tylosin rates.
- Additional leaching events.
- Increased efficiency of extraction of tylosin from soil.

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Fate of Veterinary Antibiotics in Manure Lagoons

Basic Information

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Descriptors:	
Principal Investigators:	Say Kee Ong

Publication

Fate of Veterinary Antibiotics in Manure Lagoons

Say Kee Ong, Ph.D. and Tom Moorman, Ph.D.

Problem and Research Objectives

Much of the current research on manure management is focused on the reduction of odor and the proper disposal and treatment of manure. An area that needs attention is the fate of veterinary antibiotics in manure management systems. Antibiotics are commonly and heavily used in livestock production to prevent and cure sick animals and to improve the life expectancy and weight gain of the animals.

The fate of these chemicals is of environmental importance as it has been shown that highly resistant pathogens may develop within the manure management facilities and that these chemicals may interfere with the endocrine system of various aquatic species. Currently, research on the fate of these compounds in the manure management system and in the environment is very limited. The objectives of this study are to investigate the fate of two common antibiotics, tylosin and sulfamethazine, used in the swine industry. The focus will be on the sorption and degradation of these antibiotics in manure lagoons under anaerobic and aerobic conditions.

Methodology

The proposed research consists of analytical methods development, batch sorption studies and batch degradation studies. The antibiotic to be tested for this period is tylosin, a major antibiotic used in the swine industry. Manure will be obtained from two lagoons identified as slurry LR and slurry WTC. The manure will be characterized by measuring the slurry pH, total organic carbon, total dissolved solids, potassium, sodium, and ammonia.

A key aspect of studying antibiotics in the environment is the ability to analyze the antibiotics in various media and in low concentrations. Extraction of antibiotics from both liquid and sludge from waste manure using suitable solvents will be tested. To assess the extraction of the solvents, sludge or liquid manure will be spiked with a known amount of antibiotic and the recovery of the antibiotic determined. Triplicate samples will be used to assess reproducibility. The antibiotics will be analyzed using liquid chromatograph and liquid chromatograph-mass spectroscopy (LC-MS).

Batch sorption experiments will be conducted according to the American Society of Testing and Materials E1195-01 (ASTM, 2002). In a typical test, seven to eight sets of vials will be prepared with a given mass of manure in each vial along with liquid manure. The vials will be spiked with tylosin. Sodium azide will be added to each vial to inhibit microbial degradation. Each set of vials consists of either duplicate or triplicate vials to check for reproducibility of the sorption experiments. The amount sorbed will be estimated from the difference between the initial and final concentration of antibiotic in solution.

Anaerobic degradation studies will be conducted using a series of 120 mL serum bottles containing sludge from manure lagoons. The serum bottles will be spiked with a given amount of antibiotic, and the vials will be purged with nitrogen to ensure dissolved oxygen is removed. The vials will then be sealed using crimp-typed aluminum caps and Teflon-coated rubber septa. At different times, vials will be sacrificed and the concentrations of the antibiotics in both liquid and solid phases will be analyzed. The parent compound remaining and metabolites, if any, will be determined using LC-MS. Aerobic degradation experiments will be similarly conducted.

Principal Findings and Significance

Analytical Methods Development. Three different solvents' compositions were tested: methanol, methanol/acetontrile/0.1 M ascorbic acid (45:45:10, v:v:v), and acetonitrile/isopropyl alcohol. The average recoveries of tylosin from slurry LR and WTC as a percentage of the tylosin added using acetonitrile/isopropyl alcohol were 99% and 93%, respectively for vials sacrificed within 0.5 hours of spiking. For slurry LR, recoveries averaged about 41% after 24 hours for acetonitrile/isopropyl alcohol extraction. Methanol and acidified methanol each gave an average 38% recovery after 24 hours. Adding KOH to acetonitrile/isopropyl alcohol produced a higher proportion of tylosin B to tylosin A in the extracts. Of the three extraction solvents tested, acetonitrile/isopropyl alcohol was found to be the better extractant.

Anaerobic Studies. For both slurry LR and WTC, there was a rapid loss of tylosin (60% to 85%) within the initial 24 hours, after which the loss of tylosin slowed down. The rapid loss of tylosin within the first 24 hours may be due to sorption of tylosin to the solids. Substantial sorption of tylosin (90%) has been reported within 1–6 hours after spiking in soil and manure mixtures (Ingerslev and Halling-Sørensen, 2000). Half-lives may be used to compare the differences in the various treatments, but since the loss of tylosin was very rapid, the time for 90% disappearance of tylosin was chosen as a comparison. The estimated time necessary for 90% tylosin loss was 40 and 310 hours for slurry LR and WTC, respectively. The 90% disappearance times for slurry LR and WTC with azide were 90 hours and 500 hours, respectively indicating that faster degradation occurred in the unamended slurries.

Aerobic Studies. As in the anaerobic studies, there was a rapid loss of tylosin but with a lower amount of residual tylosin remaining at the end of the 72 hours. Less than 1% of the tylosin added remained after 12 days of aeration in slurry LR. The 90% disappearance time for aerated slurry LR was 12 hours as compared to 40 hours for the anaerobic slurry LR. For slurry WTC, the 90% disappearance times were 26 hours and 310 for the aerobic and anaerobic slurries, respectively.

Loss of tylosin in manure slurries can be attributed to biotic and abiotic degradation and to sorption (i.e. the formation of non-extractable bound residues). Biodegradation and abiotic degradation may occur but strong sorption to slurry solids was likely the primary mechanism of tylosin loss. Residual tylosin continued to persist in the slurry after 8 months of incubation indicating that tylosin degradation in lagoons is incomplete and that tylosin residues will be carried over to agricultural fields if the manure is land applied.

Tylosin Degradates. A degradate eluting at 20.6 minutes appeared within 12 hours after spiking in all unamended and azide-amended anaerobic and aerobically incubated assays. Neither aeration nor sodium azide affected the amount of degradate production. The degradate compound was not detected in slurry source materials before tylosin addition or in tylosin tartrate standards at pH 7.0 but did appear in sterile lagoon liquids and water at pH 9.2. The degradate's peak mass response was 934.5 mass units (mu) and its major fragmentation ion was 772.5 mu when analyzed with LC-MS-MS under positive ionization mode.

Publications

A paper was submitted to the Journal of Water Environment Federation for review and publication. Part of this work will be presented as a poster at the WEFTEC Conference in October 2004 and platform presentation at the American Association of Microbiology in September 2004.

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Effects of Grazing Management on Sediment and Phosphorus Losses from Pastures

Basic Information

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Principal Investigators:	James Richard Russell, Thomas M Isenhart, John L. Kovar, Steven K. Mickelson, Wendy J. Powers, Richard C Schultz

Publication

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2. Haan, M.M., J.R. Russell, W. Powers, J.L. Kovar, J.L. Boehm, S. Mickelson, S.I. Ahmed, and R. Schultz. 2003. Impacts of cattle grazing management on sediment and phosphorus loads in surface waters, IN Proceedings of the Second National Conference on Grazing Lands. December 7-10. Nashville, TN.
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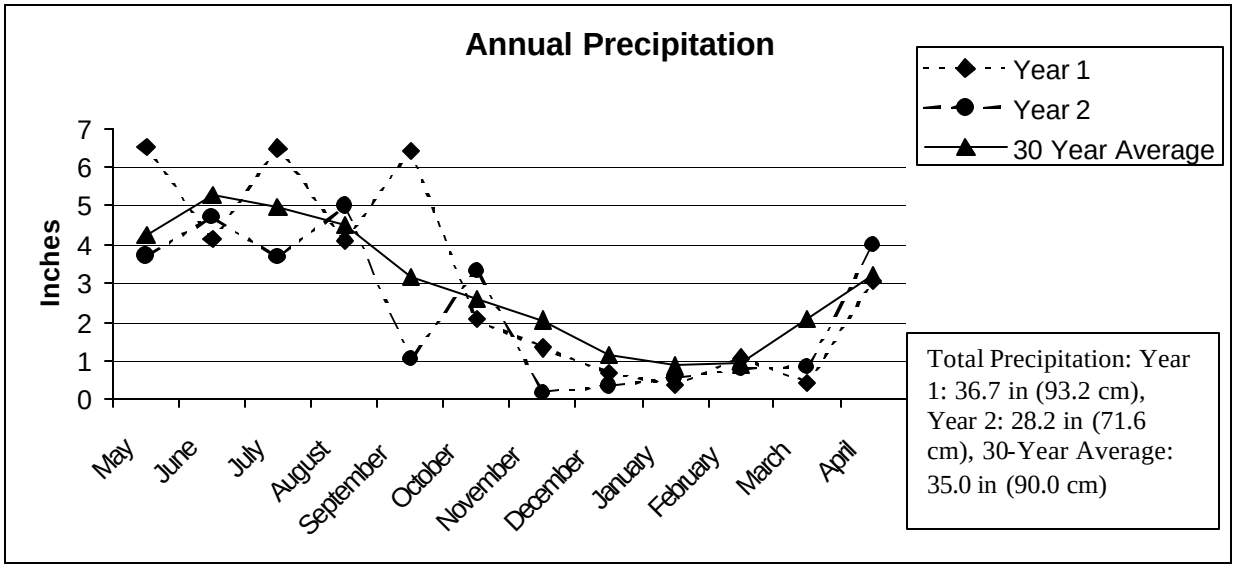
Effects of Grazing Management on Sediment and Phosphorus Losses from Pastures

James R. Russell, Wendy Powers, Richard C. Schultz,
Tom Isenhart, Steve Mickelson, and John Kovar

Materials and Methods

Site Description. The research was conducted at the Iowa State University Rhodes Research and Demonstration Farm (42°00' N, 93°25' W). Pastures were located on hills with slopes up to 15° and were primarily composed of smooth bromegrass (*Bromus inermis*). Average annual rainfall for the area is 35 in. (90 cm) (Figure 1).

Figure 1. Annual Precipitation.



Three blocks of approximately 6.8 ac (2.75 ha) were subdivided into five 1-ac (0.4-ha) paddocks, with an 18-ft (6-m) wide lane at the top of the hill for cattle movement and a 30-ft (10-m) wide buffer area at the bottom of the hill. Prior to the initiation of grazing in 2001, soil samples were collected to depths of 0 to 2.5 in (0 to 5 cm) and 2.5 to 5 in (5 to 10 cm) to determine soil P and K levels. Diammonium phosphate was applied in the spring of 2001 so that all pastures were at least at an optimum level (11–15 ppm P) of P. Soils in all paddocks contained an optimum level (81–120 ppm K) or greater of K; therefore, no additional K was applied. In both years, urea was applied at a rate of 180 lb/ac (200 kg/ha) before the start of grazing in the spring and 100 lb/ac (115 kg/ha) at the initiation of the forage stockpiling period, in August, to all pastures. Sandbags were placed around the perimeter of the pastures and between each paddock to prevent contamination from runoff by natural rainfall events from outside the experimental area and between neighboring paddocks. Prior to the initiation of the study, the research area was managed for hay harvest and moderate grazing of beef cattle.

Grazing Management. Grazing treatments were randomly assigned to each of the 5 paddocks in each plot. Treatments included: an ungrazed control (U), summer hay harvest with winter stockpiled grazing to a residual sward height of 2 in (5 cm; HS), continuous stocking to a residual sward height of 2 in (5 cm; 2C), rotational stocking to a residual sward height of 2 in (5 cm; 2R), and rotational stocking to a residual sward height of 4 in (10 cm; 4R). Grazing was initiated on May 29, 2001, and May 7, 2002, with 3 mature Angus cows (body weight 1430±185 lb (647±84 kg) in 2001 and 1350±207 lb (613±94 kg) in 2002) in each grazed paddock. Cattle received no supplemental P while stocked on pastures.

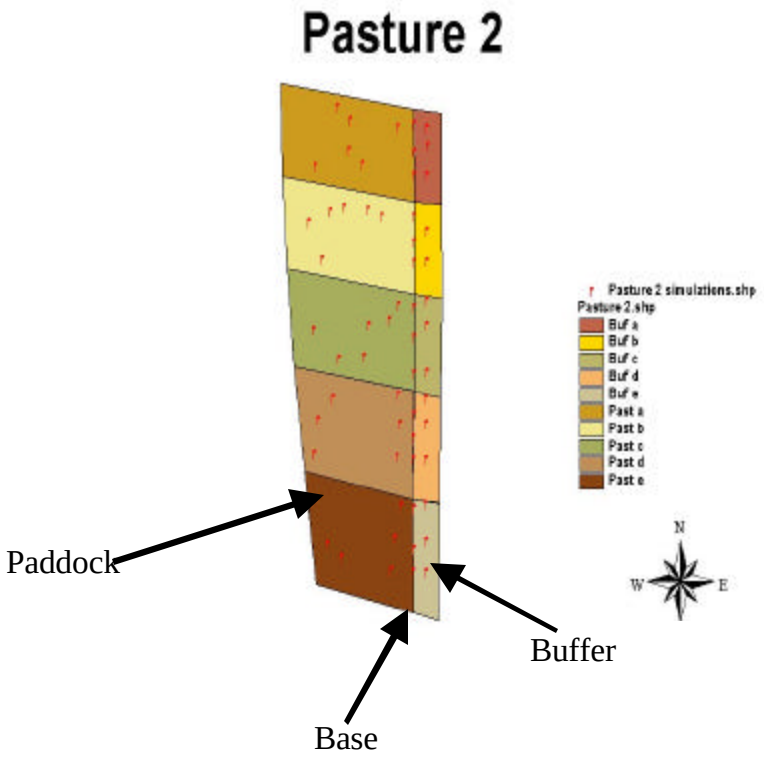
In the continuous stocking system, cattle were removed from the paddocks after the sward height decreased to 2 in (5 cm). Paddocks were allowed a rest period of 7 to 10 days to limit regrowth and thereby simulate continuous stocking. In the rotational stocking systems, cattle were removed from the paddocks after the sward height decreased to 2 or 4 in (5 or 10 cm). Paddocks were allowed rest periods of 35 days to allow plant regrowth. Forage sward heights were measured with a raising plate meter (8.8 lb/yd^2; 4.8 kg/m^2) twice weekly during the grazing seasons. During the 2001 grazing season, mean total grazing days were 199, 153, and 117 cow-days/ac (491, 360, and 274 cow-days/ha), and, during 2002 grazing season, mean total grazing days were 162, 128, and 104 cow-days/ac (400, 316, and 257 cow-days/ha) for the 2C, 2R, and 4R stocking systems, respectively.

First-cutting hay was harvested from the HS treatment in June of 2001 and 2002, yielding 2375 and 3326 lb/ac (2660 and 3624 kg/ha) forage dry matter, respectively. Regrowth from these paddocks was clipped in early August of each year to initiate forage stockpiling, but the yield of clipped forage was inadequate to harvest. Paddocks in the HS system were stocked in mid-November of each year, with animals that had been used during the summer grazing period, and grazed to a residual sward height of 2 in (5 cm), allowing grazing for 19 and 24 cow-days/ac (47 and 59 cow-days/ha) in 2001 and 2002, respectively.

Rainfall Simulations. To determine sediment and P loss in water runoff, rainfall simulations were conducted 4 times per year for 2 years (2001 and 2002). Simulations were conducted in the late spring, mid-summer, and autumn of each year and early spring of the following year. Six simulation sites were selected within each paddock; 3 within a low slope range (1° to 7°) and three in a high slope range (7° to 15°). Six simulation sites were selected within the buffer zone below each paddock. Three of these sites were at the base of the paddock and 3 were 30 ft (10 m) within the buffer strip (Figure 2). Rainfall simulation locations were identified with the geographical positioning system (GPS) so that the same locations could be used during each sampling period. Rainfall simulators were 5.4 ft^2 (0.5 m^2) and assembled so that the uphill side of the simulator was 3.3 ft (1 m) high (Bowyer-Bower, and Burt, 1989). Each rainfall simulation ran for 1.5 hours at a precipitation rate of 1.6 gal/10 min (6 L/10 min) corresponding to a rainfall rate of 2.8 in/hr (7.1 cm/hr). The water source used was rural water that had been filtered through an additional 0.45 µm filter, to remove particulate matter. During simulations, the amount of rainfall and runoff was measured at 10-minute intervals, and a sample of runoff was collected and added to a composite sample that was used to determine total sediment, total P, and total soluble P. Surface roughness was measured by digital photography of a 41-pin meter with a length of 6.5 ft (2 m; Russell et al., 2001), and ground cover was determined by the percentage of pins on the pin meter striking plant material. During simulations, soil samples were taken

adjacent to each site at depths of 0–2 in and 2–6 in (0–5 cm and 5–12 cm), for determination of Bray-1 P and soil moisture. Penetration resistance was measured at 1.4-in (3.5-cm) intervals to a depth of 14 in (35 cm) using a Bush Recording Penetrometer; readings from the 0 to 5 in (0 to 10 cm) depth, 5 to 8 (10 to 20 cm) depth, and 8 to 14 (20 to 35 cm) depth were averaged for statistical analysis. Sward height was measured using a rising plate meter (8.8 lb/yd^2; 4.8 kg/m^2), and a forage sample was clipped from a 2.7-ft^2 (0.25-m^2) area adjacent to the rainfall simulation site to determine the mass of forage dry matter.

Figure 2. Block 2 Layout.



Laboratory Analysis. Water samples were analyzed for sediment, total P, and total soluble P. Sediment was determined by filtering the water sample through a 0.45 µm filter (APHA, 1995). Total P was determined by digestion followed by the Ascorbic Acid Method (Hach, 2002). Total soluble P was determined by filtering through a 0.45 µm filter followed by digestion and the Ascorbic Acid Method.

Soil samples were analyzed for P and moisture. Phosphorus levels were determined using the Bray-1 P procedure (Frank et al., 1998). Soil moisture was determined by drying samples at 105° C for 24 hours. Surface roughness was calculated as the standard deviation in pin height determined by image analysis.

Results and Discussion

Grazing Effects in Paddocks. The proportion of rainfall lost as runoff was less (P<0.05) in the U paddocks than in all other treatments during both years 1 and 2. In year 1, the proportion of rainfall lost as runoff was greater (P<0.05) in the late spring (36%) than in mid-summer (11.8%), autumn (13.1%), or early spring (7.1%) across all treatments. Similarly, in year 2, the proportion of rainfall lost as runoff in late spring (19.4 %) was greater (P<0.05) than the mid-summer (7.5%), autumn (11.8%), and early spring (12.6%) periods.

There were no differences in mean concentrations of sediment in runoff from paddock between stocking treatments in either year**.** Mean total P concentrations in the runoff were greater in paddocks with the 2C and 2R treatments than other treatments in both years (P<0.05). Mean sediment and total P concentrations did not differ between months in year 1, but total soluble P concentrations were greater (P<0.05) in the late spring than the other sampling periods.

Table 1. Annual sediment, total P and total soluble P in runoff from paddocks grazed by different systems.

	Sediment, lb/ac (kg/ha)		**Total P, lb/ac (kg /ha)**		**Total soluble P, lb/ac (kg /ha)**	
	Year 1[b]	Year 2	Year 1	Year 2	Year 1	Year 2
U[a]	10.2 (11.4)	4.3 (4.8)[c]	0.005 (0.06)[c]	0.03 (0.03)[c]	0.04 (0.04)[c]	0.02 (0.02)[c]
HS	30.8 (34.5)	15.9 (17.8)[c]	0.20 (0.23)[c,d]	0.09 (0.10)[c]	0.17 (0.19)[d]	0.04 (0.04)[c,d]
2C	54.7 (61.2)	105.5 (118.2)[d]	0.37 (0.41)[d]	0.36 (0.40)[d]	0.26 (0.29)[e]	0.12 (0.13)[c,d]
2R	55.3 (61.9)	27.2 (30.5)[c]	0.37 (0.41)[d]	0.19 (0.21)[c]	0.31 (0.35)[e]	0.15 (0.17)[d]
4R	41.3 (46.2)	15.9 (17.8)[c]	0.23 (0.26)[c,d]	0.08 (0.09)[c]	0.18 (0.20)[d,e]	0.04 (0.04)[c,d]

[a] U = Ungrazed, HS = Summer Hay Harvest/Winter Stockpile Grazing, 2C = 2 inch Continuous Grazing, 2R = 2 inch Rotational Grazing, 4R = 4 inch Rotational Grazing.

[b] Different superscripts within the same column denote a difference, (P<0.05).

In year 2, mean sediment and total P concentrations in runoff did not differ between sampling periods. However, total soluble P concentration in runoff was less in the early spring than it was in other sampling periods.

Losses of total P were greater (P<0.05) from paddocks with the 2C and 2R treatments than U paddocks in year 1, while the HS and 4R treatments were intermediate and not significantly different from any of the other treatments. Total P losses were greater (P<0.05) from 2C treatment than from all other treatments in year 2. Losses of total soluble P were lower (P<0.05) from U paddocks than from other treatments in year 1. In year 2, the 2R treatment had greater (P<0.05) total soluble P losses than U, with the HS, 2C, and 4R intermediate to, and not significantly different from, either the U or 2R treatments. In years 1 and 2, 89% and 76% of the total P in the runoff was in the form of total soluble P. While these differences seem to be related

to forage height and cover, the paddocks grazed to 2 in (5cm) by continuous or rotational stocking had greater cow days per acre which likely contributed greater fecal P excretion per acre.

High slope areas had a greater percentage of rainfall lost as runoff than low slope areas in both years (21.2 vs. 14.6% in year 1 and 16.0 vs. 9.8% in year 2) across all treatments and months ($P<0.05$). There was no effect of slope on sediment or total P and total soluble P concentrations or total and total soluble P losses in runoff for either year. Sediment loss from high slope areas was greater ($P<0.05$) than from low slope areas in year 1 (13.1 vs. 6.5 lb/ac; 14.7 vs. 7.3 kg/ha) across all treatments. There was no significant effect of slope on sediment loss in year 2.

In both years, sward heights of the grazed paddocks were greatest in the early summer period ($P<0.05$). By later sampling periods, the paddocks had been sufficiently grazed to reach their prescribed forage sward height.

Soil moisture in the upper 2 in (5 cm) was greater in year 1 than in year 2 ($P<0.05$), 23.3% and 20.5%, respectively. Soil moisture contents were greater in the U paddocks (24.6 and 22.1% for year 1 and 2, respectively) than in all other paddocks ($P<0.05$) in both years, with no difference in soil moisture between the other treatments (23.1, 23.8, 23.0, and 21.8% in year 1 and 19.6, 20.8, 20.9, and 20.0% in year 2 for 2C, 2R, 4R, and HS, respectively). In both years, soil moisture followed the same trends with soil moisture highest in the late spring, lowest in the mid-summer, intermediate in the autumn, and high in early spring ($P<0.05$). Soil moisture contents were 27.5, 16.2, 22.9, and 26.6% in year 1 and 24.2, 13.6, 21.9, and 23.8% in year 2 for late spring, mid-summer, autumn, and early spring, respectively.

Mean penetration resistance in the 0 to 5 in (0 to 10 cm) depth for the four sampling periods in year 1 was lowest for the U treatment (45.1 lb-force; 20.5 kg-force), intermediate for the HS (51.7 lb-force; 23.5 kg-force) treatment, and greatest in the summer grazing treatments (55.0, 57.4, 57.6 lb-force for the 2C, 2R, and 4R, respectively; $P<0.05$; 25.0, 26.1, and 26.2 kg-force for the 2C, 2R, and 4R, respectively), but did not differ between summer grazing treatments.

In year 2, mean penetration resistance in the 0 to 10 cm depth for the U paddocks was lower ($P<0.05$; 54.6 lb-force; 24.8 kg-force) than all other treatments. However, there were no differences in penetration resistance between paddocks with different forage utilization systems (67.3, 74.6, 73.7, and 75.9 lb-force for the HS, 2C, 2R, and 4R treatments, respectively; 30.6, 33.9, 33.5, and 34.3 kg-force for the HS, 2C, 2R, and 4R treatments, respectively). Mean penetration resistance in the 5 to 8 in (10 to 20 cm) depth was unaffected by treatment in either year, averaging 59.4 and 76.3 lb-force (27.2 and 34.7 kg-force) across all treatments for year 1 and 2, respectively. Similarly, mean penetration resistance in the 8 to 14 in (20 to 35 cm) depth was unaffected by treatment in either year, averaging 62.9 and 86.9 lb-force (28.6 and 39.5 kg-force) for year 1 and 2, respectively.

Surface roughness did not differ by treatment or time in either year. Soil Bray-1 P concentrations in the upper 2 in (5 cm) were 20 to 25 ppm at the initiation of the experiment and did not differ between treatment or sampling period in either year. However, total P losses during rainfall simulations were greater from simulation sites that had greater soil Bray-1 P concentrations.

Averaged across months, surface cover in ungrazed paddocks was greater ($P<0.05$) than paddocks in which forage was harvested either as hay or grazed. In both years, surface cover in the 2C paddocks was lower than paddocks with other treatments ($P<0.05$). Mean surface covers were 99.0, 93.5, 82.8, 89.9%, and 93.3% in year 1 and 99.1, 95.5, 89.1, 91.4, and 94.1% in year 2 for the U, HS, 2C, 2R, and 4R treatments, respectively.

Erosion of sediment was not different between treatments in year 1 (Table 1). In year 2, the 2C paddocks contributed greater amounts of erosion ($P<0.05$) than the other treatments. The greatest amount of erosion occurred in the late spring period across all treatments in both years ($P<0.05$). Of the pasture physical characteristics measured, sediment loss was most highly correlated with percent surface cover: ($Y=794.1-17.81\ X + .096\ X^2$, $r^2=0.3362$) where Y is the sediment loss in lb/ac/simulation and X is the percentage of ground covered with plant material [($Y'=889.2 - 19.95\ X + 0.108\ X^2$,), where Y' is the sediment loss in kg/ha/simulation and X is the percentage of ground covered with plant material].

Buffer Effects. Mean sediment concentrations in the runoff were not affected by simulation location (paddock, base, buffer) or month in either year. However, mean total P and total soluble P concentrations in runoff were greater ($P<0.05$) in the paddocks than at the base of the paddock or 30 ft (10 m) in the buffer in both years. Over the two years, mean concentrations of total and total soluble P from paddocks were 49.5% and 47.4% greater, respectively, ($P<0.05$) than the mean values within the buffers. This result may indicate that grazing will increase the amount of P that is available for transport within a pasture, but it rapidly becomes immobile again in ungrazed buffer areas. However, there were no significant grazing treatment by location interactions for losses of sediment, total P, or total soluble P; these differences seem to be factors other than grazing.

