PROJECT NAME: Distribution and Fate of Background and Bioavailable Metals in Oregon Agricultural Soils, Plants and Waters

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2. PROJECT RATIONALE

Background levels of metals in Oregon soils that receive fertilizer treatments are not well understood. The effects of fertilizer use and the long-term effects on biota uptake and on surface and ground waters are also not well understood for Oregon soils. In addition, to understanding background levels, bioaccumulation, bioavailability and partitioning are keys to truly understanding risk. Bioavailability of metals is the accessibility for biological assimilation and possible toxicity. Federal and state regulatory agencies typically rely on analytical methods that entail vigorous extraction of matrices with strong acids. The relevancy of such methods to the toxicity is often not considered, thus decisions are based on data that is often not relevant for prediction of potential exposures and risk. The evidence is compelling that the quantities recovered by vigorous extraction/digestion fail to predict bioavailability of the compounds[1]. Regulatory agencies have recognized the importance of determining bioavailable versus total contaminant concentration**;** US EPA has allowed certain regions to develop site specific criteria based on bioavailable levels of priority pollutants[2].

Health/Risk/Fate- *Depends on Chemical form*: For chemical contaminants, aquatic toxicity data, water quality criteria and threshold limit values are based on dissolved concentrations and not total metal levels[3,4]. For example, a study of copper distribution and water effects ratios were recently performed under the auspices of EPA Region 2 for New York/New Jersey[5]. Based on this work (i.e. bioavailable copper) revised criteria were proposed and adopted. The modified criteria saved costly remediation efforts in NY/NJ. Presented below one can see how different conceptual[6] approaches can lead to significantly different estimates of exposure. To efficiently generate quantitative exposure estimates and to accurately characterize risks posed by metals, bioavailability needs to be considered as early as possible in the risk assessment[6]. However, the assessment of hazards posed by contaminated soils has been hampered by the lack of simple realistic procedures that assess the rates and extent to which metals can be released from soil particles and supplied to biota.

Estimated Exposure ⇑

Compound Release	Analytical Chemistry	Biological Response
	Non-recoverable	Not Environmentally Available
LOADING	Analytically Recoverable	Not Environmentally Bioavailable
		Not Pharmacologically Bioavailable
		BIOLOGICALLY RECOVERABLE

Background on Bioavailable Metal Methods:

DGT (diffusive gradients thinfilms) are simple, precision devices that accumulate dissolved substances in a controlled fashion. Conventional analyses back in the laboratory provide the in-situ concentrations at the time of deployment. The device uses a layer of Chelex resin impregnated in a hydrogel to accumulate the metals. The resin-layer is overlain by a diffusive layer of hydrogel and a filter. Ions have to diffuse through the filter and diffusive layer to reach the resin layer. The concentrations of metal ions in the sediment adjacent to the device are lowered. This can induce supply of metal ions from the soil phase to solution in the layers of sediment near the device. The total metal accumulated during the deployment is measured. DGT measures directly the mean flux of labile species to the device during the deployment. This can be interpreted directly as the mean concentration of labile metal at the interface between the device surface and the sediment, during the deployment. For the situation where supply from soil particles to solution is rapid, this interfacial concentration is the same as the concentration of metal in bulk pore-water. For a given device and deployment time, the interfacial concentration can be related directly to the effective concentration of labile metal[9], C_E. C_E represents as a concentration the supply of metal to any sink, be it DGT or an organism, that comes from both diffusion in solution and release from the soil phase.

Relevance to sediment quality regulations
The effective concentration, C_E, measured by DGT has been shown to correlate very well with uptake by biota[9,7]. DGT mimics the main mechanism of uptake by lowering the concentration locally and inducing diffusive supply and release from the solid phase. Although this is a dynamic measurement that depends on both the rate of transport and the rate of release, it can be used to provide an effective concentration, C_E. C_E is a measure of what the solution concentration would have to be to produce the observed accumulation of metal if there was no supply from the solid phase. C_E may therefore be related through water quality toxicity tests to a quality standard. C_E is measured directly and simply. It automatically accounts for all sediment properties, including pH and organic matter content.

Metals in Soils and Plant Uptake
The first application of DGT in soils showed that in soils where sludge had been applied, Cd and Zn were present in two separate pools with different kinetic availabilities[8]. A follow-up study of plant uptake of Cu, Cd, Co, Zn, Pb and Ni at different moisture contents[7] showed that the change in plant uptake with moisture content was more closely related to the observed change in DGT uptake than to soil solution concentration. It has been shown that measurements of C_E in a wide range of soils contaminated to various extents with Cu were a very good predictor of Cu uptake by plants.

Kinetic and thermodynamic constants
The extent of release of metal from the soil depends on the rate constant for transfer from soil to solution and the size of the labile pool of metal in the solid phase. The **distribution coefficient, K_d,** for the labile metal can be related directly to the labile soil phase pool size. By deploying DGT for different times in soils where the concentrations of metals in the pore-waters are separately measured, it is possible to provide direct estimates of K_d and the re-supply rate constant.