In year 1, the proportion of rainfall lost as runoff was greater ($P<0.05$) in the paddocks than at the paddock base and at 10 meters within the buffer. In year 2, the proportion of rainfall lost as runoff was greater ($P<0.05$) in the paddocks and at the paddock base than at 10 meters within the buffer. In year 1, runoff was 17.1, 11.7, and 8.6% of applied and in year 2, runoff was 12.8, 12.8, and 7.5% of applied from the paddock, base of the paddock, and 30 ft (10 m) within the buffer, respectively. These differences can partially be attributed to the differences in soil slope, soil texture, and forage composition that exist between locations.

In year 1, there was no difference in sediment loss between the paddock and the two locations within the buffer (Table 2). In year 2, sediment loss was greatest from the paddock, lowest from 30 ft (10m) within the buffer, and intermediate at the base of the buffer.

As a result of differences in rainfall infiltration and the total P concentration of runoff, total P flows from the paddocks were 3.5 and 7.0 times greater ($P<0.05$) than those from the paddock base and in the buffer in year 1 and 2.0 and 4.0 times greater ($P<0.05$) than those from the paddock base and in the buffer in year 2. Amounts of total soluble P in the runoff were 3.0 and 24 times greater in the buffer and at the base of the paddock than in the paddock in year 1.

Table 2. Sediment, Total P, and Total Soluble P losses within the paddocks, at the base of the paddock, and 30 ft (10 m) within the buffer.

	Sediment, lb/ac (kg/ha)		**Total P, lb/ac (kg /ha)**		**Total soluble P, lb/ac (kg /ha)**	
	Year 1[b]	Year 2	Year 1	Year 2	Year 1	Year 2
Paddock[a]	39.3 (44.0)	34.3 (38.4)[a]	0.25 (0.28)[a]	0.14 (0.16)[a]	0.21 (0.24)[a]	0.07 (0.08)
Base	24.6 (27.6)	18.9 (21.2)[a,b]	0.07 (0.08)[b]	0.07 (0.08)[b]	0.07 (0.08)[b]	0.03 (0.03)
Buffer	18.2 (20.4)	9.3 (10.4)[b]	0.04 (0.04)[b]	0.04 (0.04)[b]	0.01 (0.01)[b]	0.02 (0.02)

[a] Paddock = Average across all paddocks, Base = At the paddock-buffer interface, within the buffer, Buffer = Within the buffer, 30 ft (10 m) down slope from the paddocks

[b] Different superscripts within the same column denote a difference, ($P<0.05$).

Across treatments, mean forage sward heights in paddocks, at the paddock base, and 30 ft (10 m) in the buffer strip were 3.7, 6.8, and 7.1 in (9.4, 17.3, and 18.1 cm) in year 1 and 4.8, 9.0, 9.7 in (12.2, 22.9, and 24.6 cm) in year 2 ($P<0.05$).

Penetration resistance in the upper 10 cm of soil was greater in the paddocks than at either the paddock base or 30 ft (10 m) in the buffer strip ($P<0.05$) for all sampling periods except late spring of year 1. In both years at all locations and depths, penetration resistance was low during the late spring, increased to a maximum in mid-summer, decreased to an intermediate level by autumn, and had returned to late spring levels by early spring. These differences not only represent treatment effects, but are also influenced by soil moisture and texture differences between location and sampling periods.

Sequestration of phosphorus with iron mine tailings

Basic Information

Title:	Sequestration of phosphorus with iron mine tailings
Project Number:	2003IA39B
Start Date:	3/1/2003
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	IA 2nd
Research Category:	Water Quality
Focus Category:	Water Quality, Nutrients, Wetlands
Descriptors:	water quality, lakes, eutrophication, nutrients
Principal Investigators:	Ed Brown

Publication

1. Clayton, M.E., S. Liegeois, and E.J. Brown, 2004. Phosphorus sequestration in lake sediment with iron mine tailings. Soil and Sediment Contamination. (In press)
2. Schwemm, A., R. Pasker, M. Clayton, and E.J. Brown, 2003. Phosphorus sorption by sediments from wetlands in the Cedar River watershed. 26th Annual Midwest Environmental Chemistry Workshop, Iowa City, IA.
3. Boyce, Matthew D., Heather J. Bailey, Mohammad Z. Iqbal, and Edward J. Brown, 2003. Lateral and vertical distribution of phosphorus in a northeast Iowa wetland system. Geological Society of America, 37th Annual Meeting, Kansas City.
4. Brown, E.J., S. Liegois, and M. Clayton, 2003. Sequestration of phosphorus with iron mine tailings. Iowa Academy of Science Annual Meeting, Des Moines, IA.
5. Schwemm, A., R. Pasker, M. Musgrave, and E. Brown, 2003. Transport of phosphorus through a wetlands system. UG Summer Research Program, University of Northern Iowa, Cedar Falls, IA.
6. Schwemm, A., R. Pasker, M. Clayton, and E. Brown, 2004. Phosphorus sorption to wetlands sediments. Fourth Annual Water Monitoring Conference, Iowa Department of Natural Resources.

Sequestration of Phosphorus with Iron Mine Tailings

Edward J. Brown

Problem and Research Objectives

Orthophosphate (PO_4^{3-}) is found in surface and ground waters as a result of the natural weathering and solution of minerals; soil erosion and transport; use of soluble phosphate compounds in detergent manufacture, water treatment and industry; and soil fertilization. Controlling the total load of phosphorus in a lake is critical to controlling eutrophication since phosphorus is usually the biomass limiting nutrient in natural aquatic ecosystems. The phosphorus cycle does not allow atmospheric venting (as the nitrogen cycle does), so phosphorus tends to accumulate in the sediments of lakes. In a healthy, well aerated lake, this does not cause a problem because the phosphates precipitate or are tightly adsorbed to common minerals in the sediments and are thus unavailable for biological uptake. In oxygen-depleted waters, however, the internal loading of phosphorus results from a problem known as the "phosphorus trap." Phosphorus accumulated in the sediments is mobilized through dissolution or desorption to the aqueous phase under low-oxygen conditions in the sediments, resulting in a stimulation of biomass production and ultimately a further decrease in the levels of oxygen in the water column and sediments. If phosphorus in lake sediments can be sequestered in a form which is not released under anaerobic conditions, internal phosphorus loading would be reduced. Iron, in both the ferric (Fe^{3+}) and ferrous (Fe^{2+}) oxidation states, is known to react with phosphate (PO_4^{3-}), leading to precipitates and hydrous ferric oxides that tie up the phosphorus so that it becomes unavailable for the growth of plants in general and algae in particular. Fe^{3+} is the most common metal in soils and rocks and is the form of iron that is primarily found in both aerobic water and many naturally occurring minerals, including hematite.

In this project, we investigated whether this principle can be applied to runoff catchment basins and constructed wetlands for treatment of phosphate pollution in lakes and other surface waters. In this case, large amounts of ferric iron as contained in low cost mine tailings would serve as the biological oxidant for sediment organic material as well as serving as a phosphate sink. We hypothesized that the iron would be reduced by anaerobic bacterial respiration because Fe^{3+} is an electron acceptor in a wide variety of film-forming bacteria and the biological reduction of Fe^{3+} is a major mechanism leading to the production of ferrous iron in natural systems. The potential of the Fe^{3+}/Fe^{2+} couple is very electropositive, and, because of this, Fe^{3+} reduction can be coupled with oxidation of several organic and inorganic electron donors during anaerobic respiration. We hypothesized that the soluble ferrous iron that would be produced could then react with phosphate present in the sediment/water interface or be re-oxidized by other soluble electron acceptors (i.e. nitrate), leading to precipitates that immobilize phosphorus in the sediment.

Methodology:

Ore from the Cuyuna iron range in east-central Minnesota contains multiple carbonate micronodules comprised of a rhodonite core surrounded by calcite, rhodochrosite, and

hematite (Fe_2O_3). Therefore, iron mine tailings from the Cuyuna range contain various amounts of hematite. Hematite has a simple, repeating crystalline structure: iron and oxygen atoms coordinate to form two-dimensional layers, leaving small open spaces between them. Hematite can fix atoms or molecules when they are introduced into those spaces by absorption, or molecules can adsorb to the surface of the mineral. In either case, the attraction can be physical or chemical, involving ionic or covalent bonds. Furthermore, it has been shown that reactions of water with hematite can involve dissociation of water, resulting in the formation of surface hydroxyl groups which may increase its ability to sorb compounds such as phosphates. Additionally, depending on its chemical environment, hematite can also make available iron and oxygen ions for chemical reactions such as oxidation/reduction. In particular, hematite releases iron ions in aqueous solutions.

In this study, we designed experiments to investigate whether the oxidized iron in mine tailings will serve as electron acceptor (oxidant) for anaerobic respiration of organic matter, become soluble, and then immobilize sediment phosphate. The experiments were designed to measure sediment phosphorus available for algal uptake and growth in Silver Lake, Iowa. This phosphorus includes soluble reactive phosphorus (SRP) in sediment slurries as well as phosphorus released from sediments after extraction with dilute acid.

Principal Findings and Significance:

Iron mine tailings from the Cuyuna Range in Minnesota containing hematite proved to be effective for preventing the release of phosphate into aqueous solution from phosphorus-laden Silver Lake sediments, even under anaerobic conditions. In both of the treatments in which anaerobic conditions were initiated biologically, the release of bioavailable phosphorus was significantly reduced in the sediments mixed with hematite as compared to sediment solutions alone, even though the dissolved oxygen concentrations were similar to those of the nitrogen purged system. The fact that the best results were seen in samples in which glucose was added to stimulate the growth of microorganisms suggests that the process of phosphorus sequestration was microbially mediated. We suggest that the hematite in the mine tailings served as an electron sink for microbial respiration, but that the reduced iron released into solution continued to sequester phosphorus, either as it re-oxidized, forming hydrous ferric oxide complexes containing phosphorus (HFO-P), or through precipitation as vivianite. The nature of the iron-phosphorus compound(s) formed in these reactions will be investigated in year 2 of this study.

Achievements & Awards

During the first year of this project, we proved the concept that ferric iron present in hematite can be reduced to ferrous iron in anaerobic waters through anaerobic respiration by certain microorganisms and that this ferrous iron can react with P or can be re-oxidized chemically or biologically back to ferric iron which forms P-sequestering particulate hydrous ferric oxides (HFO). The results will assist in formulation of pollution reduction and remediation plans for eutrophic lakes in Iowa and other locations where P is the major pollutant.

Water Quality, Nutrient Loading and Mosquito Production in Northeastern Iowa

Basic Information

Title:	Water Quality, Nutrient Loading and Mosquito Production in Northeastern Iowa
Project Number:	2003IA40B
Start Date:	3/1/2003
End Date:	2/29/2004
Funding Source:	104B
Congressional District:	IA 1st
Research Category:	Not Applicable
Focus Category:	Nutrients, Wetlands, Ecology
Descriptors:	
Principal Investigators:	DAVID RANDY MERCER, DAVID RANDY MERCER

Publication

Water Quality, Nutrient Loading and Mosquito Production in Northeastern Iowa

David R. Mercer

Problem and Research Objectives

The influences of agricultural chemicals and human activities upon Iowa's water bodies may produce unexpected consequences. One possible outcome is the increase in mosquito production and associated risks of disease transmission. Higher levels of nutrients in water bodies may directly or indirectly augment mosquito populations if larvae feed upon nutrients or bacteria that result from runoff. Our objectives include assessment of water properties in a wide variety of natural and artificial pools and containers with an attempt to explain mosquito production. We will construct predictive models that will estimate the risks of disease transmission by mosquitoes, especially in relationship to human populations and activities.

Methodology

We conducted field surveys of putative mosquito developmental sites for correlation with biological (i.e., dead organic matter, total heterotrophic bacterial, plant and animal populations), chemical (i.e., pH, dissolved nutrients, electrical conductivity, and total alkalinity), and physical (i.e., depth, turbidity, and dissolved oxygen) properties of water bodies to identify characteristics that correlated with mosquito production. We conducted mosquito adult trapping in representative habitat types throughout Black Hawk and Buchanan Counties, Iowa. Ground-generated data are being incorporated into a Geographic Information Systems (GIS) model (ArcGIS v8.1 Mapping Software) together with land feature (i.e., soil types, land cover, land usage) and human demographic data. Using on-the-ground and remote sensing procedures, we are building a predictive model that will estimate mosquito production and the associated risks of disease transmission in Black Hawk County. We will verify our model using data from neighboring Buchanan County. We are paying particular attention to human activity and land use patterns in constructing our model.

Principal Findings and Significance

During the period of the grant, we sampled >350 putative larval developmental sites and trapped >16,000 adult mosquitoes. Although mosquito identification and data analysis are now nearing completion, to date we have demonstrated that mosquito numbers were significantly correlated with bacterial and dissolved phosphate concentrations in putative developmental sites. Using all field variables measured, we were able to explain 86% of mosquito production from sample sites in a multiple regression model. Our GIS model was able to identify urban and rural regions of risk, especially for West Nile virus transmission to older residents of Black Hawk County.

Data were complete enough to characterize mosquito production from Beaver Valley Wetlands, a reconstructed palustrine wetland in Black Hawk County that is managed by the Black Hawk County Conservation Board. Likewise, we were able to account for 70%

of mosquito production from this wetland during the study. We identified 10 mosquito species emerging from Beaver Valley Wetlands. These species ranged from probable vectors to species that rarely bite humans. The majority of mosquitoes and the greatest risk of disease transmission were associated with several temporary pools. In our manuscript we suggest that although relatively few mosquitoes were produced by the wetland, spot treatment using *Bacillus thuringiensis* var. *israelensis* might significantly reduce mosquito production while not affecting wetland services or non-target species. Furthermore, we suggest that remote sensing might be useful in identifying problematic microhabitat types and sources of nutrients that would affect the wetlands. This manuscript will be submitted for publication within the next few days.

Finally, during the funding period, we were able to compare dissolved nitrate and dissolved phosphate chemical titration strips used in field studies with standard laboratory protocols. We determined that the nitrate test strips were reliable for field assessment of nutrients in mosquito developmental sites whereas phosphate test strips were not reliable for limited sampling. A student (supported by ISWRRI funds) and I are developing this manuscript for publication.

Amplification and Attenuation of Tetracycline Resistance in Soil Bacteria: Aquifer Column Experiments

Basic Information

Title:	Amplification and Attenuation of Tetracycline Resistance in Soil Bacteria: Aquifer Column Experiments
Project Number:	2003IA49B
Start Date:	8/1/2002
End Date:	7/31/2004
Funding Source:	104B
Congressional District:	IA 1st
Research Category:	Not Applicable
Focus Category:	Agriculture, Groundwater, Non Point Pollution
Descriptors:	
Principal Investigators:	Pedro J Alvarez

Publication

Amplification and Attenuation of Tetracycline Resistance in Soil Bacteria: Aquifer Column Experiments

Pedro J. Alvarez and Jerald L. Schnoor

Problem and Research Objectives

Antibiotic resistance is a public health concern of great urgency due to a growing inefficacy of antimicrobial agents to treat infectious diseases. This is mainly due to the propagation of antibiotic resistance genes among bacteria, which is exacerbated by the potential overuse of antimicrobials in humans and the intensive use of antibiotics in animal agriculture for non-therapeutic purposes such as growth promotion and disease prevention (Mellon et al., 2001). Recent studies have found that antibiotic resistance genes occur in bacteria in the environment as a direct result of animal agriculture (e.g., swine production facilities) and that soil and groundwater in the vicinity of such facilities may be *potential sources of antibiotic resistance in the food chain* (Chee-Sanford et al., 2001). However, genes have not yet been considered as environmental pollutants, and little is known about the fate and transport of antibiotic resistance genes when released to the environment as a result of direct runoff, groundwater infiltration from lagoons, or manure spreading activities. Critical knowledge gaps include the rate and extent of gene propagation (including bacterial migration and inter-specific gene transfer from enteric to soil bacteria) and the effect of environmental factors such as soil characteristics and water chemistry on the persistence of antibiotic resistance. Learning about these issues is important to assess the impact of antibiotic resistance genes on public and environmental health and to determine the need for regulatory action in states where animal agriculture is common.

This study addresses the effect of antibiotic exposure (e.g., tetracycline) on indigenous soil microorganisms in simulated runoff infiltration conditions. The main goals are to:

Short Term

1. Characterize the fate and transport of TC in soil
2. Determine the effect of TC on development of resistant strains

Long Term

1. Monitor development of resistant strains
2. Genotypic characterization of resistant strains
3. Model the resistance gene transfer

Methodology

General Approach. Flow-though columns packed with soil were used to mimic runoff and infiltration of TC-contaminated agricultural drainage and to evaluate changes in the total heterotrophic and Tet^r microbial populations during and after sustained TC exposure. Acetate, which is a common product of animal waste breakdown and is likely to be present in farm runoff, was added as a carbon source. A control column without TC was also run. Emphasis was placed on enumerating and characterizing bacteria in the column effluent to focus on mobile bacteria with a higher potential to reach a human recipient.

Along with population enumerations, TC and acetate concentration profiles were monitored along the length of the columns to investigate TC stability and acetate utilization patterns. Tet^r microorganisms isolated from the effluent of the TC-enriched column were identified by genetic analysis and screened for the tet-determinants responsible for TC resistance. The recovery of the microbial populations after TC exposure ceased was also characterized by monitoring the percentage of Tet^r heterotrophs in the column effluent.

1. Flow-through Columns: Two cylindrical, 30-cm long flow-through glass columns (Kontes Glass Company, Vineland, NJ) were modified with six sample ports located at 2, 5, 9, 14, 19, and 24cm. from the bottom inlet of the column. Inlet and outlet three-way valves were placed at the respective locations. The columns were secured in a vertical position and tightly packed with soil (University of Iowa Softball Field). The columns were wrapped in aluminum foil to minimize algal growth and possible antibiotic photodegradation.

Two-L reservoir bottles were equipped with 3-hole caps (Kontes Glass Company, Vineland, NJ) and wrapped in aluminum foil. Masterflex Neoprene® tubing (Cole-Parmer Co.) and a Masterflex peristaltic pump (Cole-Parmer Co.) were used for the feed solution delivery. The pump flow rate was adjusted to achieve a column flow rate range between 3.0–4.0 mL/hr. The flow rate for the control column (TC -) was approximately 3.4 mL/hr and 3.6 mL/hr for the exposed (TC +) column. Bromide tracer studies were conducted on both columns prior to addition of the feed solutions.

The feed solution for both columns consisted of synthetic ground water (von Gunten and Zorbist, 1993) as nutrient source and sodium acetate as a carbon source (10 mg/L). In addition, one feed solution was amended with tetracycline-hydrochloride (T3383, Sigma Co.) at 10–50 mg/L.

2. Concentration Profiles: The concentrations of acetate and tetracycline were monitored along the column length (inlet, outlet, and sample ports). Standard curves for both chemicals were prepared monthly to ensure measurement accuracy. Acetate concentrations were measured via an anion chromatograph equipped with an auto-sampler apparatus (Alltech 570), an IonPAc AS14 column (Dionex), and a conductivity detector (Dionex). Tetracycline content was analyzed via a manual injection HPLC pump (Alltech 426) equipped with a HPLC column (Supelco, Discovery C8, 59353-U) and a variable wavelength detector (Dionex).

3. Microbial Counts: Initially, agar plate counts for the enumeration of microbial populations were performed. The effluent from both columns was collected and 100 µL were streaked onto the R2A agar plates with the intent to quantify the total heterotrophic populations. R2A plates enriched with tetracycline (50 mg/L) were also streaked with the column effluent in order to quantify the antibiotic-resistant microorganisms. Several attempts with this method yielded irreproducible results.

A modified MPN 96-well plate technique was adapted for microbial enumeration of the column effluent. Growth media containing tryptic soy broth (TSB) solution was used for the enumeration of the total heterotrophic population, and TSB enriched with tetracycline (50 mg/L) was used for the antibiotic-resistant microorganisms. This quantification was based on visual scoring of growth induced TSB-turbidity development and subsequent statistical analysis.

4. *Genetic Analysis:* Effluent from the TC-exposed column was used for the isolation of antibiotic-resistant strains. TC-enriched (50 mg L^{-1}) R2A agar plates (Difco Laboratories) were streaked with column effluent (0.1 mL) and incubated at 30°C 2–5 days, depending on the growth rates (appearance of colonies). Individual colonies were re-streaked onto TC-enriched R2A agar plates, incubated, isolated, and re-streaked a second time in order to ensure strain "purity."

Bacterial DNA was extracted from selected colonies with kits according to manufacturers' protocols (Qiagen). A Mastercycler® thermocycler device (Eppendorf) was used for the Polymerase Chain Reaction (PCR) gene detection techniques. PCR amplification was performed on the extracted DNA according to the protocols provided in the reaction kits (PanVera). The final concentrations of the PCR reagents in a 50 µL reaction mixture were: 1.25U DNA polymerase (Ex Taq), 1X reaction buffer, 200 µM doexynucleoside triphosphate, and 0.2 µM primers (forward and reverse). Primers were constructed (according to Table 1) for the following tet-determinants coding for Ribosomal Protection Proteins (RPP): TetB(P), Tet(M), Tet(O), Tet(Q), Tet(S), Tet(T), Tet(W), and OtrA. The amplification was performed as previously described by Aminov, et al. (2001). Briefly, the cycle steps were: (1) an initial denaturation at 94°C (5 min) followed by 25 cycles at 94°C (30s); (2) annealing at 30s and 30s extension (72°C); and (3) extension at 72°C (7 min). The annealing temperatures for each primer are shown in Table 1. Reaction products were analyzed by electrophoresis on a 1.2% (wt/vol) agarose gel containing ethidium bromide.

Table 1. PCR Primers targeting ribosomal protection protein (RPP) classes (Source: Aminov *et al*., 2001)

Tet-determinant targeted	Primer Sequence	Amplicon size (bp)	Annealing Temperature (°C)
TetBP-F	AAAACTTATTATATTATAGTC	169	46
TetBP-R	TGGAGTATCAATAATATTCAC		
TetM-F	ACAGAAAGCTTATTATATAAC	171	55
TetM-R	TGGCGTGTCTATGATGTTCAC		
TetO-F	ACGGARAGTTTATTGTATACC	171	60
TetO-R	TGGCGTATCTATAATGTTGAC		
OtrA-F	GGCATYCTGGCCCACGT	212	66
OtrA-R	CCCGGGGTGTCGTASAGG		
TetQ-F	AGAATCTGCTGTTTGCCAGTG	169	63
TetQ-R	CGGAGTGTCAATGATATTGCA		
TetS-F	GAAAGCTTACTATACAGTAGC	169	50
TetS-R	AGGAGTATCTACAATATTTAC		
TetT-F	AAGGTTTATTATATAAAAGTG	169	46
TetT-R	AGGTGTATCTATGATATTTAC		
TetW-F	GAGAGCCTGCTATATGCCAGC	168	64
TetW-R	GGGCGTATCCACAATGTTAAC		

Primers targeting efflux-pump tet-determinants were constructed according to Table 2. Amplification conditions were as described by Furushita et al., (2003) and included 30 cycles of 60s at 94°C, 45s at annealing temperatures shown in Table 2, and 90s at 72°C followed by a final extension of 300s at 72°C. PCR reaction products were purified with the QIAquick® PCR Purification Kit (Qiagen) according to manufacturer's protocol. Restriction digests of the PCR products were attempted with the following endonucleases (to identify the following *tet* determinants): *SmaI* (TetA), *SphI* (TetB, TetD, TetY), *SalI* (TetC), *NdeII* (TetE, TetH, TetJ), and *EcoRI* (TetG). The amplicons were analyzed by gel-electrophoresis as described above.

Table 2. PCR Primers targeting efflux-pump determinants
(Source: Furushita *et.al.* 2003)

Tet-determinant targeted	Primer Sequence	Amplicon size (bp)	Annealing Temperature (°C)
TetA-F	CGCYTATATYGCCGAYATCAC	417	55
TetA-R	CCRAAWKCGGCWAGCGA		
TetB-F	GGDATTGGBCTTATYATGCC	967	50
TetB-R	ATMACKCCCTGYAATGCA		
TetC-F	CGCYTATATYGCCGAYATCAC	417	55
TetC-R	CCRAAWKCGGCWAGCGA		
TetD-F	GGDATTGGBCTTATYATGCC	964	50
TetD-R	ATMACKCCCTGYAATGCA		
TetE-F	GGDATTGGBCTTATYATGCC	650	50
TetE-R	AWDGTGGCDGGAATTTG		
TetG-F	TATGCRTTKATGCAGGTC	917	50
TetG-R	GACRAKCCAAACCCAACC		
TetH-F	GGDATTGGBCTTATYATGCC	650	50
TetH-R	AWDGTGGCDGGAATTTG		
TetJ-F	GGDATTGGBCTTATYATGCC	650	50
TetJ-R	AWDGTGGCDGGAATTTG		
TetY-F	TATGCRTTKATGCAGGTC	911	50
TetY-R	GACRAKCCAAACCCAACC		

Principal Findings and Significance

Initially, tetracycline concentrations were monitored at inlet and outlet of the exposed column. Approximately 97% of the initial tetracycline was degraded within the column length (Figure 1).

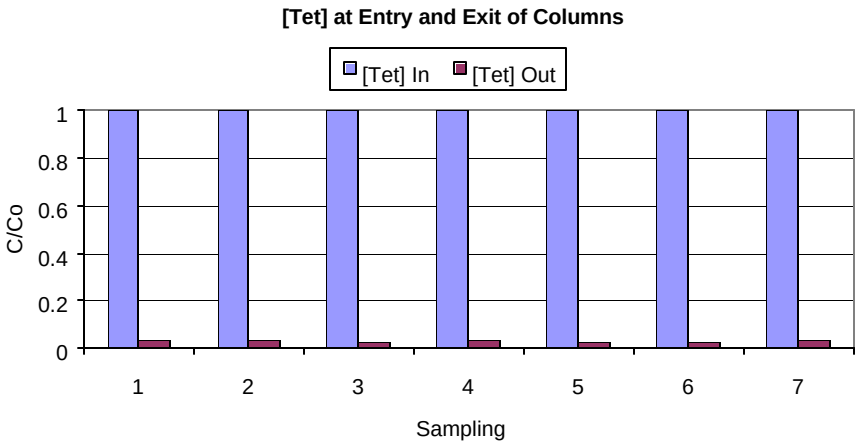


Figure 1: Inlet and outlet standardized tetracycline concentrations

Tetracycline and acetate concentration profiles along the length of the columns (Figure 2) were monitored in order to determine the antibiotic degradation behavior within the column and monitor microbial utilization of the carbon source.

A significant decrease in the aqueous TC concentration occurred near the inlet, and only trace amounts of TC (approximately 4 percent of influent concentration) were detectable throughout the length of the column. TC removal was presumably due to abiotic degradation upon contact with soil under neutral or mild alkaline conditions (pH=6.5–9.0). Furthermore, although TC is very soluble in water (S=1,700 mg/L, log k_{ow}=-1.19), sorption by other mechanisms than hydrophobic partitioning (e.g., cation bridging at clay surfaces and surface complexation) probably contributed to TC removal from the aqueous phase. It is unlikely that TC removal was due to microbial degradation because very fast TC removal (96% within one minute) was observed in batch studies where TC (100 mg/L, pH=4) was added to soil slurries (pH=7).

Assuming that acetate consumption is indicative of microbial activity, acetate profiles for the two columns suggest decreased microbial presence within the TC-enriched column. The presence of fewer microorganisms within the column could be attributed to the antibiotic dosages and the selective pressure exerted on the indigenous strains.

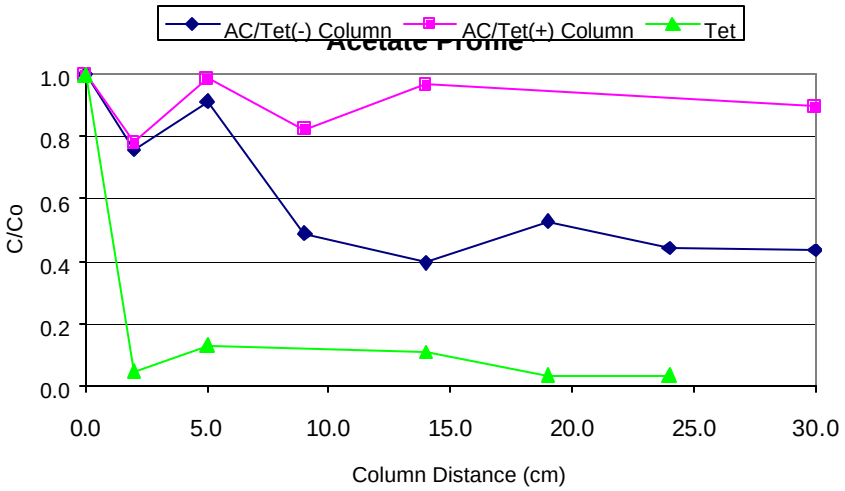


Figure 2: Tetracycline and acetate concentration profiles (12/2002)

TC exerted a significant bacteriostatic effect, decreasing the MPN concentration of total heterotrophs eluting from the TC-amended column by one order of magnitude compared to the control (Figure 3A). Nevertheless, the effluent concentration of Tet^r bacteria was significantly higher for the TC-amended column than for the control ($p<0.05$) (Figure 3B). Thus, TC exerted selective pressure for the development and maintenance of antibiotic resistance in soil bacteria, even though, to the best of our knowledge, potential Tet^r gene donors such as enteric Tet^r bacteria that could be excreted by farm animals were not initially present in this soil. Whereas TC concentrations decreased rapidly near the inlet, some TC degradation products such as 5a-6-anhydrotetracycline and 5a,6-anhydrochlorotetracycline (none of which were examined) are known to retain antimicrobial properties (Halling-Sorensen et al., 2002). Thus, it is plausible that TC degradation products, which may result from abiotic reactions, also contributed to the selective pressure for Tet^r bacteria.

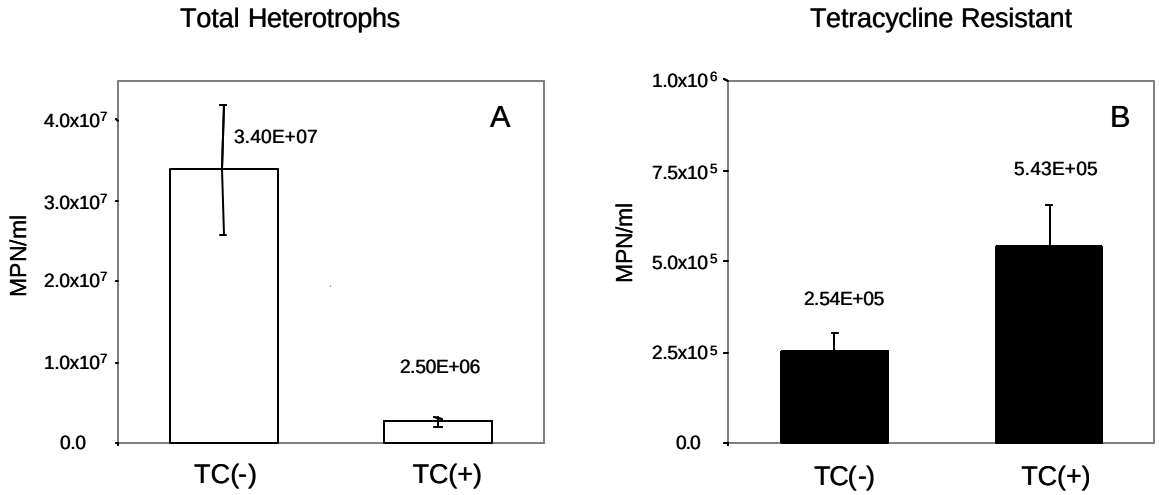


Figure 3: Effluent microbial concentrations from two columns. The TC(+) column was exposed to tetracycline during 300 days, whereas the TC(-) column served as an unexposed control. The concentration of total heterotrophs was significantly lower in the TC(+) column (panel A), while Tet^r bacteria concentrations were significantly higher ($p<0.05$) (B).

Tetracycline has been reported highly unstable in light conditions due to photodegradation characteristics. Batch experiments were performed to assess the stability of an aqueous tetracycline solution under light and dark conditions. Two 100mL solutions were prepared with one beaker completely covered in aluminum foil, simulating dark conditions, and the second beaker exposed to environment light. Surprisingly, no statistical differences were observed for the degradation rates of the two solutions, with approximately 85% of both solutions remaining after 400 hours (Figure 4).

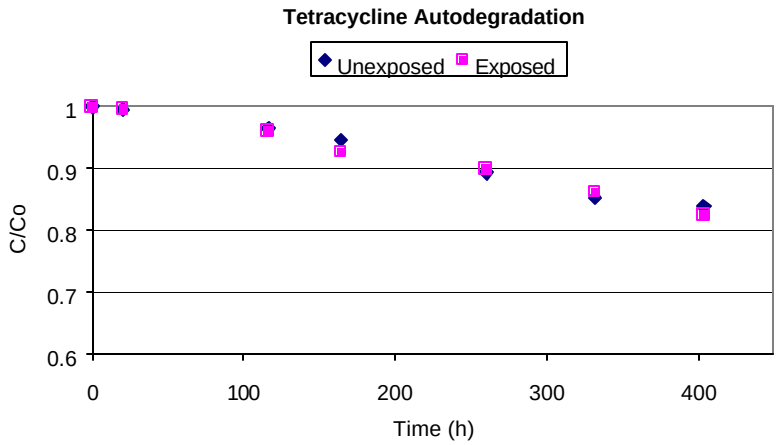


Figure 4: Tetracycline degradation under light (exposed) and dark (unexposed) conditions

To further investigate the behavior of tetracycline when in contact with soil environments, batch studies were performed with the same soil source that was used for the column packing. 250-mL amber-glass reaction bottles were filled with 100-mL of di-water and 10 grams of soil. The solution was mixed thoroughly by vigorous shaking, and the initial pH was measured (pH≈7). A 100 mg/L tetracycline solution was prepared, and the pH was also measured (pH≈4). Fifty mL of the TC solution were added to the soil mixture and shaken immediately. The pH and the tetracycline concentrations of the resulting solution were measured within 1 minute of the TC addition. Approximately 96% of the initial antibiotic was removed—degraded upon contact with the soil—which concurs with the tetracycline degradation behavior observed in the tet-enriched column (Figure 2). Along with the disappearance of the antibiotic, a rise in pH of the solution is observed, suggesting some form of alkaline hydrolysis as the mechanism of tetracycline degradation (Figure 5).

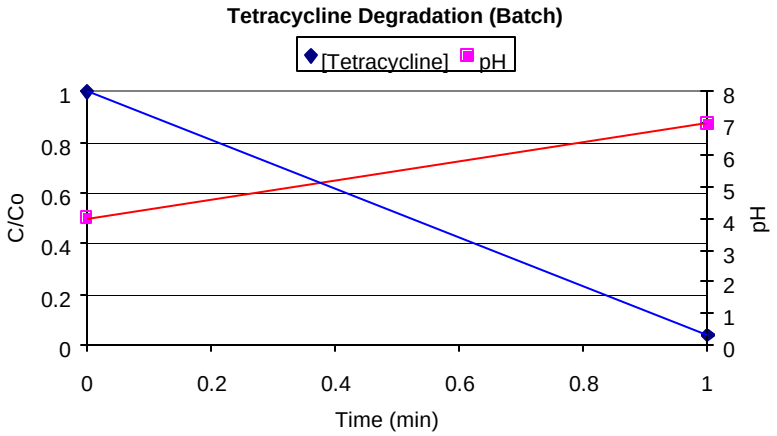


Figure 5: Batch degradation of TC and pH behavior

TC addition to the treated column was stopped after 300 days. Following a two-week lag, this resulted in a significant decrease in the percentage of Tet^r heterotrophs, from about 25% after sustained TC exposure to the pre-exposure and control levels (1 to 2%) within 30 days (Figure 6). This trend was due both to a rebound of total heterotrophs (with a related increase in acetate consumption) as well as to a significant decrease in Tet^r bacteria concentration. It should be pointed out that the concentration of total heterotrophs eluting from the column previously enriched with TC did not reach the same levels eluting from the control column within the 30-day monitoring period, possibly due to some residual antibiotic activity. Nevertheless, Figure 6 suggests that discontinuing TC exposure or curtailing its use should enhance natural attenuation mechanisms that mitigate the spread of resistance vectors.

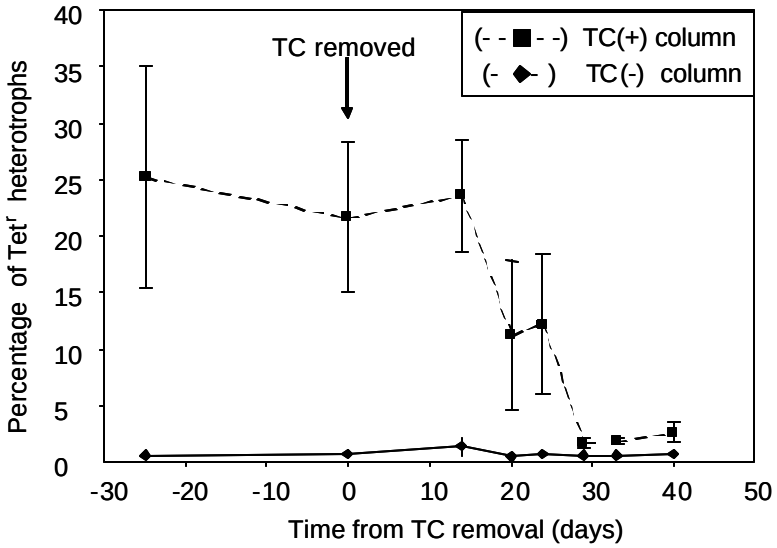


Figure 6: Decrease in the percentage of Tet^r heterotrophs after TC removal from column feed solution.

Tet^r bacteria were isolated from the TC-enriched column effluent by plating on Tet-R2A agar. Such isolates represent mobile bacteria that could reach human recipients. Two types of Tet^r microbial colonies were consistently detected and isolated. The first isolate was identified on the basis of its 16S rRNA sequence (using the BLAST database) as *Burkholderia cepacia*, which is a common soil bacterium. The second isolate was identified on the basis of its 26S rRNA sequence (using the NTBI database) as *Rhodotorula mucilaginousa*, which is a fungus. The identity of this yeast was confirmed by analysis with an API 20 C AUX yeast system kit. Both identifications were performed by Microbial Insights Inc. (Rockford, TN).

Burholderia cepacia was screened for 17 tet-determinants, coding for both ribosomal protection proteins and efflux pumps, and was found to carry an efflux pump gene (TetA or TetC) (Figure 7). Discerning whether the determinant was TetA or Tet C was not possible because the same primer was used for both genes, and restriction digest attempts of the amplicon did not result in detectable fragments.

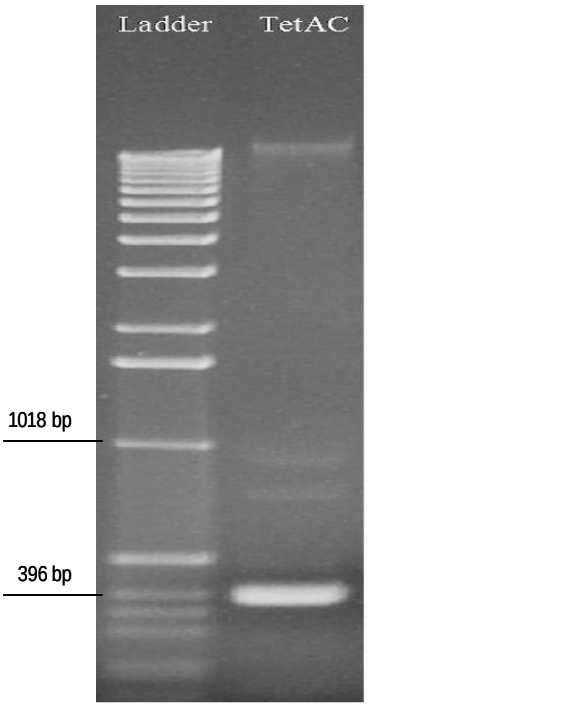


Figure 7: PCR amplification of the TetA/C gene determinant of *B. cepacia*, coding for an efflux pump. Amplicon size of approximately 417 bp along with 1 Kb ladder are presented.

The development of TC resistance in soil bacteria exposed to TC suggests that indigenous soil microorganisms may serve as reservoirs for the propagation (and possibly the amplification) of antibiotic resistance and potentially pose a direct hazard to public health. *B. cepacia* has been a focus of attention due to its (opportunistic) pathogenic characteristics (Kiska et al., 1996, Govan et al., 1996). Yet, *B. cepacia* has also received considerable attention due to its broad substrate specificity for application in

bioremediation processes (Bourquin et al., 1997, Steffan et al., 1999). Thus, the correlation between such a widely employed bacterium and its potential for disease propagation due to acquired antibiotic resistance may, in the future, influence the selection process and feasibility of some bioaugmentation practices.

Interestingly, the fungal microorganism (*R. mucilaginosa*) was only detected on R2A plates during the TC-enrichment period. This yeast was never detected in the effluent from the control column without TC or in the treated column effluent after TC exposure ceased. Since yeasts are relatively insensitive to TC (e.g., TC is produced by a yeast, *Saccharomyces*), we speculate that *R. mucilognosa* proliferation during TC exposure was due to the inhibition (or death) of bacteria that were antagonistic to this yeast. This implies that TC exposure might affect microbial community structure, not only through its direct bacteriostatic effect, but also indirectly by influencing microbial interactions among different populations.

This study investigated the phenotypic response of soil microbial communities exposed to selective pressure by tetracycline. Sustained exposure to TC resulted in a significant increase in the concentration of tetracycline-resistant soil bacteria, as well as a large decrease in the concentration of total heterotrophs. This suggests that TC release to the environment by animal agriculture is conducive to the development and amplification of antibiotic resistance, with soil bacteria serving as resistance reservoirs for Tet^r continuance. Nevertheless, removing the selective pressure by TC resulted in phenotypic shifts that returned the microbial community to initial conditions within one month, which implies that discontinuing TC exposure or curtailing its use should enhance natural attenuation mechanisms that mitigate the spread of resistance vectors.

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Spatial and temporal patterns in precipitation and dry-fall deposition of Nitrogen and Phosphorus in Iowa: implications for nutrient transport and water quality

Basic Information

Title:	Spatial and temporal patterns in precipitation and dry-fall deposition of Nitrogen and Phosphorus in Iowa: implications for nutrient transport and water quality
Project Number:	2003IA59B
Start Date:	4/1/2003
End Date:	3/31/2004
Funding Source:	104B
Congressional District:	IA 4th
Research Category:	Not Applicable
Focus Category:	Geochemical Processes, Water Quality,
Descriptors:	
Principal Investigators:	John Downing, John Downing

Publication

Spatial and Temporal Patterns in Precipitation and Dry-fall Deposition of Nitrogen and Phosphorus in Iowa: Implications for Nutrient Transport and Water Quality

John A. Downing

Problem and Research Objectives

This research focuses on two priority areas: Nutrient Management, and Animal Waste and Water Quality. The increased mobility of nutrients (N and P) through atmospheric transport derives from the alterations of land use through agriculture and other landscape changes. Both particulate and gaseous losses to the atmosphere from row-crops and animal agriculture can contribute to local and global water quality problems. This proposal aims to fill essential information gaps by characterizing spatial and temporal patterns in the deposition of nutrients by both precipitation and dryfall.

Atmospheric nutrient (nitrogen and phosphorus; N and P) loading and transport through precipitation and dry deposition is one of the least understood, and maybe one of the most important, pathways of nutrient transport in agricultural landscapes. Atmospheric P deposition through precipitation on a lake's surface has recently been found to contribute >30% of the annual P load, single-handedly preventing eventual remediation to attain projected federal nutrient standards. The purpose of this project is to fill three essential information gaps: (1) to characterize both nitrogen and phosphorus deposition, (2) through both wet- and dry-deposition to dry- and wet-surfaces, and (3) to characterize the spatial and temporal variation of this deposition across the state of Iowa.

Methodology

We measured nutrient deposition from April 1, 2003–March 31, 2004 at five sites representing a range of landscape characteristics common in Iowa. Upon collection, samples were returned to the Limnology Lab at Iowa State University for analysis. Comparisons among types of deposition measures are being made using non-parametric equivalents of ANOVA. Temporal analyses are being made graphically as well as using multivariate methods to relate deposition to storm type, source and intensity. Spatial patterns are being characterized using kriging within geostatistical (GIS) packages. This project will allow a broader understanding of the process of atmospheric nutrient transport in agricultural landscapes and a means of evaluating the role of atmospheric deposition in water quality impairment and remediation.

Principal Findings and Significance

The principal findings have been that (1) atmospheric deposition of N and P are much more substantial than previously concluded, (2) dry deposition is several times the deposition measured in precipitation alone, and (3) local dust deposition can bias results if proper precautions are not taken. These results are highly significant to water quality investigations in that they show a new, major pathway of nutrient transport that can negatively influence water quality. The results in (3) indicate that most atmospheric deposition measurements made using previous standard methods may be in error.

Relationship of Nitroso Compound Formation Potential to Drinking Source Water Quality and Organic Nitrogen Precursor Source Characteristics

Basic Information

Title:	Relationship of Nitroso Compound Formation Potential to Drinking Source Water Quality and Organic Nitrogen Precursor Source Characteristics
Project Number:	2002IA16G
Start Date:	9/1/2002
End Date:	8/31/2004
Funding Source:	104G
Congressional District:	first
Research Category:	None
Focus Category:	Water Use, Toxic Substances, Agriculture
Descriptors:	None
Principal Investigators:	Richard Louis Valentine

Publication

13. Title: Relationship of Nitroso Compound Formation Potential (NCFP) to Drinking Source Water Quality and Organic Nitrogen Precursor Source Characteristics

14. Statement of Critical Regional or State Water Problem:

Recent research indicates that certain disinfection practices may result in the formation of significant amounts of N-nitrosodimethylamine (NDMA), and quite likely other nitroso compounds in drinking water. These compounds are believed formed when chlorine is added to water containing ammonia, and certain organic nitrogen compounds ("precursors"). Measurements in several drinking water distribution systems suggest that unprotected sources receiving point and non-point waste discharges are particularly susceptible to their formation, especially when chloramination is practiced.

The formation of NDMA and possibly other nitroso compounds in drinking water is an emerging concern because they are generally carcinogenic, mutagenic, and teratogenic (O'Neill et al., 1984). For example, the nitrosamine, N-nitrosodimethylamine, NDMA ($(CH_3)_2NNO$) is a particularly potent carcinogen. Risk assessments from California's Office of Environmental Health Hazard Assessment (OEHHA) and US EPA identify lifetime de minimis (i.e., 10^{-6}) risk levels of cancer from NDMA exposures as 0.002 ppb (2 ng/L) and 0.0007 ppb, respectively. In February of 2002 the California Department of Health Services established an interim action level of 0.01 ppb (10 ng/L) in drinking water.

Many drinking water sources in the Midwest and other parts of the country are unprotected receiving point and non-point waste discharges. Municipal and industrial waste discharges, and those associated with agricultural practices, are potentially important sources of the organic nitrogen precursors required for the formation of nitroso compounds. These waters are correspondingly expected to be susceptible to nitroso compound formation from chlorination and especially chloramination. This may limit the use of some water sources for drinking water or restrict treatment options that otherwise have desirable characteristics. Initial observations indicate that some consumers are being exposed to undesirable levels of NDMA. Organic nitrogen is therefore not a simple benign pollutant typically associated with nutrients as generally thought..

A need exists for an improved understanding of the nature and extent of this potential problem. Work is especially needed that relates nitroso compound formation potential to source water quality and origin of organic nitrogen precursors, watershed uses, and to biogeochemical processes that could influence the quantity and types of nitroso compounds potentially produced.

15. Statement of Results or Benefits:

This project will obtain information on 1) quantities and selected types of nitroso compounds that could be formed in drinking source waters, 2) the relationship of their formation potential to source water quality and organic nitrogen precursor source characteristics, and 3) the influence of several biogeochemical processes that may influence their formation.

The anticipated benefits from this study are several fold. First, the results of the proposed study will provide a link between ambient water quality and its use for public supplies. For example, source water quality my limit the suitability of some commonly used disinfection practices such as chloramination. This is particularly important given that chloramination is being widely adopted as the preferred method of disinfection in distribution systems of utilities that cannot meet newly established EPA rules on the formation of halogenated DBPs when free chlorination is practiced. Information gained in this study will also be important in making

decisions about the need for comprehensive occurrence and risk assessments, and if warranted, the need to minimize the occurrence.

Identifying specific factors making waters susceptible to nitroso compound formation will aid in assessing the potential extent of the problem and point to mitigation strategies. For example, these might involve organic nitrogen control either through treatment modifications or source control, careful selection of drinking source waters and well locations, and modification of waste and agricultural waste management practices. Clearly, consideration of the potential for nitroso compound formation should be one aspect of any discussion relating drinking water quality to point and non-point pollution, including that from to agriculture and concentrated animal feeding operations.

16. Nature, Scope, and Objectives

Nature and Scope. Nitroso compounds are a class that includes numerous carcinogens, mutagens, and tetraogens. Approximately 300 of these compounds have been tested, and 90% of them have been found to be carcinogenic in a wide variety of experimental animals (Magee, 1982; O'Neill, 1984). Until recently it was believed that the occurrence of nitroso compounds in drinking water and wastewater was due to contamination of the source water. Recent laboratory and field studies, however, show that N-nitrosodimethylamine (NDMA), a particularly potent carcinogen, can be produced as a consequence of drinking water and wastewater disinfection (California DHS, 2002; Najim and Trussell, 2001; Choi and Valentine, 2002; Mitch and Sedlak, 2002). Specifically NDMA is produced by reactions of chlorine in water containing both ammonia, and certain organic nitrogen compounds. It is therefore a newly recognized "disinfectant by-product" (DPB). Formation of other types of nitroso compounds by this mechanism is suspected because of structural and reactivity similarities. Furthermore the occurrence potential appears significant in particular, in unprotected drinking source waters receiving a variety of waste discharges. These are likely the source abundant organic nitrogen precursors and ammonia.

It is hypothesized that water supplies receiving municipal and industrial waste discharges and in particular, agriculture related wastes are particularly susceptible to nitroso compound formation particularly if chloramination disinfection is practiced. It is also proposed that NDMA be but one representative of a new class of disinfectant by-products, the nitroso compounds, many of which are of a health, concern. The quantities and types in drinking source water will depend then on the nature of the inputs of organic nitrogen (both quantities and origin) as well as to processes that can affect the specific types and concentrations of precursor compounds.

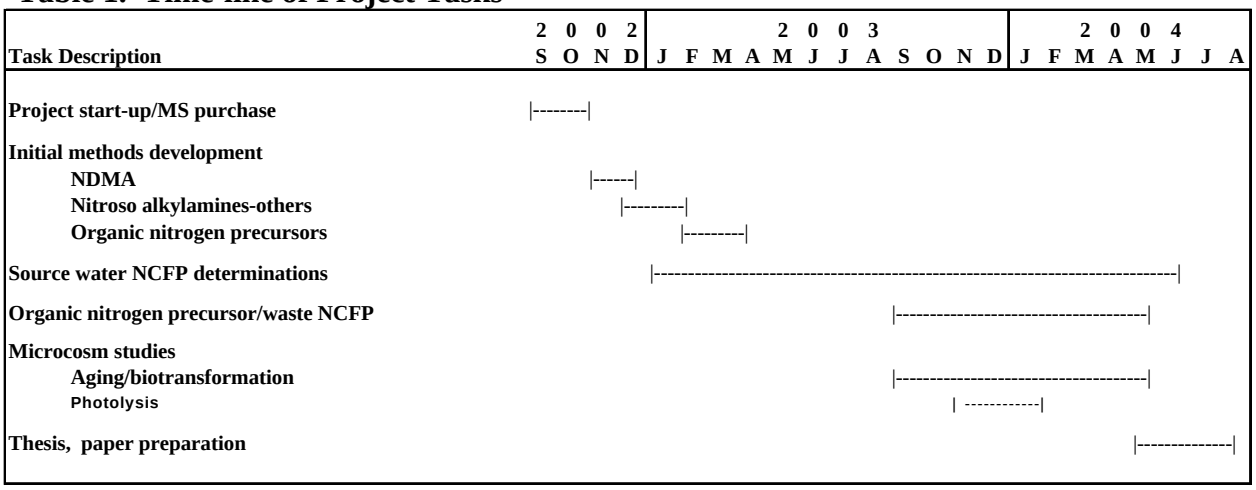
There will likely be a genesis of nitroso compound formation potential dependent on such factors as biodegradation and photolysis. Biological processes in particular will be important as it is well established that soluble microbial products include significant amounts of organic nitrogen which differs in characteristics from material consumed for growth and energy requirements (Parkin and McCarty, 1981). These products include many substances that could act as nitroso compound precursors. Alternatively, biotransformation may reduce the NCFP if the characteristic precursors are highly degradable. Passage of the water through an aquifer will also influence nitroso compound formation potential through biological and possibly geochemical processes. Many organic nitrogen compounds also exhibit high light absorption characteristics and hence high potential for direct photolysis and transformation to other compounds.

Objectives. Based upon the ascertained research needs, the following specific objectives of this research study have been formulated with respect to the relationship of source water quality and nitroso compound formation potential (NCFP) as a newly recognized disinfectant by-product:

1. Characterize the nature of the NCFP (types and quantities), in a variety of "susceptible" surface and groundwater drinking source waters, and examine the relationship of NCFP to source water quality and land usage.
2. Characterize the NCFP of a variety of organic nitrogen sources (municipal effluents, CAFO lagoons etc).
3. Conduct microcosm studies to evaluate the influence of natural transformation processes (photolysis, biological transformation) on the NCFP.

Time-line. This study will be conducted over a period of 2 years starting September 1, 2002 (Table 1). We will initially focus on developing analytical methods for several different nitrosamine compounds and their precursors during the first 4-5 months. Once we have established analytical methods and reliable method detection limits, we will be able to the measure nitroso compound formation potential in various water samples as discussed in the next section. Source water sampling will be coordinated with ongoing USGS activity. We anticipate collecting these samples and measuring NCFP immediately upon their receipt on a monthly basis over about a 12-16 month period. Studies involving sources of organic nitrogen precursors will not depend on the USGS scheduling. It is anticipated that this work, along with microcosm studies can be conducted during the second year in parallel with the source water studies (months 12-21). The last three months will be used to focus on data interpretation, and thesis and journal paper preparation.

Table 1. Time-line of Project Tasks



17. Methods, Procedures, and Facilities:

NCFP determination. Three standardized tests will be used measure NCFP. One will measure the formation potential from reaction of preformed monochloramine (NCFP-M), the second will measure the formation potential from free chlorine (NCFP-FC), and the last will measure the formation potential from reaction of nitrite (NCFP-N). It is anticipated that the formation potential from reaction of monochloramine will yield the most nitroso compounds. The NCFP-FC test will be conducted since ambient levels of ammonia can be quite large in some samples leading to formation of monochloramine. The NCFP-N test will be conducted to measure direct nitrosation. It is anticipated that this will yield comparatively small amounts of nitroso compounds. However, several organic nitrogen precursors, such as Thiram (Graham et al, 1995), are known to react rapidly with nitrite, and therefore reaction of nitrite should be evaluated. In general the importance of nitrite would also be minimal, as most drinking sources do not contain very much nitrite although partial nitrification in some well water, and in distribution

systems sometimes is observed.

All samples will be adjusted to pH 8.3 by using acid or base addition. In the NCFP-M test, preformed monochloramine will be prepared at an ammonia to chlorine molar ratio of 2:1. An aliquot will be added to the stirred samples at a concentration of 4 mg/L as chlorine (0.05 mM). In the NCFP-FC test, hypochlorous acid (free chlorine) will be added at 4 mg/L as chlorine, and in the NCFP-N test, sodium nitrite will be added at 2 mg-N/L. Solutions will be incubated in the dark at 20 ^{o}C for 24 hours and 72 hours then nitroso compounds will be quantified. Relatively concentrated wastes (precursor sources) may have to be diluted with purified water before measurements can be made. Initial concentrations of nitroso compounds will be measured in the source waters as part of the control to account for background levels (Nikaido et al. 1977).

NDMA will be measured in all samples. In addition, a select number of other nitroso compounds will be determined. Table 2 is a list of candidate nitroso compounds and their organic nitrogen precursors that will be considered. They all are of health concern and are likely to be of environmental importance since the precursors are relatively common constituents of wastewater and river/lake water (Sachter et al, 1997; Nikaido et al, 1977). Studies will initially focus on the formation of several alkyl nitrosoamines because it is believed that they will react via the proposed pathway, and because we do not anticipate any analytical difficulty due to their similarity with NDMA. We will consider adding additional compounds when we determine our analytical capabilities with regards to them.

Table 2. List of candidate nitroso compounds and their organic nitrogen precursors

Nitroso compound	Organic Nitrogen Precursor
N-Nitrosodimethylamine	Dimethylamine
N-Nitrosodiethylamine	Diethylamine
N-Nitrosoethylmethylamine	Ethylmethylamine
N-Nitrosodi-n-butylamine	Di-n-butylamine
N-Nitrosodi-n-propylamine	Di-n-propylamine
N-Nitrosodibutlylamine	Dibutylamine
N-Nitrosodiphenylamine	Diphenylamine
N-Nitrosopiperidine	Piperidine
N-Nitrosomorpholine	Morpholine
N-Nitrosopiperazine	Piperazine
N-Nitrosopyrrolidine	Pyrrolidine
N-Nitroso-N-ethylurea	Ethylurea
N-Nitroso-N-methylurea	Methylurea

Source water studies. A variety of ongoing USGS studies will allow them to provide samples for determination of the nitroso compound formation potential as well as information on water quality (nitrogen, phosphorus, dissolved organic carbon, suspended solids) and land usage. In particular, the USGS has generated a large amount of information to be used in this study as a guide in selection of surface sampling sites and for the interpretation of results. A recent report by Becher et al (2001) summarizes surface water quality from 1996 to 1998 at 11 intensively monitored locations in four watersheds. In addition, it provides information on land usage. Differences in water quality are attributed largely to agricultural practices which it is hypothesized, will be reflected in differences in nitroso compound formation potential.

Surface water continues to be assessed by monthly collection of samples from small streams such as the Wapsipinicon River near Tripoli, to large rivers such as the Mississippi River at Clinton, IA. Although most land is used for row-crop agriculture in Iowa, the effect of concentrated animal facilities (CAFO's) on stream quality is being investigated by the USGS by

sampling the South Fork of the Iowa River. Point source inputs to streams are being evaluated by collection of samples in and near municipal sewage outfalls to evaluate the occurrence of organic waste-water contaminants. We anticipate obtaining samples at approximately 11 surface sampling locations on a monthly basis.

A special activity will involve measuring the NCFP along the course of a river. The Iowa River will probably be used for this with sampling at approximately 10-15 locations, including the influent and effluent to the Coralville reservoir. This will result in information on the impact of a large reservoir, and multiple inputs of point and non-point wastes, as well as on the fate of organic nitrogen compounds and NCFP.

As part of ongoing USGS projects, samples are collected from municipal wells to assess the ambient quality of water for public supply. Municipal wells are completed in variety of different aquifers that range from shallow vulnerable alluvial to deep protected bedrock aquifers. Water-quality of shallow alluvial aquifers will be evaluated by collection of samples from monitoring wells at approximately 10 locations over a 12 month period. Samples will be simultaneously obtained from nearby surface source waters for comparison.

Samples will also be obtained at two municipal utilities practicing chloramination to compare the NCFP test results with what is actually being produced in their distribution systems after treatment. One site will be Cedar Rapids, which obtains water from shallow alluvial wells on the Cedar River, and where measurements in the distribution show significant formation of NDMA. Another will utilize surface water from the Mississippi River such as Burlington, Iowa.

Precursor sources. Samples will be obtained from several municipal wastewater treatment facilities practicing different treatment methods. These will include conventional activated sludge, trickling filter, lagoons, and extended aeration activated sludge. Samples will also be obtained samples from several lagoons used to treat animal wastes at CAFO sites (with the assistance of Dr. Melvin Stewart at Iowa State University). The idea is to directly determine the contribution of these materials to the NCFP and to provide material for microcosm studies. Ideally these will come from locations potentially impacting source waters used in this study.

Microcosm studies. Microcosm studies will examine nitroso compound formation potential genesis as a function of water age in several (6-10) source water samples and river water diluted wastes. Each microcosm will be a four-liter sample, stored in the dark at 20 ^{o}C. We will age these one week to two months. It is hypothesized that biological activity will influence the extent and distribution of nitroso compound formation. Aging may favor the ultimate formation of NDMA while more complicated nitroso compounds may predominate in less aged water. Conversely, biodegradation of precursors compound may cause a reduction in formation potential. Some samples will also exposed to simulated sunlight using a Suntest solar simulator to examine the influence of sun on NCFP. Only a limited number of studies will be conducted with source water due to equipment limitations.

Facilities and analytical systems. The USGS will provide extensive information on water quality (organic nitrogen, TOC, inorganic nitrogen) of the samples provided to Iowa. This information is obtained as part of their ongoing studies using their own facilities and standard protocols. Additional analytical work will be conducted in the Environmental Engineering and Science Laboratories (EESL) at the University of Iowa. Most of the functions of the EESL take place in the facility (4800 ft^2, opened Summer 2001) located in the Seamans Center for the Engineering Arts and Sciences. Some projects are supported at the division of the EESL located at the University Water Treatment Plant - a 2,400 ft^2 facility designed for both research needs and teaching purposes. The laboratory is fully equipped with a wide selection of modern analytical equipment. It is managed by a full-time laboratory director (Dr. Craig Just) who is responsibile for student training, equipment maintenance, methods development, and most importantly, laboratory QA/QC.

It is anticipated that the general methodology developed for the analysis of NDMA at the University of Iowa will be extended for the analysis of a variety of specific nitroso compounds (Taguchi et al., 1994). In this approach the nitroso compound will be determined by an isotope dilution gas chromatography/mass spectrometer (GC/MS) method. Prior to extraction, all 1 L samples are dosed with the deuterated nitroso compound as an internal standard if available. Otherwise deuterated NDMA will be used as an internal standard. Isotopically labeled nitroso compounds in methanol other than NDMA, seem unfortunately, to be available only for a few precursor candidates. To the 1 L sample is added 200 mg of carbonaceous adsorbent (Ambersorb 572, Aldrich) and then the sample is shaken for 1 hour at 200 rpm. The Ambersorb beads are vacuum filtered onto a glass fiber filter, and dried in air for 30 minutes. Beads are transferred to a 2-mL amber vial where beads are soaked with 0.5 mL of methylene chloride for 20 minutes before analysis. A 95 μL aliquot of methylene chloride extract is injected into GC/MS (Varian) equipped with Large Volume Injector (Optic2). The nitroso compound will be quantified based on the mass detection of the characteristic molecular ion. The MDL at the 99% confidence level for NDMA is expected to be approximately 2.0 ng/L.

Specific organic nitrogen precursors will be determined by GC-MS methodology (Sachter et al, 1997) or by HPLC. Total organic nitrogen will be measured using the Kjeldahl method (APHA, 1993). Nitrate and nitrite will be determined by ion chromatography using a Dionex IC, and total organic carbon by Shimadzu TOC 4000 analyzer (APHA, 1993).

18. Related Research:

Environmentally oriented NDMA occurrence and formation studies have been generally empirical in nature and have focussed primarily on determining if, not how, nitrosamines may be formed in water. All have assumed that nitrite is a required reactant. Lab based studies usually involve its addition along with an appropriate organic nitrogen containing precursor. This is based upon the widely held belief that the primary formation mechanism involves the classical acid catalyzed nitrosation. This reaction is however, also biologically catalyzed and likely will account for any background levels of nitroso compounds in the untreated waters (Ayanaba and Alexander, 1974; Nikaido et al, 1977).

The recent concern about the occurrence of NDMA originated when it was found in highly purified wastewaters intended for recycle and reuse. It was found at alarming levels, sometimes exceeding 1000 ng/L. Its formation as a consequence of some treatment step was suspected because it was absent in the plant effluent. Subsequent observations found it in some treated drinking waters while absent in the influent streams (California Department of Health Services, 2002). Based upon suspected linkage to disinfection practices, Najim and Trussell (2001) showed formation of over 16 ng/L in one chloraminated drinking water and approximately 400 ng/L in a filtered and chlorinated tertiary waste water. They hypothesized that NDMA formation was somehow related to the common practice of chlorination or chloramination (formation of chloramines by reaction of chlorine and ammonia).

Recently Choi and Valentine (2002) and Mitch and Sedlak (2002) independently proposed a novel mechanism to describe NDMA formation in chlorinated and chloraminated water containing DMA as a model precursor. Choi and Valentine (2002) also developed a kinetic reaction model (Figure1) of use in making predictions about NDMA formation. The proposed mechanism is based largely on studies of hydrazine formation (a rocket fuel) and its oxidation (Lunn et al, 1991; Castegnaro et al, 1986; Cahn and Powell, 1954). The key reactions include the formation of monochloramine from the initially added HOCl (Reaction 1), the reaction of chlorine with DMA to form dimethylchloramine, DMCA (Reaction 2), and the slow transfer of active chlorine from monochloramine to DMA to form more DMCA (Reaction 3). Formation of NDMA is initiated by the formation of 1,1-dimethylhydrazine (UDMH) intermediate from the

reaction of DMA with monochloramine (Reaction 4), followed by the oxidation of UDMH by monochloramine to NDMA (Reaction 5). Figures 2 and 3 show a close correspondence between measured NDMA concentrations and that predicted by the comprehensive model in chlorinated and chloraminated water containing DMA.

It should be noted that addition of nitrite to DMA containing solutions yielded very little NDMA. This shows as expected, that classical nitrosation is not likely an important formation mechanism involving simple organic amines at drinking water conditions unless some unrecognized catalytic process is occurring. Fast reactions of nitrite with some "unusual" precursors such as Thiram (Graham et al., 1995), a pesticide cannot be ruled out however. The general importance of this possibility needs to be evaluated in control studies along with formation by the direct chlorine reaction route.

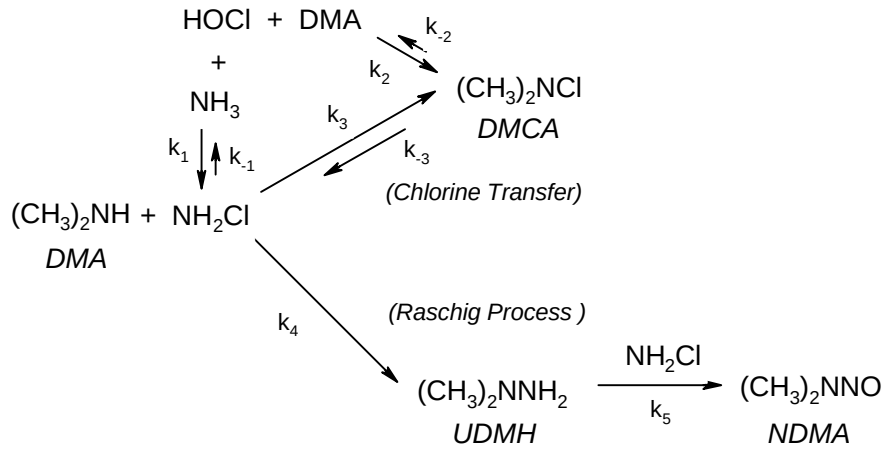


Figure 1. Mechanism of NDMA formation in chlorinated waters containing DMA and ammonia.

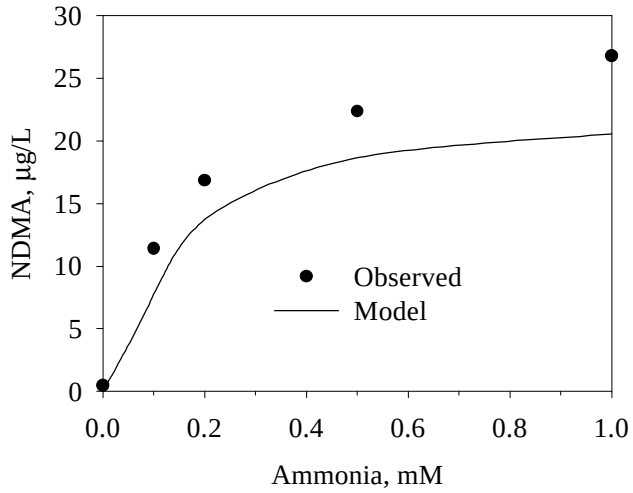


Figure 2. NDMA formation after 24 hours contact. Addition of 0.1 mM HOCl to a mixture of 0.1 mM DMA and ammonia. pH 7.0 ± 0.1; 4 mM bicarbonate buffer. Ionic strength was adjusted to 0.05 M with NaCl. Temperature 25°C.

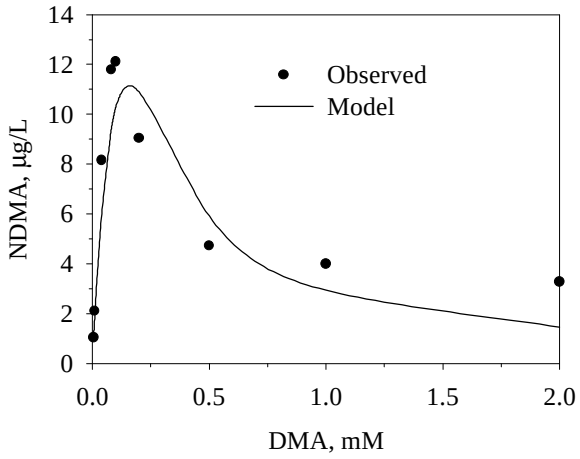


Figure 3. NDMA formation after 24 hours as a function of DMA concentration. Preformed monochloramine concentration was fixed at 0.1 mM. The pH was adjusted to 7.0 ± 0.1 using 1 mM bicarbonate buffer. Temperature 25 ^{o}C.

The rationale for the applicability of this mechanism to the formation of some other types of nitroso compounds is based upon precursor structural similarities (Rowe and Audreith, 1956). For example diethylamine and dibutylamine should yield formation of N-nitrosodiethylamine and N-nitrosodimethylamine by the same mechanism. This however, has not been demonstrated either in the field or in the lab. In addition, no studies have been conducted examining nitroso compound formation by this mechanism in waters thought susceptible as described in this proposal. Studies on the genesis of NCFP and the influence of natural attenuation processes are a logical extension of recent work.

The PI is currently participating in an American Water Works Association Research Foundation (AWWARF) sponsored project surveying NDMA occurrence in treated drinking water in the US and Canada. An important finding is that the highest NDMA formation appears to occur in chloraminated waters obtained from unprotected and "susceptible" sources (unpublished data). For example, concentrations increased from <1 ng/L in the raw water to 6 ng/L in the effluent, and then to approximately 20 ng/L in the distribution system of the City of Cedar Rapids, Iowa. The source is a shallow alluvial well on the Cedar River.

The proposed project will build upon this work and studies recently supported by an ISWRRI seed grant that ends in May 2001. The proposed study does not duplicate any work in these other projects, which were instrumental in developing the hypotheses in this proposal.

19. Training Potential:

This project will support two doctoral graduate students or one doctoral and one master's student at the 1/2 time level for two years. These students will work both with Professor Valentine as well as help in sampling under the direction of Stephen J. Kalkhoff (see section 20). In addition, one undergraduate student from our undergraduate environmental engineering program or from chemistry will be hired for each of the two summers. We anticipate having work-study funds or funds from the Research Experience for Undergraduates-REU program to augment this.

20. Statement of Government Involvement:

The PI will work very closely with Stephen J. Kalkhoff, Chief, Eastern Iowa Basins, National Water-Quality Assessment Program (NAWQA) study unit. He will participate in planning, sampling, and in collaboration on resulting papers. Working together with the two students, he will facilitate the integration of this project with several ongoing USGS studies.

First, the U.S. Geological Survey will provide historical data, ongoing water-quality monitoring data, and ancillary information as part of the collaborative efforts in this project. Data collected as part of the Eastern Iowa Basins (EIWA) study unit of the National Water-Quality Assessment Program (NAWQA) and other historical data indicate that organic compounds including nitrogen containing compounds vary seasonally. Dissolved organic nitrogen concentrations generally are greatest during snowmelt and rain events in early spring when runoff from the land surface is occurring. As intuitively expected, greater concentrations of organic compounds occur in streams draining watersheds with large numbers of concentrated animal feeding facilities. Because of the NAWQA's program needs to relate water quality to natural and human factors that effect it, the U.S. Geological Survey has also compiled a variety of ancillary data, land use, hydrology, etc for the sampling sites that would be available to help understand the nitroso formation potential of water resources in Iowa. This information will provide guidance in the selection of source water sampling locations and times.

The U.S. Geological Survey District is also involved with several projects to investigate water-quality conditions in Iowa and is collecting data from a variety of environments for which a determination of nitroso compound formation potential and precursors would be value. For example, samples are collected from municipal wells to assess the ambient quality of water for public supply, surface water is assessed by monthly sampling of several streams and rivers, and point source inputs to streams are being evaluated by collection of samples in and near municipal sewage outfalls to evaluate the occurrence of organic waste-water contaminants. This extensive sampling effort will allow the U.S. Geological survey to both provide analytical data on organic nitrogen concentrations and provide samples for determination of the nitroso compound formation potential.

21. Information Transfer Plan

The primary form of information transfer will be through peer-reviewed publications in nationally recognized journals, and presentations at national and regional meetings of several organizations (e.g. AWWA, WEF). Subject matter would be relationship of water quality to nitroso formation potential, sources of nitroso compounds precursors, influence of aquifer passage on the NCFP, correlation of land use practices with NCFP, and the NCFP of various wastes.

The intended audience will be 1) water treatment and supply professionals, 2) state and federal government agencies involved with water quality (e.g. Iowa DNR, EPA, USGS), and 3) members of the agricultural community who must deal with ag waste issues. Dr. Melvin Stewart, Department of Agriculture and Biosystems at Iowa State University has agreed to facilitate potential presentations sponsored by our extension service.

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An Integrated Immunological-GIS Approach for Bio-monitoring of Ecological Impacts of Swine Manure Pollutants in Streams

Basic Information

Title:	An Integrated Immunological-GIS Approach for Bio-monitoring of Ecological Impacts of Swine Manure Pollutants in Streams
Project Number:	2002IA25G
Start Date:	9/15/2002
End Date:	9/15/2005
Funding Source:	104G
Congressional District:	Iowa's 3rd district
Research Category:	None
Focus Category:	Non Point Pollution, Water Quality, Methods
Descriptors:	None
Principal Investigators:	James A. Roth, Dusan Palic, Bruce Willard Menzel, Clay Lynn Pierce

Publication

1. Title

An Integrated Immunological-GIS Approach for Bio-monitoring of Ecological Impacts of Swine Manure Pollutants in Streams

2. Statement of critical regional or State water problem

Thirty years after enactment of the Clean Water Act, 40% of our nation's rivers, lakes, and coastal waters are still considered unfit for fishing, swimming, drinking or aquatic life. The U.S. EPA identified agricultural operations as the primary cause of non-point source pollution in the nation's impaired rivers and lakes. At least 10 % of the nation's impaired river miles are affected by pollution from livestock operations (3.). In portions of the Midwest, confinement livestock operations are a particular problem in this regard. Spills and dumping of fecal material occurred over one thousand times at feedlots in the ten Midwest U.S. states between 1995 and 1998. Over the past two decades, swine production in the U.S. has increasingly shifted to a large-scale, corporate model. Today, two percent of the hog operations in the U.S. produce over 46% of the total hog population (26.). In Iowa, the largest swine production state, about 21 million tons of manure are produced annually, chiefly at large-scale confinement operations (pers. comm., Dr. Jeffery Lorimor, ISU Department of Agricultural and Biosystems Engineering). Commonly, the manure from such operations is held in earthen lagoons for anaerobic decomposition prior to application as a fertilizer to crop fields. As older-style lagoons age, their failure and leakage is a growing problem. Improper application of the fertilizer and its runoff from cropland also result in manure delivery to local waters. Because of its volume, composition, and handling methods, therefore, swine fecal contamination is a serious threat to environmental quality in regional waterways and especially in Iowa (1.). Local citizens are becoming increasingly intolerant of the environmental cost of confinement livestock production (36.)

Cases of massive deaths of aquatic organisms, often referred to as fish kills, are an extreme manifestation of the ecological impact of fecal contamination. Typically, they result from high concentrations of toxic ammonia contained in the manure or from depletion of dissolved oxygen in the water caused by decomposition of the pollutant. From 1995 to 1998, over 13 million fish were killed in more than two hundred documented manure spills in the Midwest (35.). In the past 20 years, fecal pollution was the single most important cause of 495 documented fish kills in Iowa, accounting for over one-quarter of the diagnosed cases, and hog manure was the primary pollutant (2.). Although cases of acute toxicity of manure pollution capture the headlines, there are other impacts that may be of equal, or perhaps greater, long-term ecological importance. For example, fecal pollutants contain nutrients, especially nitrogen and phosphorus compounds, microorganisms and other materials that upset ecological processes and impair water quality for human uses (13.). Additionally, exposure to sub lethal pollutant concentrations can interfere with normal life processes of wildlife such as feeding, reproduction, defense and disease resistance. This can result in gradual declines and even extirpations of animal populations and communities. Such chronic effects of manure pollution are poorly known, because of the difficulty of measuring them and placing them in ecological context. Moreover, low-level delivery of fecal pollutants can portend larger catastrophic inputs, for example, when a gradually leaking storage lagoon eventually bursts or an erosive, manure-fertilized crop field receives heavy rainfall.

State and federal agencies engaged in reducing non-point source water pollution are interested in obtaining new technologies for identifying, measuring and anticipating pollution occurrence. Clearly, development of tools that could integrate biological and environmental information to produce site-specific predictive models for guiding pollution-prevention management practices is highly desirable. Benefits of such tools and management practices would accrue locally and more broadly throughout interstate drainage basins. The highly publicized Gulf of Mexico hypoxia situation is an example of the geographically widespread impacts of agricultural (and other) pollution in the Mississippi River basin. The proposed research would develop a novel tool that integrates molecular biological and ecological approaches to quantitatively evaluate environmental impacts of swine manure pollutants. Although the technique will be developed with specific reference to Midwestern waters, it will be more broadly applicable, both geographically and with reference to other forms of pollution that engender immune responses in animals. Thus, we believe that the technique has potential to be widely adopted by state and federal environmental management agencies.

3. Statement of results or benefits

The expected results include 1) testing, evaluation and application of techniques that quantitatively measure fish immune response to swine manure exposure, 2) development of predictive models for estimating site-specific fecal pollution potential in Midwestern warmwater stream systems based on local landscape features and farming practices, and 3) a marriage of the two techniques as a new and cost-effective pollution biomonitoring methodology. Several benefits will result from the research. First, the work will extend knowledge in basic immunobiology science. Knowledge of the fish immune system is very limited. The assays that will be tested in the project were developed for homeothermic animals - mammals and birds. Adaptation of their use for fish will contribute to knowledge of fish immune reactions. This will be valuable information, not only in the context of pollution control, but also relative to fish health. Aquaculture, or fish farming, is the fastest growing segment of American agriculture, and fish health-related issues are a major factor inhibiting its continuing development. Second, the refined immunological assay methods will provide a relatively simple and fast tool for detecting ecological impacts of low-level and long-term manure pollution. Third, applications of the technique will permit identification of livestock operations and croplands that potentially pose threats to aquatic ecological integrity from manure seepage and runoff. Fourth, the research will contribute to development of the USGS IRIS and Aquatic Gap projects that are underway at Iowa State University and partially funded by the Iowa Department of Natural Resources (see below).

4. Nature, scope and objectives of the project, including a timeline of activities

Nature: The proposed research reflects the need for integrated, multidisciplinary approaches to deal with complex environmental issues. It will combine physiological laboratory techniques, computer modeling of agricultural landscapes and non-point source pollution pathways, and field-based ecological analyses to create a new and integrated approach for evaluating impacts of livestock fecal contamination on Midwestern streams.

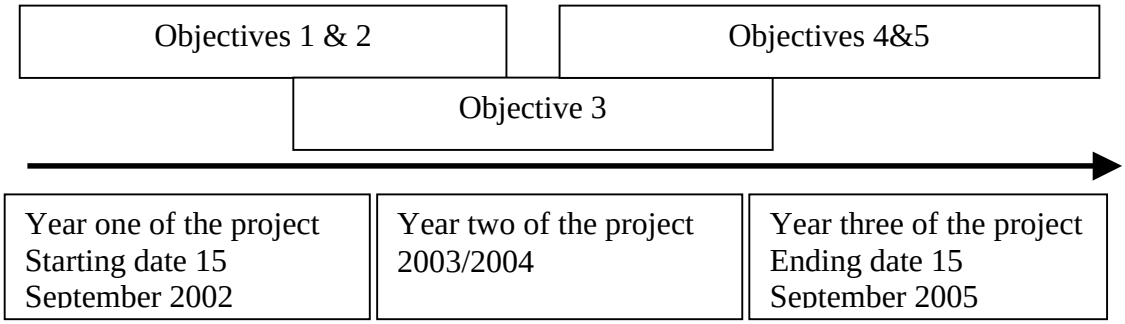
Scope: The research relates to two major priorities of the NIWR National Competitive Grants Program.

A) It will complement work by the USGS related to non-point source pollution, contributing to

development of integrated watershed decision support tools for assessing organics and microorganisms transport and fate, along with their effects on aquatic systems.
B) It promises development of a new water quality sensor technology that will be based on integrated methodologies and will provide results that are readily accessible through the Internet.

Objectives: This research is predicated on the hypothesis that low levels of swine liquid manure slurry and anaerobic lagoon liquid released to open water cause changes in immunological response in fish and increase fish susceptibility to infection.
The initial objectives, therefore, are 1) to evaluate this hypothesis through a series of laboratory immunological assays applied to the test organism, the fathead minnow (*Pimephales promelas*) and 2) to identify one or more assays for use as a bio-monitoring technique to detect ecological impact of manure pollution in nature. A subsequent task involves use of digital environmental databases that are maintained and managed by the USGS BRD Iowa Cooperative Fish and Wildlife Research Unit at Iowa State University. The objective is 3) to characterize a number of Iowa watersheds and stream systems according to their potential susceptibility to hog manure pollution and to use this information to design a water quality and fish sampling regime. Finally, water and fish collected at selected stream sites will be analyzed through a battery of chemical and immunological procedures with the objectives 4) to quantitatively measure ecological impact of manure pollution on the streams, and 5) to evaluate the utility of this approach as a biomonitoring tool for environmental protection agencies.

Suggested timeline:



5. Methods, procedures and facilities
The fathead minnow is a native Iowa species, abundant and ubiquitous in small streams (14.). Thus, it is a good choice as a representative of fish communities exposed to low level concentrations of swine manure pollutants released into Iowa waters. Moreover, it is commonly used as a standard bioassay organism in toxicological analyses, so there is substantial knowledge on its tolerance to a wide array of environmental physical conditions and pollutants (15.). Additionally, colonies can be easily established and maintained in the laboratory. Fathead minnows used for the experiment will be raised in a controlled environment, without previous exposure to swine manure.

The bacteriological composition and characteristics of swine manure slurry and lagoon liquid are generally known, a number of bacterial varieties being present (16.). During manure storage and manipulation, some bacteria die and degrade. Consequently, it is expected that a number of chemical varieties of released bacterial lipopolysaccharides (LPS) are present in both manure forms, reflective of different bacterial sources. Since thorough bacteriological analyses of the manure and water are time consuming and expensive, the LPS component of the bacterial cell wall will be used as a measure of bacterial variety and concentration. Furthermore, since LPS is considered an activator and/or promoter of phagocytic cell activity (17.), the amount of LPS in manure samples can serve as a measure of immunologically active substances present (20). The immune response will be determined by activity of phagocytic cells, through several forms of measurement. Evidence from limited research involving fish suggests that several respiratory burst activity (RBA) assays are useful for determining phagocytic function in fish, and the procedure also seems to hold promise as a bio-indicator for fish health (6.). Additionally, histological examinations of melano–macrophage centers (MMC) in liver, spleen, kidneys, intestines, skin and gills will be used to measure effects of long term exposure to manure (19.).

Geographic Information Systems (GIS) technologies provide a tool to enter, store, manipulate and integrate geo-referenced data on, for example, landscape features, water quality, and aquatic organisms (30.). The project will apply GIS technology and landscape modeling to calculate possible swine pollutant flow path patterns in Iowa watersheds having large hog confinements and in those where liquid manure fertilizer is applied on crop fields. Using this approach, we will estimate temporal and spatial distribution of manure loads and concentrations that reach receiving waters. This will provide the basis for a field sampling regime to determine actual conditions of water quality and fish communities at stream sites selected to represent a range of calculated manure pollutant loadings. Water quality data on fecal coliform bacteria, phosphorus compounds and ammonia, among others, will be evidence concerning actual loading of manure material. Ecological impact of the pollution will be evaluated by the developed immunological assays performed on wild-caught fathead minnows. Statistical comparisons will be made between the calculated and measured evidence for the pollutant to determine the accuracy and reliability of the immunological approach in actual practice. As a further check on the procedure, Index of Biotic Integrity (IBI) values will be calculated based on wild fish community parameters. The IBI is a commonly used bioindicator of stream environmental quality. It serves as a summary measure of biotic community response to pollution and other forms of habitat degradation. It is being used routinely for long-term environmental monitoring programs in Iowa and other Midwestern states. This design, therefore, will allow comparisons between this established coarse-scale environmental indicator and the experimental fine-scale immunological indicator.

The project will involve the following stages and procedures.
Stage 1 – Determine the lethal concentration for 50% of the experimental population of fish exposed to swine liquid manure slurry and swine anaerobic lagoon liquid. This procedure is necessary in order to calculate the range of concentrations that will be applied in the actual experiment, since the experimental fish should not experience any mortality during long-term toxicity tests. For this, different concentrations of the test substances, measured as LPS, will be used in static water aquaria containing 10 to 15 fish each, following standard procedural guidelines of the U.S. EPA OPPTS 885.4200 (23.). To determine LPS concentrations, the

Limulus amoebocyte lysate assay will be used (20.). In brief, five different concentrations of test substance will be used in each of two aquaria, together with a positive (concentration that causes 100% mortality) and negative (no active substance) control. Mortalities will be measured twice a day (morning/evening) and then calculated as LC 50 for 96 hours. Once LC 50 is determined, the range of sub lethal concentrations will be calculated and used in long term toxicity testing.

Stage 2 – Develop and standardize procedures for isolating phagocytic cell populations from fish tissue samples (21.) and use them for the battery of assays: iodination, ingestion and RBA (18.). Iodination assays measure the antimicrobial activity in phagocytic cells based on activity of the myeloperoxidase – hydrogen peroxide – halide ion system. Ingestion assays measure actual phagocytosis using labeled particles submitted to phagocytes. The RBA assays will include three different procedures. The procedure that shows best results with fish cells will be selected for further study: 1) *Superoxide-dismutase (SOD) inhibitable reduction of cytochrome c.* Briefly, upon appropriate stimulation, phagocytes produce superoxide anion that is converted to hydrogen peroxide in the presence of SOD. Peroxide then oxidizes p-hydroxy-phenylacetate (PHPA) in the presence of horseradish peroxidase to a fluorescent product $PHPA_2$ (18.). 2) *Oxidation of 2'7'dichloro-dihydro- fluorescine (H_2DCF).* This is based on a combination of esterase and peroxidase activity towards different reactive oxygen species produced during oxidative burst (17.). 3) *Luminol enhanced chemiluminescence.* In this assay , the phagocytic activity is measured using a set of biochemical reactions, mainly myeloperoxidase activity, again based on superoxide anion release and induced measurable chemiluminiscence (18.) The experimental fish will also undergo histological examination of MMC forming centers by standard histological methods (19.). MMC are aggregates of phagocytic cells in the sites of infection. If the stimulus for their gathering lasts long enough, melanin production by the cells increases in order to prevent accumulation of free radical byproducts of phagocyte activity. These centers are visually identified by their dark melanin-based color and are often used as a non-specific sign of chronic infection (19.).

Stage 3 – Apply assays developed in Stage 2 to fish exposed to different sub lethal concentrations of swine liquid manure slurry and anaerobic lagoon liquid calculated in Stage 1. Exposure of the fish will follow standard U.S. EPA procedures (23.). In short, a range of five different sub lethal concentrations of substance will be used in two aquaria. Each aquarium will have 150 fish at the beginning, and starting after two days of exposure, 5-10 fish will be sacrificed weekly and subjected to examination.

Stage 4 – Submit fish to a challenge test using *Aeromonas hydrophila* as the pathogen and determine relative percent survival (RPS). The challenge test is a standard procedure for evaluating immunomodulation effects of different substances (23., 24.). It consists of two steps. The first is to vaccinate the experimental animals with a commercially available vaccine. The second step is to challenge vaccinated and non-vaccinated groups with the pathogen and then measure RPS. In this study, the challenge test will be performed on groups of fish that were either previously exposed to sub lethal concentrations of manure or not so exposed. All groups will be assayed for phagocyte cell function during the challenge period.

Stage 5 – Statistical evaluation of data using appropriate methodology for finding the

differences between groups of test organisms exposed to different conditions and toxicants. Possible approaches include analysis of variance, contingency-table analysis, and two types of multivariate procedures – principal components analysis and logistic regression (25.).

Stage 6 – GIS-mediated flow path analysis of manure delivery to waterways (30.). This stage will rely heavily on cooperation with the USGS Iowa Cooperative Fish and Wildlife Research Unit (ICFWRU) and its Iowa River and Stream Information System. IRIS is a digital database for integrating physical, chemical and biological information into a comprehensive, statewide information system for interior Iowa rivers. It is linked with the Iowa Aquatic Gap program that is also managed by the ICFWRU and funded by USGS BRD. Using information in these databases, a number of Iowa watersheds will be selected that represent a range of likely hog manure influences on local waters. For each watershed, we will determine the drainage-basin morphometry and the manure production or application rate. The 500 m resolution digital elevation model (DEM) will be utilized in several steps involving delineation of the stream network, identification of stream sites for fish and water sample collection, determination of the drainage area at each site, calculation of landscape parameters that characterize this drainage area and estimation of watershed-averaged manure deposition rate. We will use ArcView software for advanced GIS geospatial analysis using raster and vector data supported by ArcGIS desktop extensions for application to the USGS Hydrologic Modeling System, HEC-HMS. In order to use this software we will formulate statistical models capable of representing the spatio-temporal variability in surface waters and build a model for predicting concentrations of manure loads under different hydrologic conditions and for different agricultural chemical application and deposition rates. We expect to utilize the Agricultural Nonpoint Source Pollution Model (AGNPS) (38.) and perhaps the Feedlot Evaluation Model (37.) as the bases for developing the new models.

Stage 7 – Field testing of methods. Samples of water and fish communities will be collected using standard techniques (34.) at stream sites determined from Stage 6 analysis. Cursory evaluation of general health status of the catch will be done, and most fish will be preserved for later laboratory inspection and enumeration, but a subsample of fathead minnows will be taken alive and assayed for immune function using methods developed in Stage 2.

Stage 8 – Integration of field data in the IRIS system, comparative analysis of the data obtained with Fish Index of Biotic Integrity (IBI) and fish innate immune system changes, and testing of the spatial/temporal prediction model for estimate of manure impact on selected sites (31.).

Facilities that will be used are the fish culture and maintenance facility in the ISU Department of Animal Ecology, immunology laboratory in the ISU Department of Veterinary Microbiology and Preventive Medicine, and the ISU Geographical Information Systems Facility.

6. Related research

The innate immune system is the collection of defense mechanisms that protect the organism against microbial infection, with no need for prior exposure to the microbe. The link between low-level environmental contamination and activation of the fish IIS has been suspected for

some time (4., 5.). The IIS has several attractive features for application to bio-monitoring. First, it promptly reacts on changes caused by interaction between the animal and its environment. Second, changes of innate immunity can influence susceptibility to disease and provide another measurement tool (6.). Third, innate immunity appears to be evolutionarily conservative (7.), so that the response towards a given pollutant by one species (of fish, for example) may reflect similar responses among other species in the local community. Therefore, techniques for measuring the IIS response potentially can be standardized and used to predict entire aquatic community responses. Finally, sampling of the different components of the IIS can provide a set of inter-related parameters that can be used to discern pattern formation in the response towards a specific pollutant (6.). A cellular component in the IIS is the phagocytic cell, a part of the immune system that actively ingests foreign particles encountered in the body or on mucosal surfaces (such as the gills of fish). An activated phagocyte releases a spectrum of different chemical compounds that can be measured in order to monitor the immune response (8.).

Fish species and communities are commonly used as bioindicators of aquatic environmental conditions (9.) for several reasons. Fishes are often abundant and are generally easy to capture. Their biology, ecology and long-term population responses to pollution are well known, and they have cultural values that Society appreciates. Use of fishes as biomarkers of innate immunity changes is based on several additional considerations. First, impairment of innate immunity is more pronounced in fish than, for example, in mammals (10.). Second, changes in fish innate immunity may be appropriate for evaluating overall condition of the aquatic environment (11.). Third, long-term bio-monitoring results can be used for evaluating potential impacts on human and ecosystem health, because the route for the toxicant often leads to humans through aquatic systems (12., 13.).

7. Training potential

A. Ph. D. graduate student – 1. Dusan Palic, DVM, will use this project as the basis of his Ph.D. dissertation in Immunobiology and Fisheries Biology. During this research he will have an opportunity to continue his scientific education through combining medical expertise with ecosystem approaches to solving large scale problems at higher levels of complexity.
B. Undergraduate students – 10. The students will be recruited from the ISU Department of Animal Ecology and other biology programs. They will be employed as hourly assistants during the academic year and in summer. Their duties will include maintaining the stock of experimental minnows, assisting with the immunological assays, collecting fish and water samples from stream sites, and performing water quality tests.

8. Statement of government involvement

Dr Clay Pierce, Assistant Leader (Fisheries) of the Iowa Cooperative Fish & Wildlife Research Unit, Biological Resources Division - U.S. Geological Survey is Co-Principal Investigator of this project. Dr Pierce has expertise in fish and stream ecology and experience with Geographical Information Systems. His responsibilities as federal collaborator in the project include assistance on proposal preparation, literature review and consulting data sources from federal project, active involvement in choosing field sites for the research using projections from path flow analysis models and evaluating field data analysis in hydroecological context. Also, Dr Pierce will take active part in coordinating implementation of collected data in IRIS

information system, presentation of the results through IRIS and the World Wide Web, and adviser role for the graduate student.

9. Information transfer plan

Information derived from the project will be transmitted to scientists, environmental managers, and the general public. Scientific audiences will be reached initially through presentations at scientific conferences. Examples include annual meetings of the Iowa Academy of Science (state level), the Midwest Fish and Wildlife Conference (regional level) and the American Fisheries Society (national level). Ultimately, articles will be published in scientific research journals such as the *Journal of Environmental Management, Fish and Shellfish Immunology,* and *Ecological Toxicology.* The research will be a registered project with the Iowa Agriculture and Home Economics Experiment Station and the Iowa Cooperative Fish and Wildlife Research Unit at Iowa State University. As such, annual and final reports will be provided for the USDA CRIS reporting system and the Unit's own annual report. In this way, project results will be available to the national and international scientific community. Our field work, especially, will involve coordination with local natural resources managers of the Iowa Department of Natural Resources, the USDA Natural Resources Conservation Service, and the USGS state office. Through these interactions, we will advise the management community about the project and its results. If the integrated biomonitoring approach is ultimately deemed feasible, workshops will be held for staffs of these agencies to introduce them to the procedures and encourage their adoption for management applications. The general public and the private mass communication sector will be informed about the project in several ways: through established public information channels of ISU Extension and the ISU Colleges of Agriculture and Veterinary Medicine, and by the IRIS WWW page: (http://madagascar.gis.iastate.edu/iris/viewer.htm).

10. Investigator's qualifications

Two page resumes for investigators are included as separate documents at the end of this proposal.

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Complementary Investigations for Implementation of Remote, Non-Contact Measurements of Streamflow in Riverine Environment

Basic Information

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COMPLEMENTARY INVESTIGATIONS FOR IMPLEMENTATION OF REMOTE, NON-CONTACT MEASUREMENTS OF STREAMFLOW IN RIVERINE ENVIRONMENT

Submitted to

Iowa Sate Water Resources Research Institute
Ames, Iowa, 50011-1010

by

Marian Muste and Ceyda Polatel
IIHR- Hydroscience and Engineering
The University of Iowa
Iowa City, Iowa 52242

Preliminary Report #1

January 2003

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1. SUMMARY OF CONDUCTED EXPERIMENTS

1.1. FACILITIES AND INSTRUMENTATION

The set of experiments proposed by Muste et. al. (2001) were conducted at IIHR-Hydroscience and Engineering laboratory in a 10m long, 0.61m wide and 0.5m deep, recirculating tilting open channel flume (Figure 1).

Laser Doppler Velocimetry (LDV) was used to measure the velocities in the water column. LDV measurements were done with a two-component fiber optic LDV system of conventional design. The LDV principles, operation and output are relatively followed the standard procedures and will not be detailed herein. At each measurement point, 15,000 samples are obtained and a standard procedure is used to determine the averages.

Large-scale particle image velocimetry (LSPIV) was used for measurements on the free surface. This method is an extension of conventional PIV for velocity measurements in large-scale flows (Fujita et al., 1998). While the image- and data-processing algorithms are similar to those used in conventional PIV, adjustments are required for illumination, seeding, and pre-processing of the recorded images.

A digital camera (Sony DCR-TRV320) is used for recordings. Two quartz-halogen photographic lamps with diffusers are used to illuminate the selected area. The transparent walls of the channel were covered by black masks so that there was a 2 cm opening at the both sides of the channel in the vicinity of the water surface plane (Figure 2). Proceeding in this way, only water surface region was illuminated and good contrast was obtained.

The seeding material for LSPIV was Styropor® expandable polystyrene which is produced by BASF with a bulk density of 12.5 kg/m^3 and diameters of 2 to 3 mm is used. Flow images recorded at 30 Hz were subsequently digitized in 640 by 480 pixels of 8-bit, gray-level resolution images and processed with the PIV analyzing software EdPIV®.

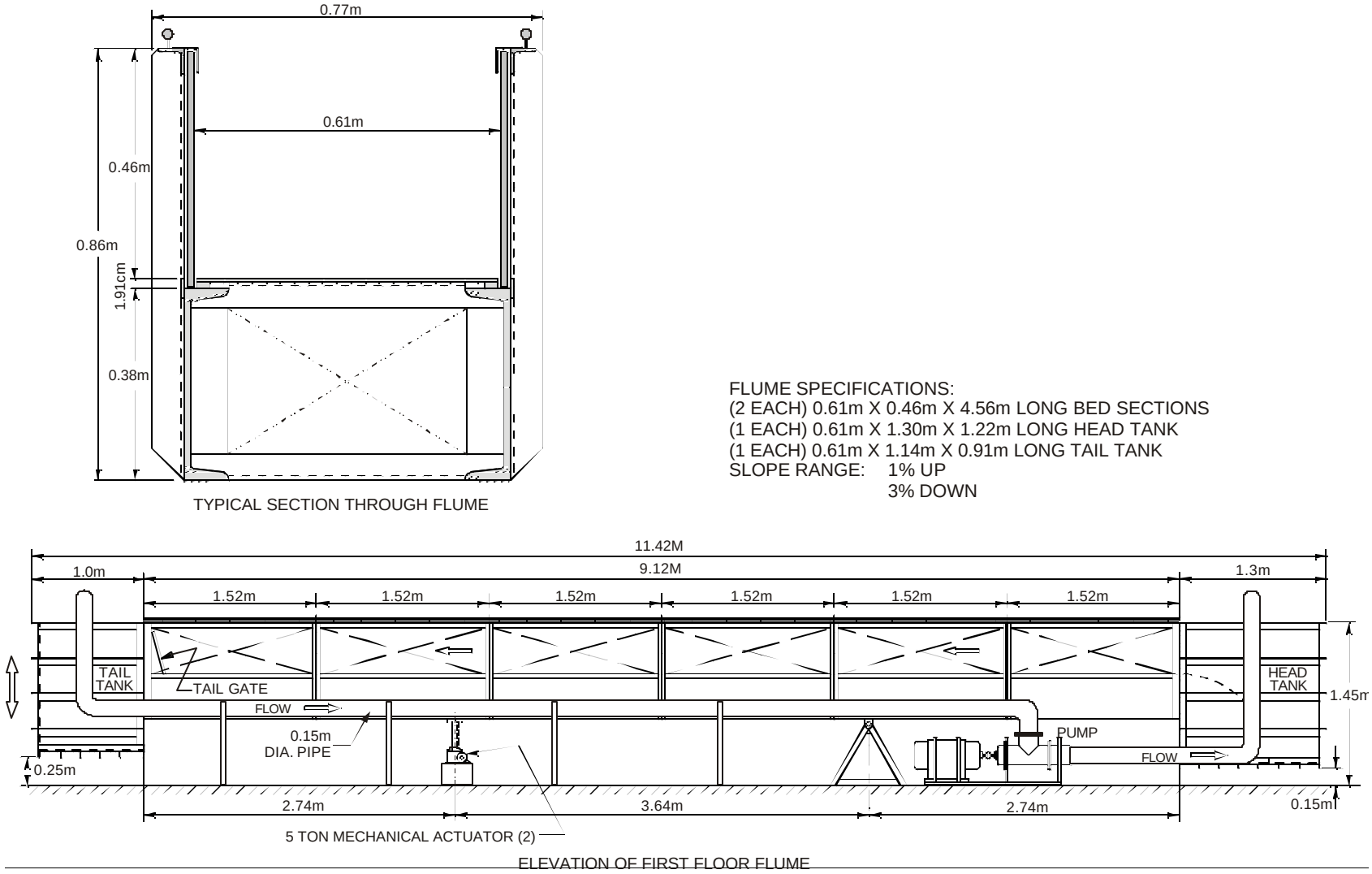


Figure 1. Open channel flume used in experiments

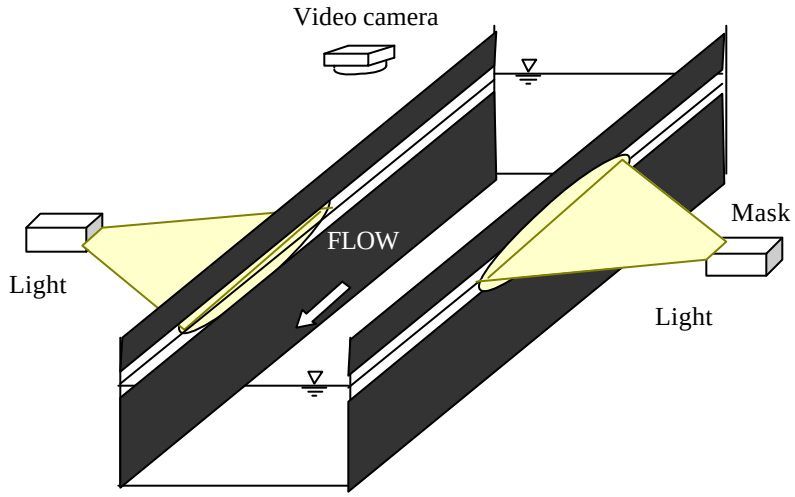


Figure 2 LSPIV system

EdPIV uses single-exposed multiple frames, as opposed to the multi-exposure single frame procedure, where several exposures can superpose on the same frame (Raffel, 1997). This approach is a straightforward application of PIV concepts to video-based recording systems. The analysis algorithm belongs to the so-called correspondence approach that performs correlation on the grey-level values contained in small regions, called interrogation areas.

The image processing algorithm used was developed by Gui and Merzkirch (2000). This algorithm implements the most recently developed PIV evaluation techniques, i.e., the central difference interrogation, continuous window shifting and image pattern correction. In essence, the algorithm finds the correlation between the image pattern enclosed in the *interrogation area* (IA) centered on a point a_{ij} in the image recorded at time t, and the IA centered at point b_{ij} in the image recorded at time $t+dt$, as illustrated in Figure 3. The correlation coefficient $R(a_{ij}, b_{ij})$ is a similarity index for the groups of pixels contained in the two compared IAs. In order to save computational time, correlation coefficients are only computed for points b_{ij} within a so-called *search area* (SA) defined around the point a_{ij}.

The two pictures in Figure 3 depict moving foam patterns on two successive video frames separated by a time interval dt. The Interrogation Area (solid square) defines the size of the foam patterns taken into account to identify the displacements. The Search Area (dotted circle) defines

the area that is searched for possible displacements. To save computation time, only a portion of the image is searched. The arrow from a_{ij} to b_{ij} represents the identified displacement.

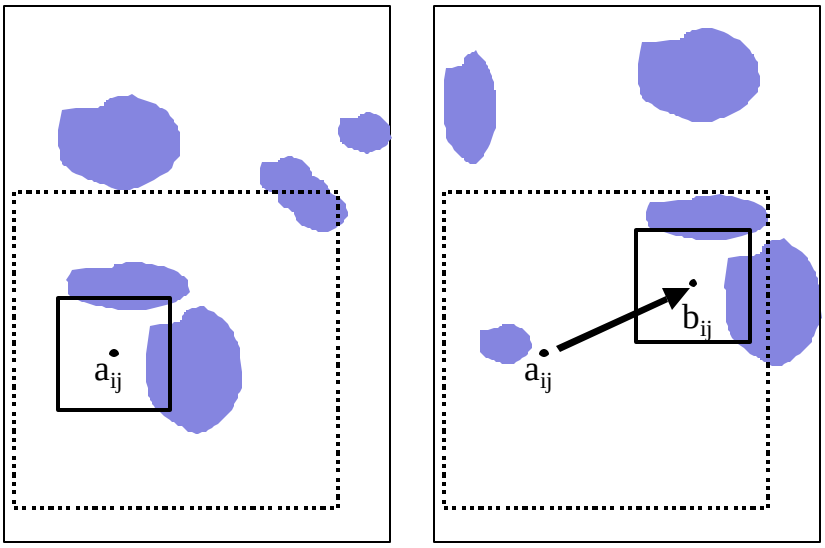


Figure 3. Sketch illustration of the algorithm used to identify the flow tracer displacement

The size and the shape of the SA are chosen on the basis of a priori knowledge of the flow field, such as the direction and magnitude of the mean flow. The most probable displacement of the fluid from point a_{ij} during the period dt is the one for which the correlation coefficient $R(a_{ij}, b_{ij})$ is maximum. A parabolic interpolation is used to determine the displacement with sub-pixel accuracy. Fujita and Komura (1994) show that particle-image displacements of about 0.2 pixels can be captured using this parabolic fitting when displacement gradients are relatively small. When several successive frames are available, as it is in our case, the most probable displacement is assessed using the maximum average coefficient of correlation computed over the complete sequence of images. Velocity vectors are derived from these displacements by dividing them by dt, the time between successive frames. The final vector field density is dependent on the choice of selection of the pitch, which defines the computational grid for the analyzed imaged area. Given the statistical approach used to determine the displacements and given the imperfections of the recorded images, it is possible to obtain spurious velocity vectors. Numerous post-processing routines are available to detect such vectors (see Raffel et al, 1998).

In our case, post-processing consisted simply of i) considering as spurious the vectors of less than 0.2 ms^{-1} and ii) interpolating linearly the missing grid points along current lines.

The code that was used for the LSPIV analysis EdPIV® has IMG correction, Boundary Mask, Background removing and error detection and error correction options. In the analysis of the data presented here, only error detecting option was used to detect erroneous vectors.

1.2. EXPERIMENTS

The experimental set conducted so far consists of 3 major flow categories:

A. Flat bed flows
B. Flow over discrete roughness elements (Ribs)
    1. k-type roughness-I
    2. k-type roughness-II
    3. d-type roughness
C. Flow over large scale roughness
    1. Smooth dune surface
    2. Roughened dune surface
        a. Wiremesh
        b. Sand

A. Flat Bed Flow

Experiments conducted with flow over flat/smooth bed is summarized in Table 1.

Table 1 Flat Bed Experiments

Code	d (m)	u_o (m/s) *	Fr	Re	AR	S_o
Ocf2-5	0.025	0.500	1.01	12500	24.4	$6.81x10^{-04}$
Ocf2-5	0.025	0.200	0.40	5000	24.4	$6.81x10^{-04}$
Ocf2-5	0.025	0.100	0.20	2500	24.4	$4.32x10^{-02}$
Ocf2-5	0.025	0.050	0.10	1250	24.4	$8.58x10^{-02}$
Ocf6	0.060	0.500	0.65	30000	10.2	$3.83x10^{-04}$
Ocf8	0.080	0.500	0.56	40000	7.6	$1.70x10^{-04}$
Ocf10	0.100	0.500	0.51	50000	6.1	$1.70x10^{-04}$
Ocf16	0.160	0.500	0.40	80000	3.8	$8.50x10^{-05}$
Ocf19	0.190	0.500	0.37	95000	3.2	$3.19x10^{-04}$

(*): Data not processed yet

In the above table, *Fr* and *Re* are Froude number and Reynolds number based on flow depth, *d*, respectively. *AR* stands for the aspect ratio, which is the ratio of channel width (61cm) to the flow depth. S_o shows the channel slope. u_o is the free surface velocity. Exact values for u_o are provided after LSPIV data is analyzed. At the present time the provided values are rough values for the cases whose LSPIV data is not analyzed.

B. Flow over Discrete Roughness Elements (Ribs)

Rectangular ribs of 1cm x 1cm in cross-section and 61cm in length are fixed over the smooth channel bottom. The placement frequency of the ribs was changed from 4.5cm to 18cm, to obtain different flow conditions. λ values of 9, 18, 4.5cm are used for the cases k1, k2 and d1 respectively.

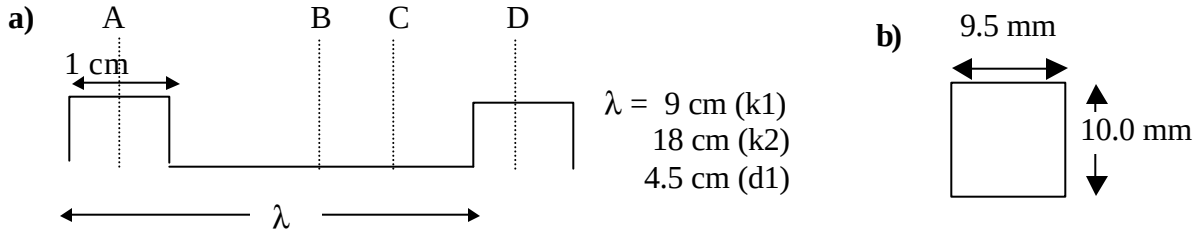


Figure 4. a) Longitudinal and **b)** cross-sectional view of ribs

Flow conditions for each flow case are summarized in following tables

Table 2 k-type roughness –I

Code	d (m)	u_o (m/s)*	Fr	Re	AR	S_o
Rib10	0.100	0.500	0.505	50000	6.10	$3.48x10^{-03}$
Rib08	0.080	0.439	0.496	35120	7.62	$3.27x10^{-03}$
Rib06	0.060	0.350	0.456	21006	10.16	***

(*): Data not processed yet

Table 3 k-type roughness -II

Code	d (m)	u_o (m/s) *	Fr	Re	AR	S_o
rib10	0.100	0.508	0.513	50800	6.10	$2.49x10^{-03}$
rib08	0.080	0.462	0.522	36960	7.62	$2.55x10^{-03}$
rib06	0.060	0.350	0.456	21000	10.16	$2.61x10^{-03}$

(*): Data not processed yet

Table 4 d-type roughness

Code	d (m)	u_o (m/s) *	Fr	Re	AR	S_o
Rib10	0.100	0.500	0.505	50000	6.10	-
Rib08	0.080	0.500	0.564	40000	7.62	-
Rib06	0.060	0.500	0.652	30000	10.16	-

(*): Data not processed yet

C. Flow over Large-Scale Roughness (Dunes)

A train of 22 dunes with a geometry shown in Figure 5 is used to investigate the flow over dunes. For the flow cases with wiremesh, a wiremesh was placed over the surface of the dunes. To obtain sand roughness, a layer of sand particles of 1.65-2mm diameter were glued over the dune surface.

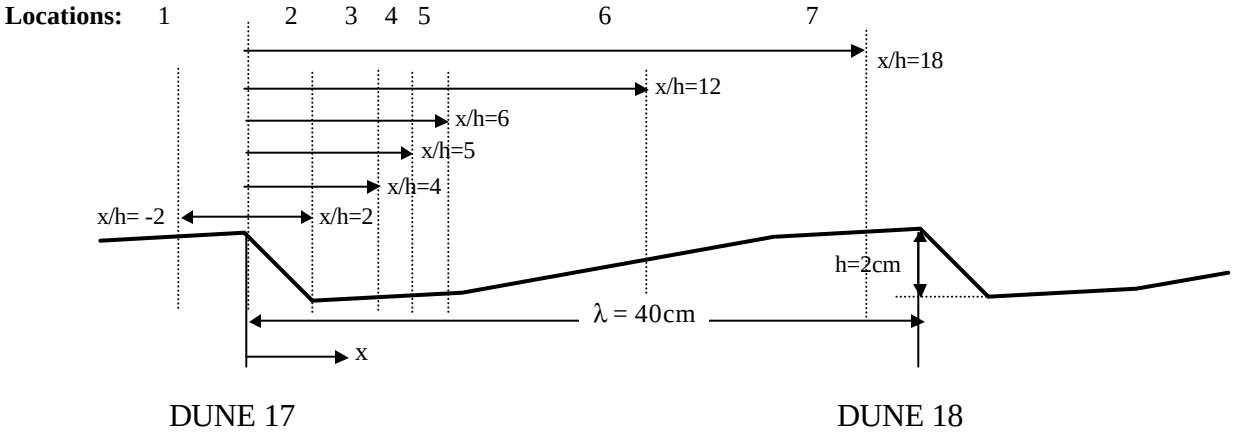


Figure 5 Geometry of dunes, and 7 locations for LDV vertical profiles

In Table 5 flow conditions obtained for experiments are shown. Experiments for Depth 3 and 6 have been discarded due to small aspect ratios.

Table 5 Flow over dunes experiments

Flow Cases	Depth at crest	u_o (m/s) *	Fr	Re	AR	S_o
Depth 1	0.118	0.5	0.465	59000	5.17	-
Depth 2	0.078	0.5	0.572	39000	7.82	-
Depth 3	0.251	0.5	0.319	125500	2.43	-
Depth 4	0.165	0.5	0.393	82500	3.69	-
Depth 5	0.06	0.5	0.652	30000	10.16	-
Depth 6	0.202	0.5	0.355	101000	3.02	-
Depth1 sand	0.118	0.5	0.465	59000	5.17	-
Depth1 Wire Mesh	0.118	0.5	0.465	59000	5.17	-

(*): Data not processed yet

1.3. EXPERIMENTAL PROCEDURE

Velocities throughout the water column, including the free surface were obtained by combining Laser-Doppler Velocimetry (LDV) and Particle Image Velocimetry (PIV) in an arrangement illustrated in **Figure 6**. LDV measures velocities in the column of water (in a vertical), while PIV measures free surface velocities. It is worth noting that that there is no better alternative instrument to measure free-surface velocity. The two non-intrusive measurement techniques are able to fully document instantaneous and mean flow characteristics at the free surface and in the water column with relatively high temporal and spatial resolutions.

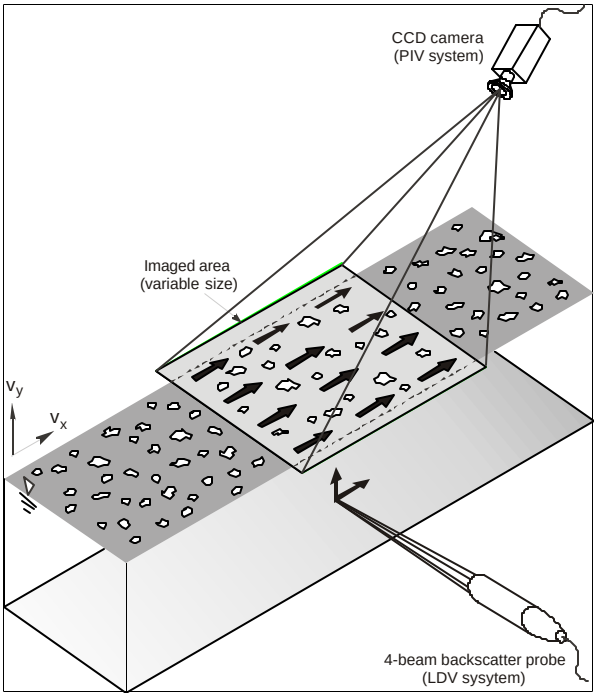


Figure 6. Laboratory experimental arrangement

In addition, video recordings of the free surface texture were taken to document any particular signature of the internal and secondary flow parameters on the aspect of the free surface.

For each flow cases LSPIV and LDV tests were conducted and the surface texture was recorded. Experiments were conducted in the following order

1. Obtaining the flow conditions: Channel slope, flow depth and surface velocity are adjusted iteratively. Starting from a rough numbers for S_o, d and u_s, the final channel slope is obtained. Flow depth is changed by adding or removing water from the channel, and surface velocity is controlled by decreasing or increasing the circulating-pump frequency.
2. LDV measurements: After the flow is set, the water is seeded and experiments are conducted.
3. Surface texture recording obtained with the camera placed above the flow and strategically positioned lights.
4. LSPIV measurements: Flow is seeded with styropor particles. Flow surface is illuminated from sides by halogen lamps with diffusers. Recording is done by a camera set above the channel.

For details of experimental settings, see attached paper (Polatel, 2003).

2. DATA ANALYSIS

The time period from the project inception was used to setup the LDV and LSPIV systems for the specific conditions required by the project, to train the research team with the instrument operation, and to conduct the experiments. Data processing is currently in progress. Analysis of the experiments will proceed using the sample analysis provided in Appendix A.

Based on the preliminary results obtained so far, a publication for the Student competition of the International Association for Hydraulic Engineering and Research (IAHR) Congress in Thessaloniky (Greece) was submitted by the PhD student conducting the research (Polatel, 2003). The submitted paper is provided in Appendix B. The main findings of the preliminary results are summarized below:

- Surface velocity reacts to changes in the channel bed variation and roughness;
- Secondary currents are ubiquitous in flume experiments and they affect selectively the velocity profiles in the water column, in direct relationship with the position of the measured vertical.
- When direct action on the free surface is absent (e.g., wind shear), the free surface appearance is mainly related to the large-scale turbulence structures acting in the flow.

3. REFERENCES

Fujita, I., and Komura, S. (1994). "Application of Video Image Analysis for Measurements of River-Surface Flows," Proc. Hydraulic Engineering, JSCE, 38, 733-738.

Fujita, I., Muste, M. and Kruger, A. (1998). "Large-Scale Particle Image Velocimetry for Flow Analysis in Hydraulic Applications," *J. Hydr. Res.*, 36(3), pp. 397-414.

Gui, L. and Merzkirch, W. (2000). " A Comparative Study of the MQD Method and Several Correlation-Based PIV Evaluation Algorithms," *Experiments in Fluids*, 28, pp. 36-44.

Raffel, M., Willert, C. and Kompenhaus, J., 1997 : *Particle Image Velocimetry*. Spring-Verlhag, 253 pp.

Muste, M., Bradley, A., Kruger, A., Cheng, R. T. (2001). "Complementary Investigation for Implementation of Remote, Non-Contact Measurements of Streamflow in Riverine Environment," USGS/National Institutes for Water Resources, *Proposal*, IIHR-Hydroscience & Engineering, Iowa City, IA.

APPENDIX A

Sample of Experimental Result Analysis

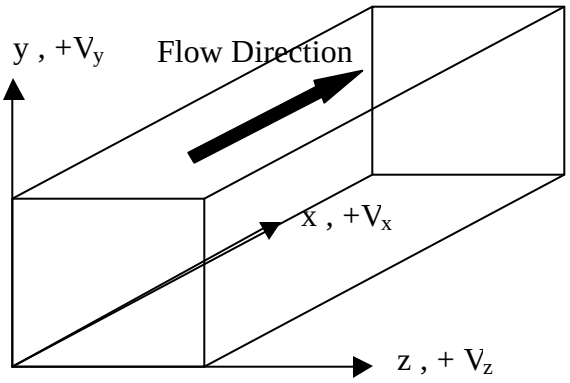
LSPIV and LDV Analysis
Flow Case: Ocf-Depth 10

A. GENERAL CONSIDERATIONS

1. Experimental conditions

Recording: manual focus
Illumination: Black panels at sides, side illumination from both sides
Flow condition: Flat smooth bed, VFD readout 24.2%, Depth 10cm, Slope 0.000573
Friction velocity, u_*=0.02m/s

2. Coordinate System



B. LSPIV RESULTS

1. Samples of de-interlaced images

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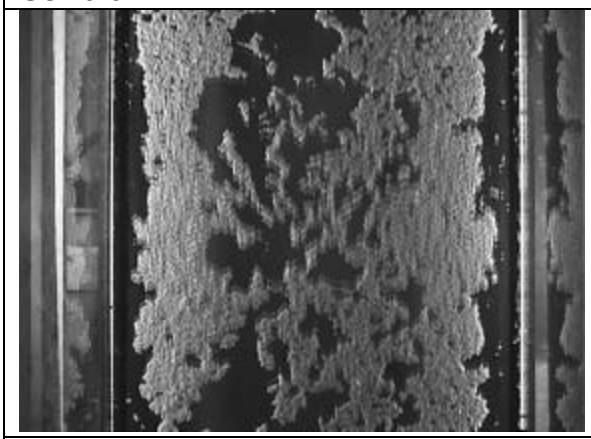	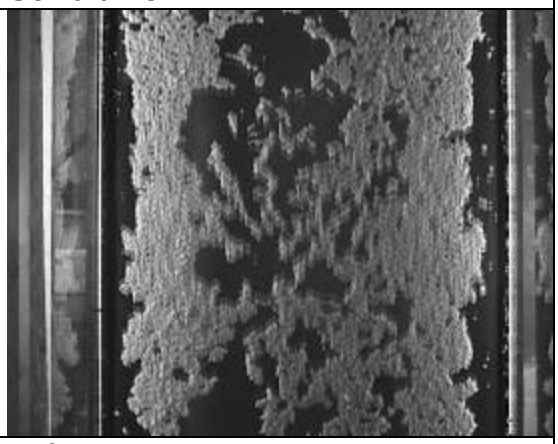
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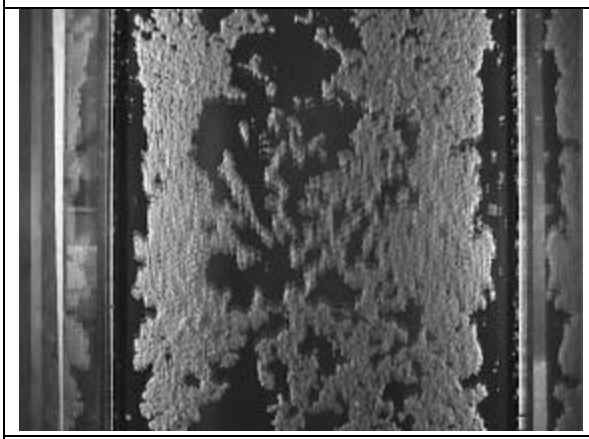
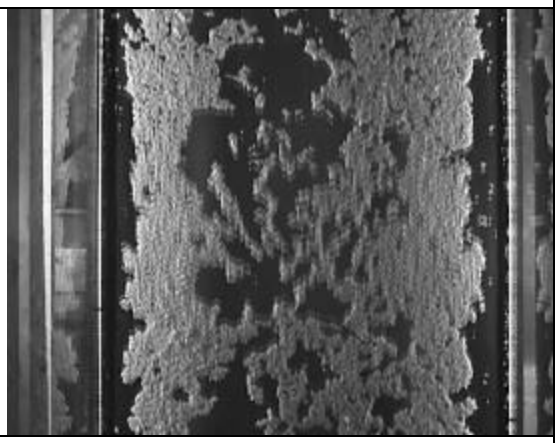
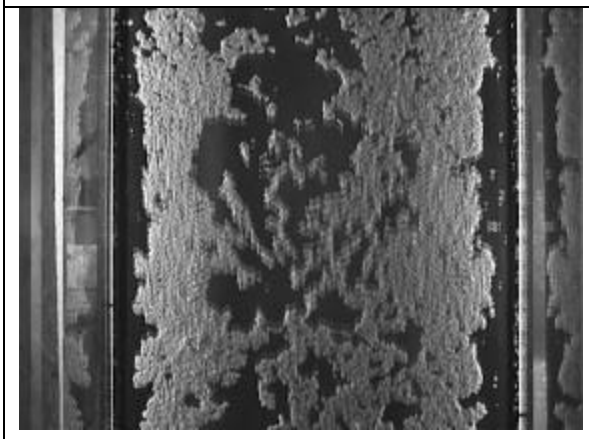
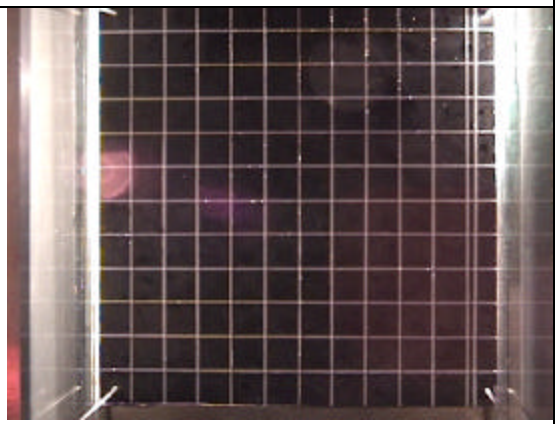
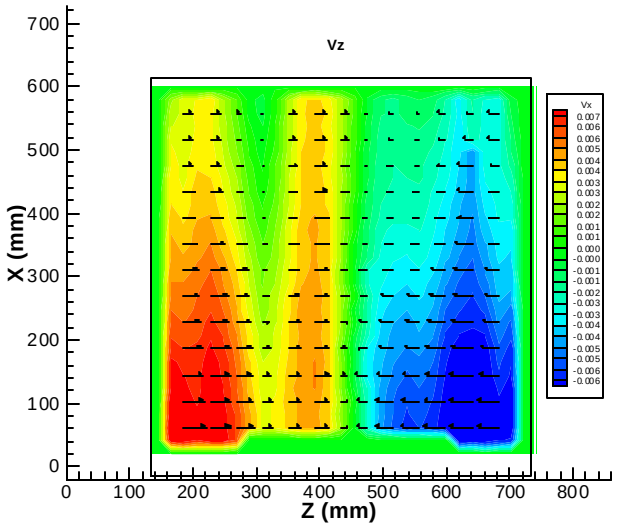
	
Ocf10r0751	**Grid**
	

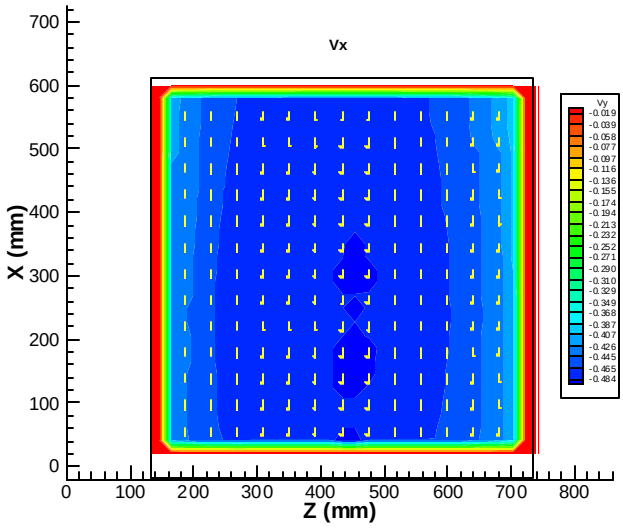
2. Mean vector fields - data for free surface at 7m from the flume entrance

PIV processing parameters: Interrogation window size: 64x64; image scaling : 774 pixels/m;

Mean Vz



1.1. Mean Vx



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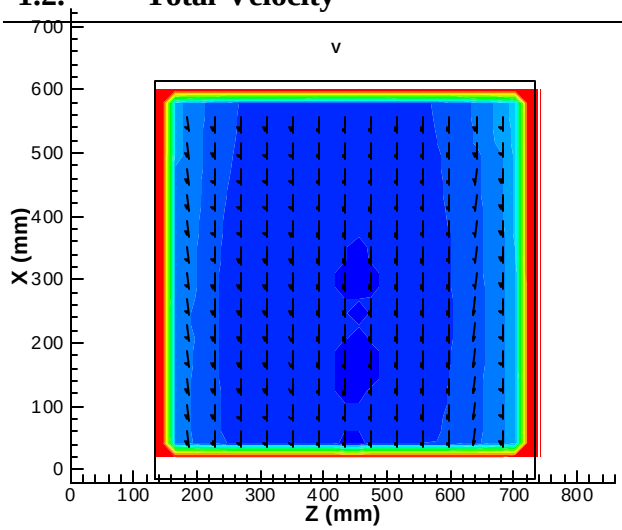
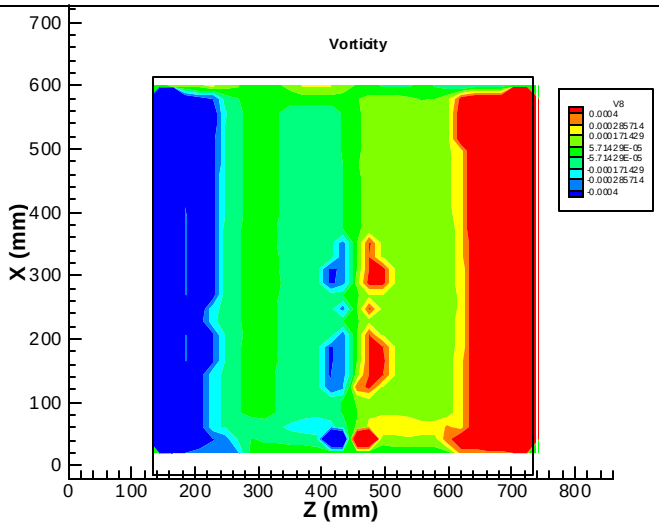
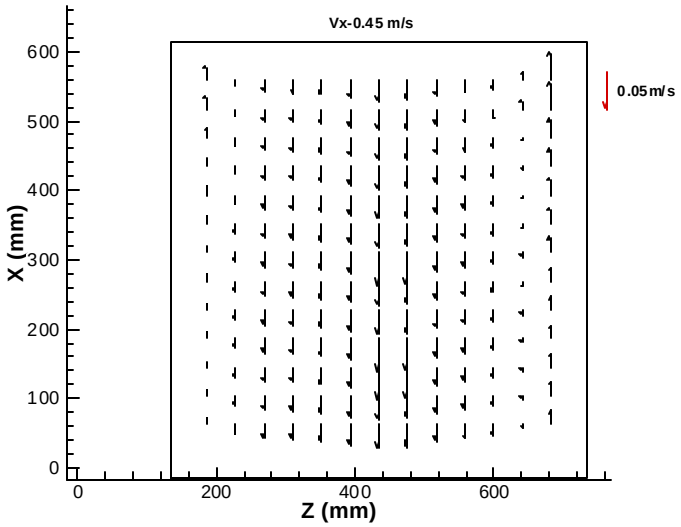


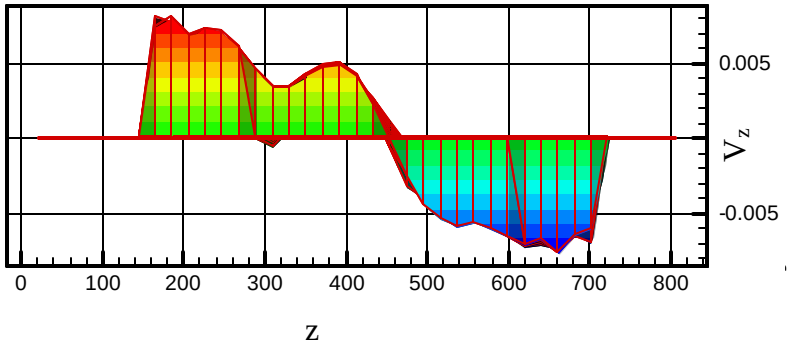
1.3. Vorticity



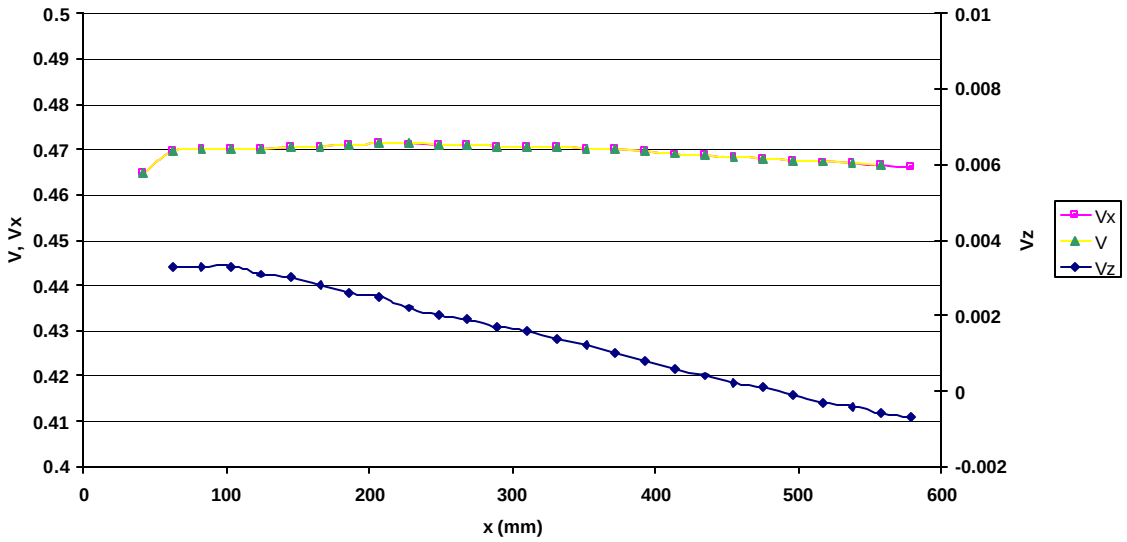
Zoomed-in Streamwise velocity component over the LSPIV test section
(0.45m/s subtracted from the mean streamwise velocity component)



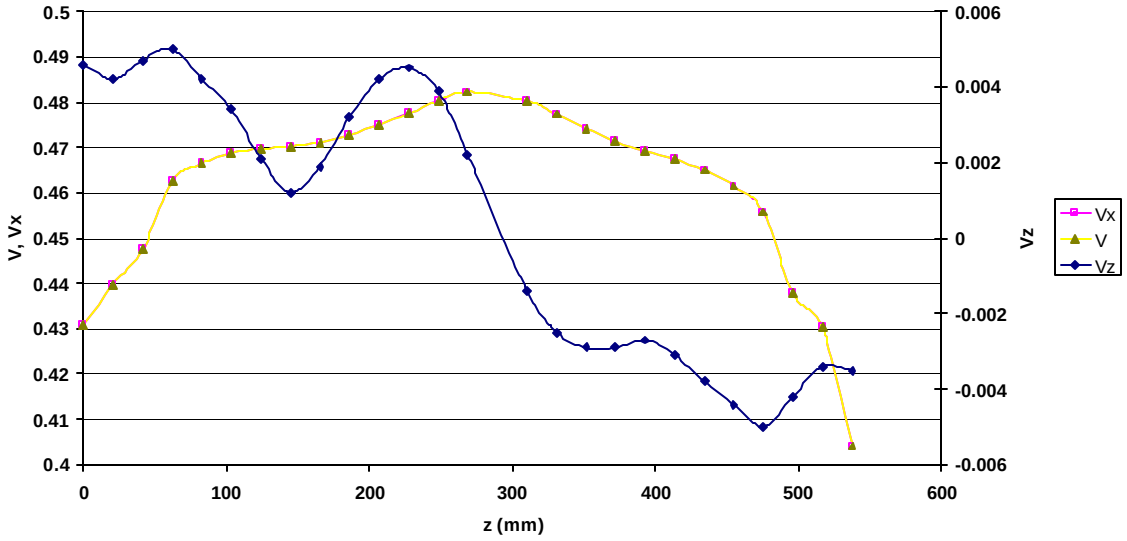
Spanwise velocity component distribution in the test section



Distribution of mean velocity profiles in the streamwise direction

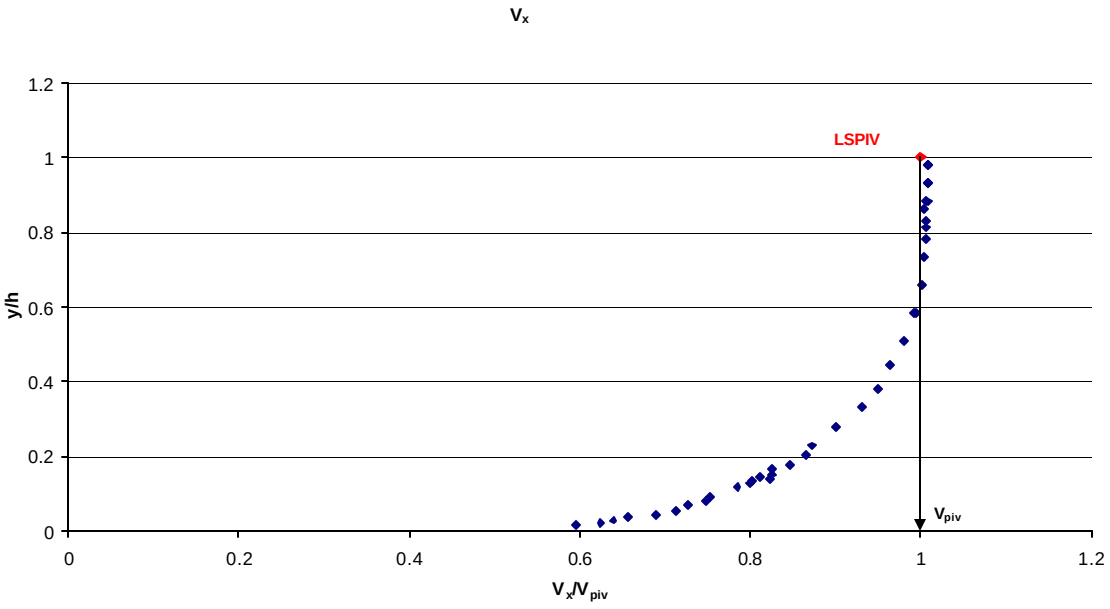


Mean velocity components in the spanwise direction

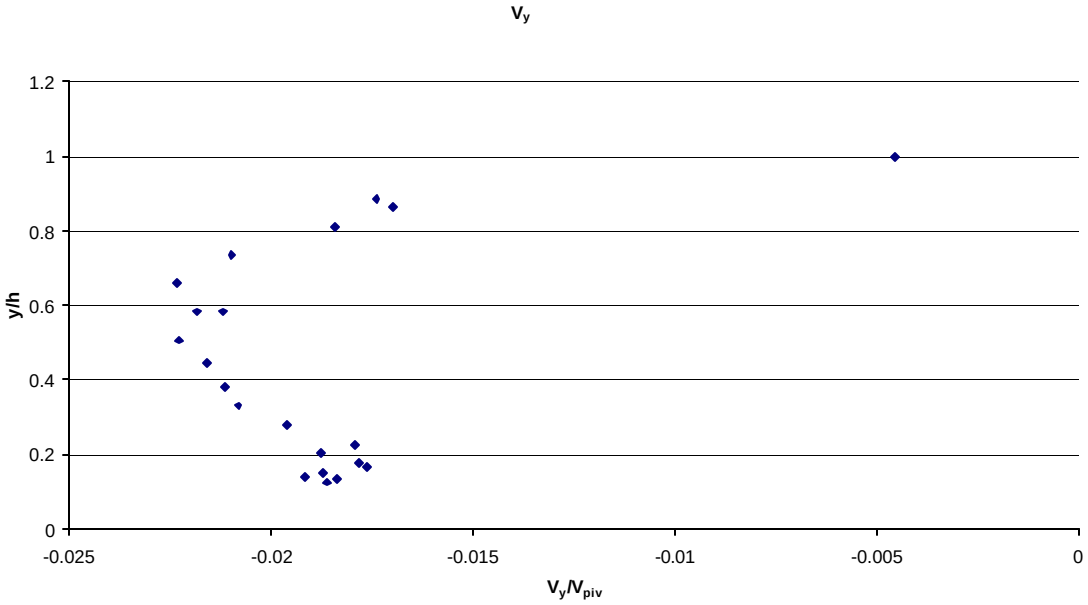


C. LDV RESULTS

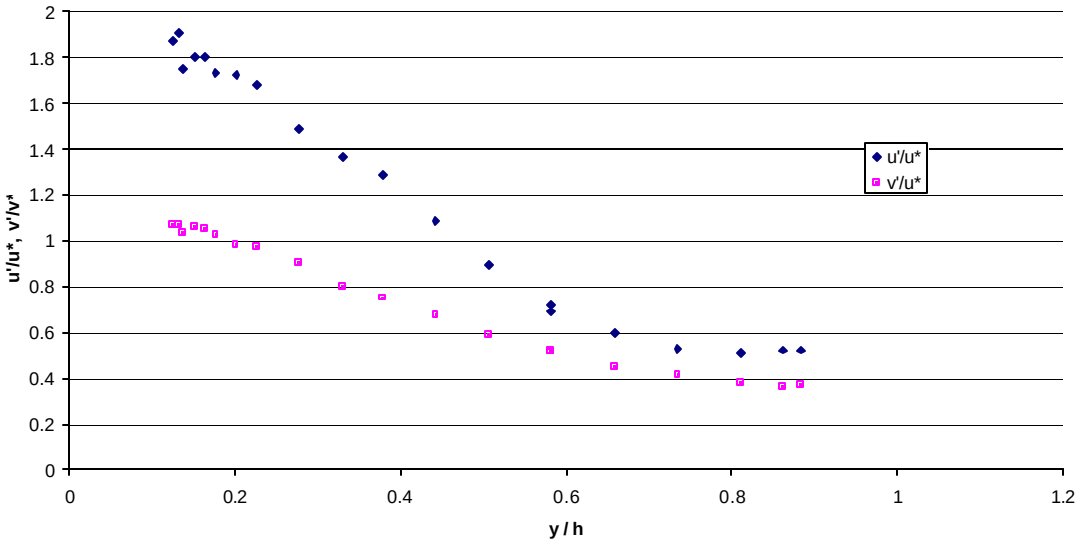
Mean streamwise velocity distribution over the depth



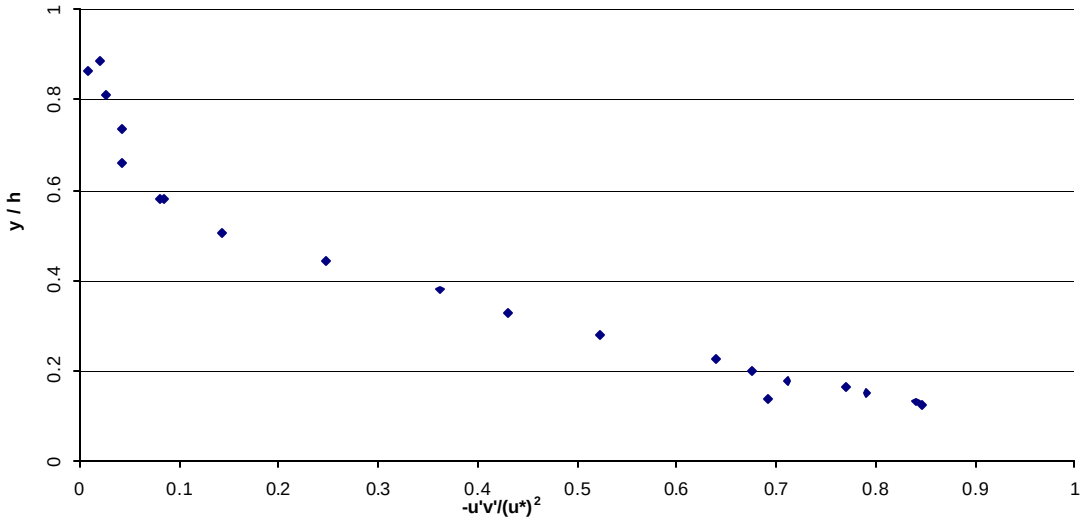
Mean vertical velocity distribution over the depth



Turbulence intensity distribution over the depth

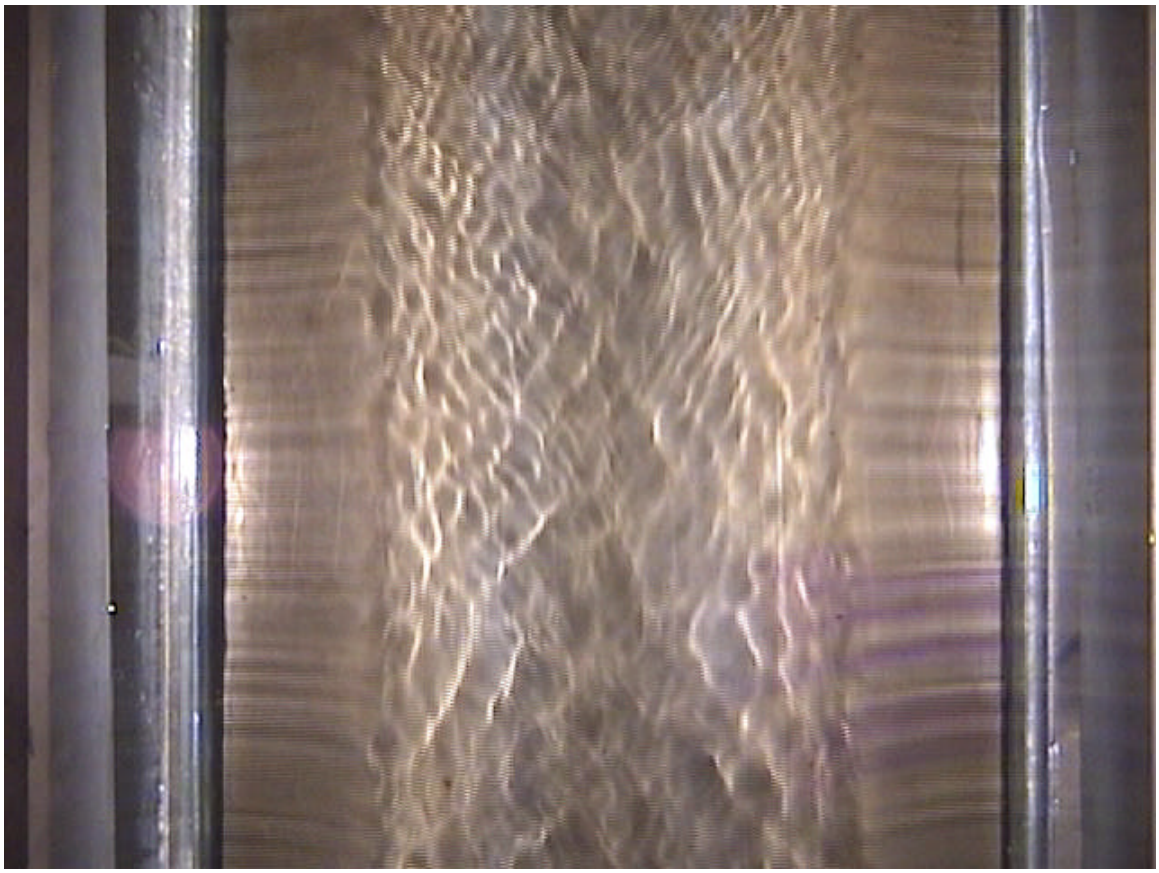


Reynolds stress distribution over the depth



D. FREE-SURFACE TEXTURE

Video frame of the free surface



APPENDIX B

Additional Reference

SIGNATURE OF BED CHARACTERISTICS ON FREE SURFACE VELOCITY IN OPEN CHANNEL FLOWS

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ABSTRACT

Discharge estimation is the essential part of any hydraulics problem related with rivers, like sediment or pollutant transport. Traditional methods, used to determine river discharge have many shortcomings. A non-contact method with lower cost, better accuracy and less hazards than that of traditional stream-gaging method are currently investigated. One of these methods, called "indexing", aims to plot all the velocity profile for known bed characteristics by using only one pointwise velocity measurement. The essential part of this method is to estimate the non-dimensional velocity distribution for given bed characteristics. Having the structure of the velocity distribution, only one pointwise velocity measurement will be enough to plot velocity profile. Taking the free surface velocity as indexing velocity has advantages in the aspect of appropriateness to non-contact measurement techniques. However, both free surface velocity and velocity profile are sensitive to many factors together with bed characteristics. From the perspective of the non-contact, remote measurements of the free surface, the effects of related parameters on the free surface velocity and velocity profile have become an important issue to be investigated. Here the summary of some preliminary results related to this topic, obtained from the ongoing studies at IIHR- Hydroscience and Engineering laboratories are presented. Among the parameters involved, bedform effect is discussed in this paper. Two tests, one for the flow on smooth straight channel bed and the other for the flow over sand dunes are presented. The results of the observations show that there is a signature of the spatial changes in the channel bed on the free surface velocity. The quantitative description of this signature is left as a future work for the subsequent publications.

Keywords: Open channel flow, Free surface velocity, Indexing, Laser Doppler Velocimetry (LDV), Large Scale Particle Image Velocimetry (LSPIV), Sand dunes.

INTRODUCTION

The idea behind the remote discharge measurements is to integrate remotely obtained bed characteristics and velocity distribution information. For these methods, it is crucial to have a unique and accurate relationship between the velocity distribution and flow conditions (secondary currents, large-scale turbulence, wind effects) and bed forms.

Extensive amount of studies in the literature is dealing with estimating the velocity distribution by using a pointwise measured velocity for a given bed configuration. In the present context, the term "indexing" is used to relate the velocity distribution over the depth to the velocity measured at a point in the water column (e.g. surface, maximum or depth averaged velocity).

Indexing has become important from the perspective of new measurement technologies that are using one point velocity measurement to characterize velocity distribution over the depth. Newly developed Large Scale Particle Velocimetry (LSPIV) (Muste et al., 2001), Radar (Cheng, 2001), Horizontal Acoustic Doppler Currentmeter Profiler (HADCP) techniques provide sufficiently accurate one-point measurements for this purpose.

Studies taking different velocities like maximum or free surface velocities as indexing velocity exist in the literature (Chiu, 2002). Taking maximum velocity as indexing velocity has some shortcomings in the aspect of uncertainties in determining magnitude of the maximum velocity together with its position.

Free surface velocity as indexing velocity on the other hand has advantages in the aspects of convenience in finding location and magnitude of the free surface velocity and appropriateness to non-contact measurements. Due to the limitations of the measurement techniques, the free surface couldn't be attained at the past. However, recently developed methods such as radars and image velocimetry methods make it possible to measure free surface velocity and subsequently determine discharges.

Another factor that underlines the need to study on relationship between free surface velocity-velocity distribution is that there are various published accounts showing that the state of the free surface (i.e., waviness, ripples) in open channel flows has peculiar relationships with the velocity distribution in the column of water below it. Even in the simpler case of open-channel flume flows on smooth beds there is no agreement on the appearance of the mean velocity profile near the free surface. This situation is explained by the sensitivity of the near-surface flow region to secondary factors such as waviness of the free surface, secondary currents, bedforms, large-scale turbulence, and sidewall (banks) effect (Muste, 2001).

From the perspective of the non-contact, remote discharge measurements, relationship between the free surface velocity and velocity distribution, and identification of effects of secondary factors on free surface velocity has become an important issue to be investigated.

For this purpose, a set of experiments is planned, in which the effects of aspect ratio, bed characteristics and wind effect will be investigated. In accordance with the motivation of non-contact measurements, both free surface velocities and velocity distributions are measured remotely in the lab. To measure the free surface velocity LSPIV method is selected since it is the

most suitable technique available. To obtain the velocity distribution LDV method is used due to its non-intrusivity, accuracy and directional sensitivity. Here, as a preliminary discussion of this extensive study, effect of channel bed configuration on free surface velocity will be discussed.

EXPERIMENTAL SETUP AND FLOW CONDITIONS

The experimental arrangement of Laser-Doppler Velocimetry (LDV) and Large Scale Particle Image Velocimetry (LSPIV) systems used to obtain velocities throughout the water column, including the free surface is illustrated in Figure 1. The camera mount and the fiberoptic-probe mount will be integrated in a platform designed and manufactured at IIHR. LDV will be used for measuring velocities in the column of water (in a vertical), while LSPIV will be used for free surface measurements. It is worth noting that that there is no better alternative instrument to measure free-surface velocity. The two non-intrusive measurement techniques are able to fully document instantaneous and mean flow characteristics at the free surface and in the water column with relatively high temporal and spatial resolutions.

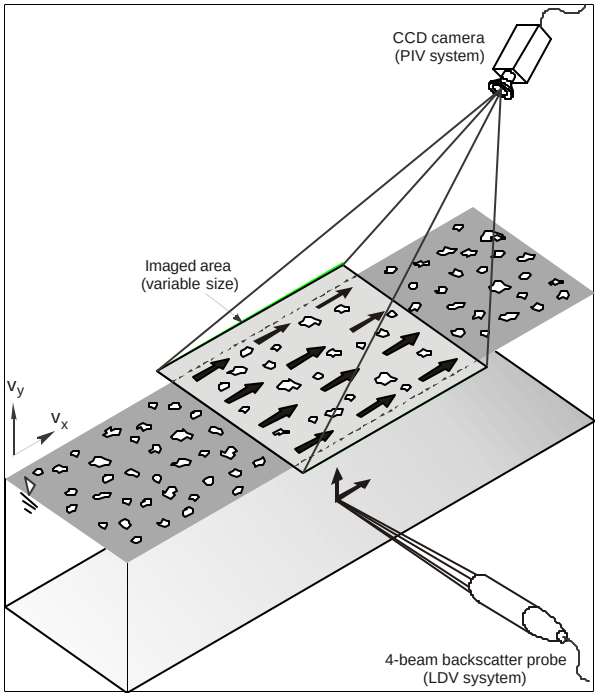

Figure 1. Experimental arrangement. Courtesy Muste et al. (2001)

The proposed set of experiments have been conducted at IIHR- Hydroscience and Engineering laboratory. Experiments were conducted in a 10m long, 0.61m wide and 0.5m deep, recirculating tilting open channel flume. Hydraulic conditions for the test conducted are summarized in Table 1. For DUNE10 case, velocity and depth at Location 1 as taken as characteristic velocity and depth.

Table 1. Hydraulic conditions

	h (m)	u_o (m/s)	Re	Fr	Aspect Ratio
Smooth Bottom (OCF10)	0.10	0.482	48200	0.49	6.1
Over Dunes (DUNE10)	0.118	0.479	56500	0.45	5.17

For the flow case DUNE10 a train of 22 dunes is used. The shape and the size of the dunes are identical to those used by Balachandar (2002) and shown in Figure 2. LDV measurements over one wavelength were done at 6 sections. LSPIV recordings also cover an area slightly larger than a dune wavelength.

LDV and LSPIV measurements are carried out in over the 17th and 18th dunes from the entrance of the channel. The measurements for OCF10 were done at the same distance from the entrance of the channel with DUNE10 case.

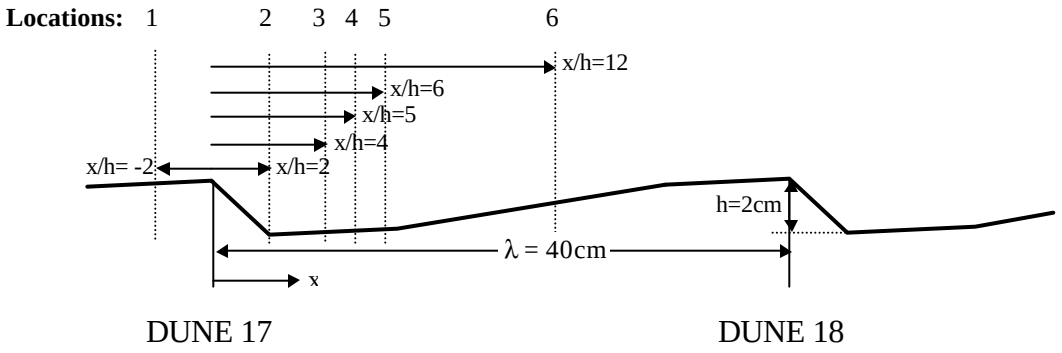

Figure 2. Geometry of dunes, and 6 locations for LDV measurements

LDV EXPERIMENTS

A two-component, two-color, fiberoptic-based LDV system (TSI 900-3) is used in the experiments. The system comprises of a L-70-2 two-watt argon-ion laser, a two component fiberoptic Colorburst™ transmitting optics, Colorlink™ receiving optics, IFA 655 signal processor (burst correlator), and FIND™ interfacing and data analysis software.

Two components of instantaneous velocities, the streamwise and the vertical, were measured with the LDV system. At each measurement point, 15,000 samples are obtained and a standard procedure is used to determine the averages. For both flow cases, LDV measurements are done at the centerline of the channel.

LSPIV EXPERIMENTS

A digital camera (Sony DCR-TRV900) is used for recordings. The imaged area set as it covers the entire area between the crests of dune 17 and 18. Two quartz-halogen photographic lamps with diffusers are used to illuminate the selected area. Flow images recorded at 30 Hz will be subsequently digitized in 640 by 480 pixels of 8-bit, gray-level resolution images and processed with the PIV analyzing software EdPIV®.

Transparent walls of the channel are covered by black masks so that there will be 2 cm openings at the both sides of the channel in the vicinity of the water surface. So only, the water surface region was illuminated by the lights.

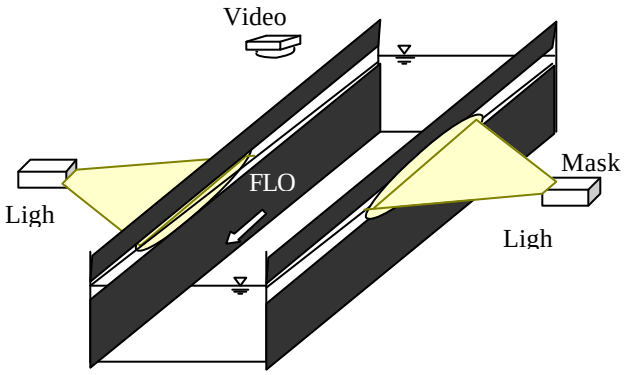

Figure 3. LSPIV

As a seeding material Styropor® expandable polystyrene which is produced by BASF with a bulk density of 12.5 kg/m^3, and diameters of 2 to 3 mm is used.

Although seeding is the very important part of LSPIV experiments, there is very little information about the behavior of floating seeds for LSPIV. Since the used seeds are very light, it is assumed that they follow the flow without disturbing it.

OVERVIEW OF RESULTS

In Figure 4, LDV and LSPIV measurements at the centerline of the channel are shown. LSPIV measurement is found as 0.482m/s, which is almost 0.02m/s smaller than the uppermost LDV measurement point, which is 0.5m/s. The mean velocity of the flow is found as 0.46 m/s.

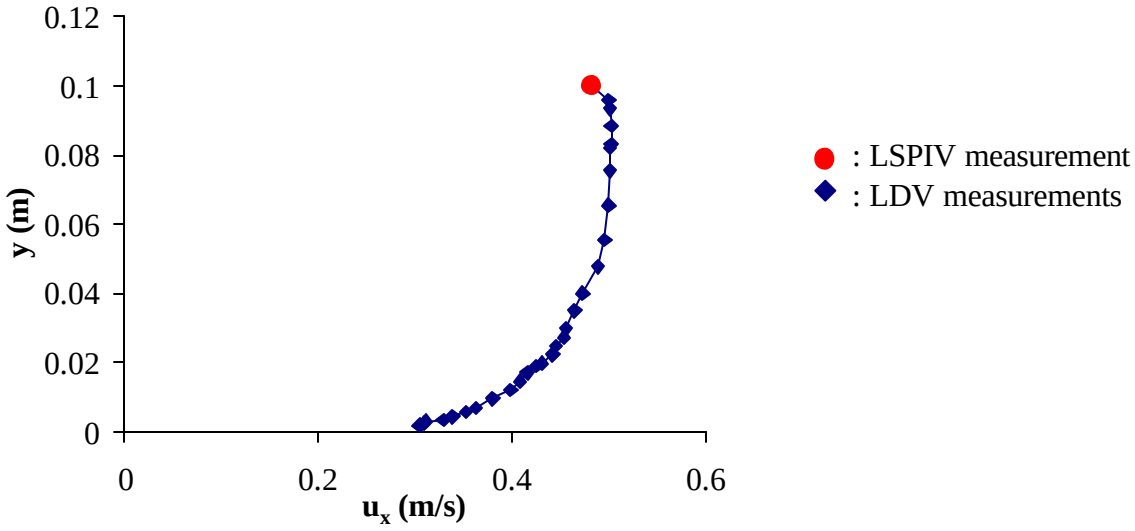

Figure 4 Case OCF10 velocity distribution in vertical direction at the channel centerline

LSPIV results for streamwise, u_x, and spanwise velocity, u_z, distributions are shown in Figure 5. The symmetry of profiles of u_x and u_z is the validation of the consistency of the LSPIV results. Symmetric nature of the u_z according to channel centerline is an indicator of secondary flow towards the centerline of the channel from the sides. There are two, close to symmetric local peak points in the distribution of u_z at each half of the channel cross-section. The discussion of possible reasons for these peaks, which also seen in DUNE10 case, is left for subsequent publications since it needs more data to be concluded.

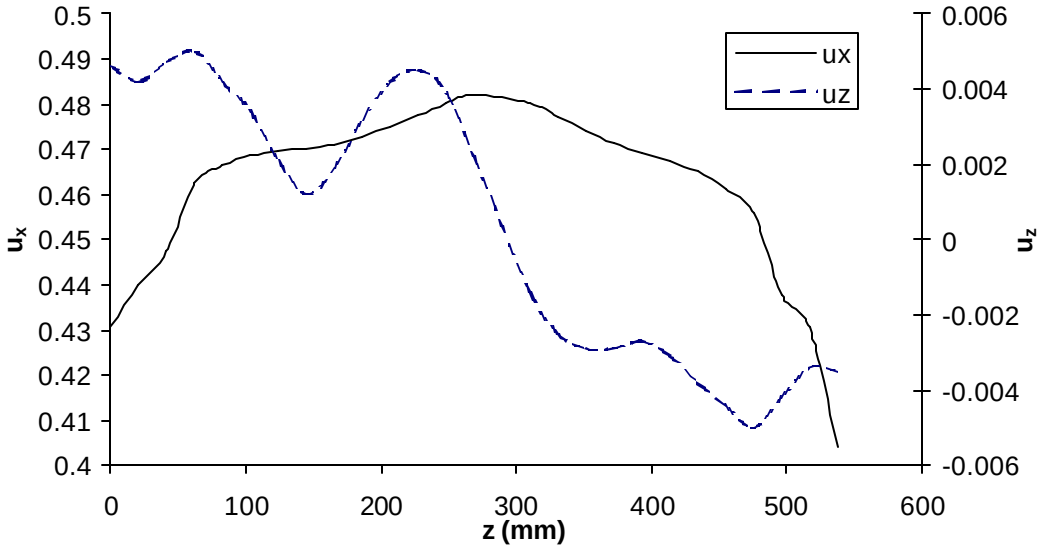

Figure 5 Case OCF10 Streamwise and spanwise velocity components along spanwise direction – LSPIV results

In figure 6, LDV and LSPIV measurements for the DUNE 10 case at the centerline of the channel are shown. The summary of the results for this case is given in Table 2. Profile shown for location 7 is the repetition of the one for location 1.

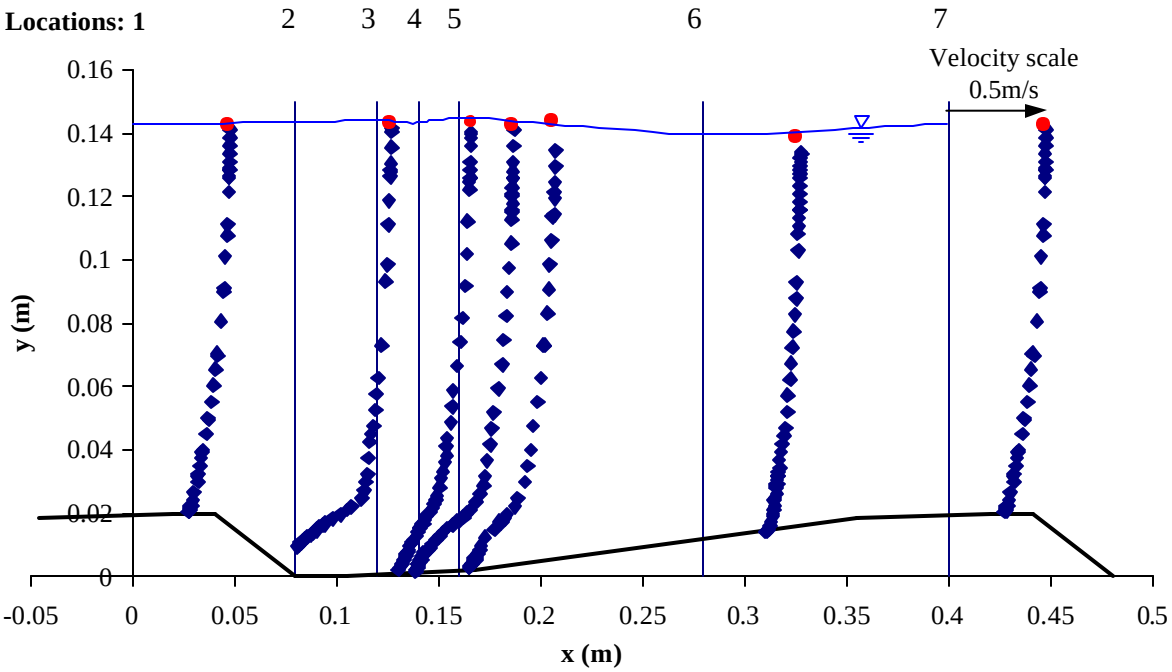

Figure 6 Case DUNE10 velocity distributions in vertical direction at the channel centerline for all 6 locations

Table 2. Summary of result for DUNE10

	u_{LDV} (m/s)	u_o (LSPIV) (m/s)	u_{mean} (m/s)
LOC1	0.475	0.456	0.422
LOC2	0.479	0.460	0.347
LOC3	0.479	0.469	0.371
LOC4	0.470	0.461	0.369
LOC5	0.459	0.461	0.378
LOC6	0.471	0.459	0.420

In Table 2, u_{LDV} shows the LDV measurements taken at the highest possible point. These values have importance in assessment of existence of bias in LSPIV values.

In Figure 7 and 8, streamwise and spanwise velocity distributionS at all 6 locations found by LSPIV are shown. Again, the symmetry in the distributions is the relative validation for the LSPIV method.

The important point that can be drawn from this graph is that even though the differences in the velocities for different locations are very small, they are following a pattern. As seen in Figure 7, velocities become larger at the locations with higher channel bottom elevation.

Another conclusion can be deduced from these graphs is, even the aspect ratio of the experimental conditions is much smaller than the values seen in natural rivers, surface velocity reacts to spatial changes in channel bottom.

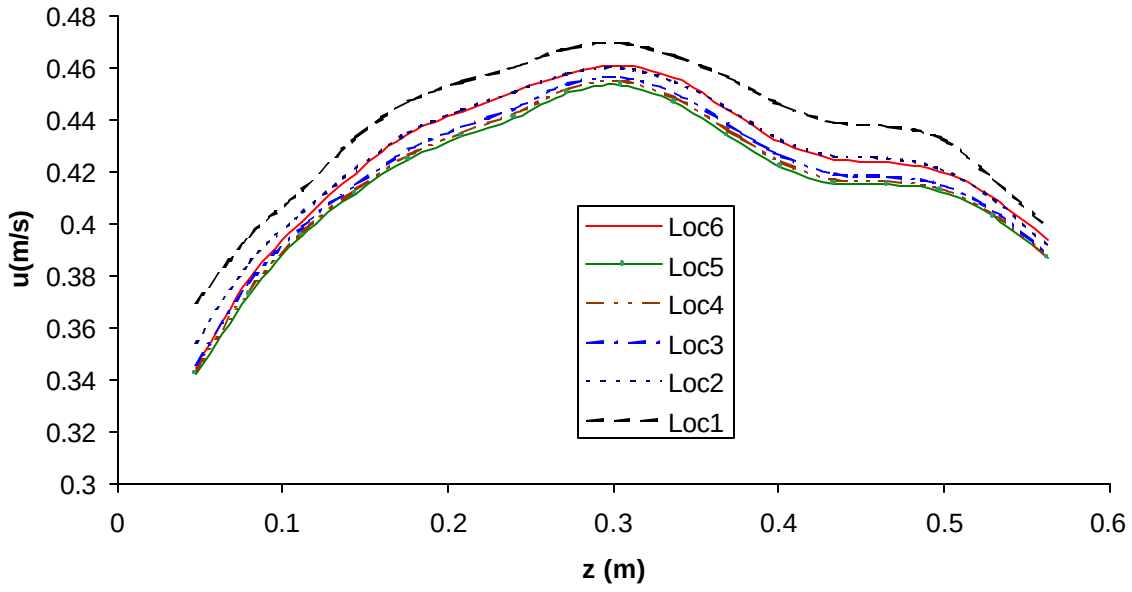

Figure 7 Case DUNE10 Streamwise velocity components along spanwise direction for all 6 locations – LSPIV results

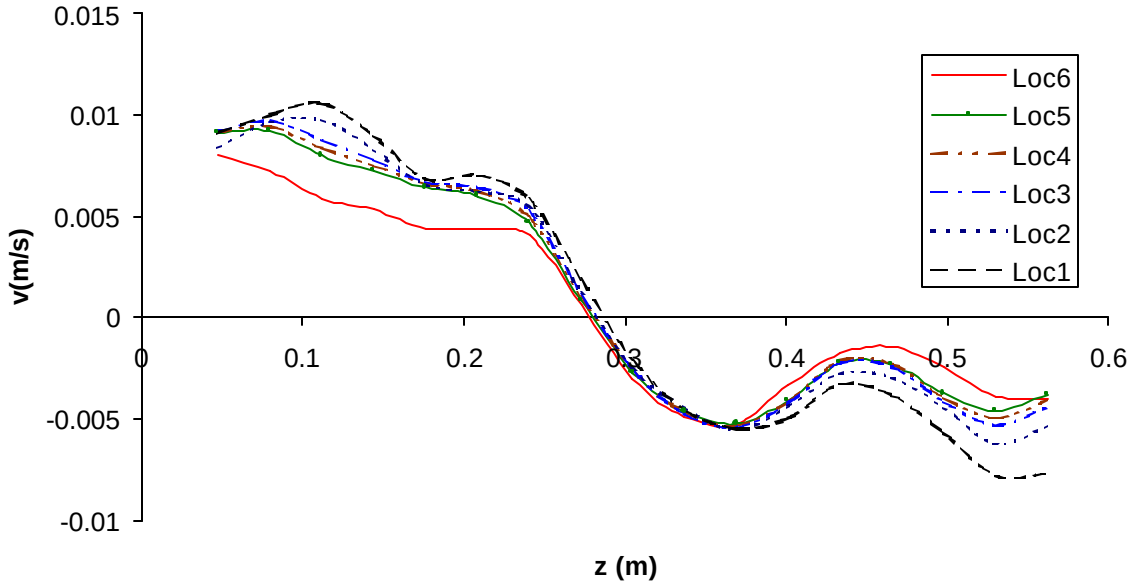

Figure 8 Case DUNE10 Spanwise velocity components along spanwise direction for all 6 locations – LSPIV results

CONCLUSIONS

The summary of some preliminary results of the ongoing studies on channel bed- surface velocity relationship is presented here. Based on the analysis the following conclusions can be drawn:

- For the given flow conditions, even though aspect ratios are smaller than the values seen in natural rivers, it is clearly seen that surface velocity reacts to spatial changes in channel bottom.
- The distribution of velocity component in spanwise direction is an indication to secondary motions in the flow.
- There might be a bias in calculated velocities by LSPIV resulted by the slip of seeding particles used. This general problem of all optically based measurement methods in which seed are used, needs further consideration.
- Consistency in the obtained data for these two flow cases is a validation of the measurement techniques used in a relative sense.

Present results show that further studies are required to define the effect of bathymetry quantitatively. It is hoped that new and useful results will be obtained by quantifying how well the seeding particles for LSPIV follow the flow; extending the range of concern of flow properties, like aspect ratio and free surface velocity for these channel bed configurations; extending the range of different channel bed characteristics and considering the other affects like, sidewalls and surface waviness on free surface. These results will be of high practical importance for the emerging generation of remote, non-contact discharge measurement technologies.

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Predicting sorption, mobility, accumulation, and degradation potential of antibiotics in Iowa's soil/water environment

Basic Information

Title:	Predicting sorption, mobility, accumulation, and degradation potential of antibiotics in Iowa's soil/water environment
Project Number:	2002IA4B
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End Date:	2/28/2005
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Descriptors:	
Principal Investigators:	Steven Fales

Publication

1. Pils, J.R.V. and D.A. Laird, 2003. Adsorption of Tetracycline and Chlorotetracycline on K- and Ca-saturated Soil Clays and Humic Substances. Agron. Abstr. S02-pils672004-O.pdf.
2. Pils, J.R.V., V.P. Evangelou, and D.A. Laird, 2002. Smectite tactoid formation induced by confined and double layer monovalent cations. Agron. Abstr. S02-pils152704-O.pdf

Predicting Sorption, Mobility, Accumulation, and Degradation Potential of Antibiotics in Iowa's Soil/Water Environment

Steven Fales

Problem and Research Objectives

Approximately 31.6 million pounds of antibiotics are used in the production of poultry (10.6 million pounds), hogs (10.3 million pounds), and cattle (3.7 million pounds) in the United States each year (Mellon et al., 2001). Over three-fourths of these antibiotics (24.6 million pounds) are given to healthy animals in low doses to promote growth (Levy, 1997). Most of the antibiotics given to farm animals are not metabolized in the body, rather they are excreted in the active form (Lee et al., 2000). The fate of antibiotics introduced into soil and aquatic environments with manure and other animal wastes is largely unknown. However, there is much concern that the presence and persistence of low levels of antibiotics in soil and aquatic environments could encourage the buildup of existing, and potentially the development of new, antibiotic-resistant bacterial populations (Henry, 2000).

In Iowa, Earthen Waste Storage Structures (lagoons) are widely used for temporary storage of liquid animal wastes with the intent of protecting surface and ground water from contamination and allowing farmers to use the wastes in a timely fashion. Liquid animal wastes are generally spread on agricultural soils both as a means of disposal of the wastes and as a nutrient source for crop production. The Iowa Dept. of Public Health (1998) found relatively high concentrations of chlorotetracycline (11 to 540 µg/L) and erythromycin (10 to 275 µg/L) in such liquid animal wastes. The report also indicated that many of the 18 *E. coli* isolates, all three Salmonella species, and an isolate of Enterococcus demonstrated resistance to one or more of the antibiotics.

The antibiotics most commonly added to livestock feed as growth promoters (1 to 100 mg per head per day) are chlorotetracycline (Aureomycin), oxytetracycline (Terramycin), and macrolide (erythromycin) (Sewell, 1993; FAC, 1998; Herman et al., 1995). The fate of these compounds in Iowa soils depends on sorption and desorption of the antibiotics on soils, leaching, and the rates of chemical, photochemical, and microbial decomposition of the antibiotics. The basic hypothesis of the study is that the fate (sorption/desorption, leaching, and decomposition) of antibiotics in soil environments is strongly influenced by the chemical reactions between the antibiotics and soil constituents.

Specific Objectives:

1. Characterize three common Iowa soils and isolate and characterize reactive soil components (clay-humic complexes, clay minerals, and humic materials) from these soils.

2. Quantify sorption of tetracycline and chlorotetracycline on the soils and soil components.

3. Determine the effects of staturating cation (Ca vs K) and ionic strength (I=0.05 and I=0.005) on sorption of tetracycline and chlorotetracycline on the soils and soil components.

4. Quantify the influence of sorption on tetracycline and chlorotetracycline degradation rates.

5. Quantify mobility of tetracycline and chlorotetracycline in soil columns.

Methodology

Soil samples, surface (0-15 cm) and subsurface (≥ 15 cm), were collected from three sites representing three different soil series and a range of soil physical and chemical properties. Both the studied soils and the general sampling locations had been previously characterized (McBride et al., 1987). Based on interviews with the landowners or operators, specific sampling sites that had never received manure applications were selected. The soils were characterized using standard analytical procedures to determine pH in $CaCl_2$, pH in KCl, pH in water, organic C, organic H, organic N, % sand, % coarse silt, % fine silt, % clay, and extractable cations (Ca, Mg, Na, and K).

Soil components were physically and chemically separated from the soils and prepared for the sorption and degradation studies. Clay-humic complexes were isolated from the soils by sedimentation (<2 µm e.s.d.). Portions of the clay-humic complexes were K- or Ca-saturated by washing in 1M KCl or 0.5 M $CaCl_2$ and then dialyzed against distilled water and freeze-dried. Other portions of the clay-humic complexes were treated with 30% H_2O_2 for removal of the humic materials before being K- and Ca-saturated, dialyzed against distilled water, and freeze-dried. Humic materials were separated from the three soils by hydrolyzing Na-saturated samples in 0.1 M NaOH under and N_2 purge. After the hydrolysis, the humic materials were separated by centrifugation, neutralized to pH 7, K- or Ca-saturated, dialyzed, and freeze-dried.

A batch equilibration technique was designed and used to measure sorption of tetracycline and chlorotetracycline on the various soils and prepared soil components. HPLC was used to quantify tetracycline and chlorotetracycline in the supernatant solutions and sorption was determined by difference. Variables tested include soil components (clay-humic complexes, clay minerals, and humic substances), saturating cation (K vs Ca), and ionic strength (I=0.05 and I=0.005).

Currently we are developing an extraction technique, which will allow bound tetracycline and chlorotetracycline to be removed from a soil or soil component. This technique is needed to quantify degradation kinetics of sorbed tetracycline and chlorotetracycline. We are also developing techniques that will allow determination of whether bound tetracycline and chlorotetracycline remain bioactive. The extraction technique is also needed for this study.

The final stage of the research will be a column leaching study. Intact soil columns treated with tetracycline and chlorotetracycline will be leached with high and low ionic strength solutions with different ratios of K and Ca. The ionic strength and the K:Ca ratios of the leaching solutions will be selected to both encourage and discourage colloid mobility. Leachate will be analyzed by HPLC.

Principal Findings and Significance

Tetracycline (TC) and chlorotetracycline (CTC) are very strongly sorbed on soil clays, soil humic substances, and soil clay-humic complexes. In agreement with previous studies, sorption of TC and CTC was found to increase with decreasing pH and to be greater in Ca-systems compared to K-systems. Also, in agreement with previous studies, we found higher sorption of CTC compared to TC, most likely due to the lower solubility of CTC. An important new finding is that TC and CTC are more strongly sorbed on soil clays than on soil humic substances, and that interactions between clays and humic substances significantly diminish sorption of TC and CTC on soil clay-humic complexes. Although TC and CTC are strongly sorbed by soil clays, humic substances, and clay-humic complexes, a portion of sorbed TC and CTC is released back into an aqueous solution through desorption. Desorption increased with increasing pH and for K-systems relative to Ca-systems. Desorption of TC was greater than desorption of CTC. Desorption of TC and CTC from the clay-humic complexes was substantially greater than desorption from either the humic substances or the soil clays. The results demonstrate that interactions between soil clays and humic substances significantly influence both the sorption and desorption of TC and CTC from soil components. One possible explanation is that humic substances compete with TC and CTC for sorption sites on soil clays. The results also suggest that soil clay-humic complexes have the potential to facilitate transport of TC and CTC to surface and ground water systems and that contaminated clay-humic complexes may act as reservoir to slowly release TC and CTC in soil and aquatic systems.

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Information Transfer Program

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	7	0	0	0	7
Masters	8	0	0	0	8
Ph.D.	1	0	0	0	1
Post-Doc.	0	0	0	0	0
Total	16	0	0	0	16

Notable Awards and Achievements

Ms. Jutta Pils, a Ph.D. candidate in Soil Science (Soil Chemistry) within the Department of Agronomy at Iowa State University, was invited to present a talk entitled "Sorption of Tetracycline and Chlorotetracycline on K- and Ca-saturated Soil Clays and Humic Substances" as part of a special Symposium honoring Dr. A.D. Karathanasis' achievements and his selection for the Heick Professor Fellowship, Lexington, KY, April 2004 (expenses paid).

Ms. Jutta Pils was awarded Sixth Place for an oral presentation at the Annual Meeting of the Soils Science Society of America, Indianapolis, IN, 2002.

Oral presentation at 51st Annual Meeting of the Entomological Society of America, Cincinnati, OH, October 2003. "Geographic Information Systems and remote sensing as predictors of West Nile virus risk in northeastern Iowa," David R. Mercer, Jeff Fisher, Sara L. Sheeley, Forest Isbell & Keri Leymaster.

Publications from Prior Projects

1. 2000IA45B ("REMOVE THIS TEST") - Book Chapters - This is only a test.