Summary of Project Rationale -<u>Conventional metals concentrations in all matrices</u> and bioavailability
Conventional metal concentrations are important to collect given the larger body of comparative data. We propose to determine metals by US EPA methods (SW-846) on Oregon soils, plants and surface and ground waters. However, the collection of biologically relevant data is also important, bioavailability, bioaccumulation and partitioning of metals under typical agronomic fertilizer applications rates and on Oregon soils needs to be a part of any risk assessment study. One excellent approach is the use of DGT where research has clearly demonstrated that insight into the supply of metals from soils can be gained by using DGT as physical surrogates for plant/organism uptake.

3. PROJECT GOALS AND OBJECTIVES

Establishing background metal levels in Oregon soils and waters
Multiple sites around Oregon, in established agricultural regions will be developed as "agricultural-fertilizer-used" sites. The following locations will be used: North Willamette Research & Extension Center (NWREC), Malheur Experiment Station (MES), Columbia Basin Agricultural Research & Extension Center (CBAREC), and Klamath Agricultural Research station (KARS). Standard grab samples will be collected at each site. Establishing background/baseline numbers will be accomplished by pulling samples from all sites prior to treatments. Samples will be pulled in August/September of 2003 to establish background/baseline at all research stations. Total recoverable metals (Cd, Pb, Ni, Hg, As) will be determined on soil, runoff (dissolved in water and sediment), surface and ground waters, see attached tables for specific sampling #s per matrix, per site, per application, per year.

Validating risk assessment input

In the human health risk assessments, the soil-water partition (K_d) is an important input parameter. All stations will have controls and three fertilizer treatments levels. With this experimental design we will have setup the opportunity to determine metal levels (ultimately K_d's) for several application scenarios. This will provide for the eventual validation of K_d's, for Oregon soils, an important input parameter of the risk assessment calculations, in a series of Oregon soils. For each soil series used at each site we will also have chemically characterized these soils so additional chemical considerations will be known for interpretation of K_d's (e.g. lime effects, pH effects, moisture, organic matter, etc.)

Factors affecting fate and transport

Paired studies will be setup at all sites, in addition at the NWREC, we will include paired sites to study the effects of liming with respect to fertilizer applications. The experiment station sites around the state have been chosen in part because they also have different soil chemistries (pH, organic matter, CEC, etc.). By utilizing the same procedures at multiple sites we will begin to understand the impact of soil chemistries on metal fate and transport due to fertilizer applications.

Fertilizer Treatments (dose study and field duplication)

In addition to control and benchmark field trials, three treatments levels will be use at each station for the study duration. The treatment levels will be determined by staff at ODA. These treatments will be duplicated at all sites (crop rotation permitting). By utilizing the same treatments at multiple sites we will begin to understand the impact of soil chemistries on metal fate and transport due to fertilizer applications from yearly high dose conditions. In addition, all study plots will be duplicated, this will allow for a better understanding of the heterogeneity of the field ecosystem within each treatment at each research station. Dose affect, soil levels, and plant uptake levels will be evaluated.

Bioavailability of metals in plants and organisms

DGT are an excellent surrogate for plant and organism uptake. Measurements of Cu as its effective concentration (C_E), its soil solution concentration, by EDTA extraction and as free Cu^{2+} in soil solution were made on 29 different soils covering a large range of copper concentrations[9]. They were compared to Cu concentrations in the plant material of *Lepidium heterophyllum* grown on the same soils. Plant concentrations were linearly related and highly correlated with C_E, but were more scattered and non-linear with respect to Cu^{2+}, EDTA extraction or soil solution concentrations. These results demonstrated that the dominant supply processes in these soils were diffusion and labile metal release, which the DGT-soil system mimics. The work showed that insight into the supply of metals from soils can be gained by using DGT as a physical surrogate for plant uptake. DGT are a better method for determining "generic" plant uptake than simply looking at one plant (crop) species, there are always outstanding questions when looking at a single crop for estimating plant uptake. For example, what portion of the plant is to be analyzed, should the plant part be based on typical human consumption or wildlife consumptions? If a test crop is used then there is always the nagging question, "but what would the metal uptake be if another crop were grown?". DGT measures what is bioavailable and therefore gives one confidence, regardless of crop what the likely metal risk presents.

Distribution of metals in soils, waters and plants –CONVENTIONAL METHODS

Conventional analytical methods (US EPA SW-846 methods) will be used to determine metal content in soils, surface waters, plants[11] and ground waters (when available). Analyses will include Cd, As, Pb, Hg, and Ni. The development of this data base will be critical to understanding the distribution of metals in Oregon soils under various defined agricultural fertilizing[10] conditions. Plant tissue will be analyzed for all heavy metals as well as about 10 other elements. Other elements are included for plant tissue analyses to discern whether synergistic or antagonistic effects occur with other essential and non-essential elements in plants[10].

Distribution of metals- speciation

Our hypothesis is that bioavailable contaminant levels are much better suited to study the true ecological impact from fertilizer applications. To test this hypothesis we will investigate the bioavailable concentrations of Cd, Pb, Hg, As(III), and Ni using DGT. Equally important are the chemical characterizations of the ecosystem (study sites), bioavailable fractions, effects of physical/chemical perturbations on the ecosystem and their effects on the bioavailable concentrations. To these ends this project will attempt to make strides towards understanding some of the critical factors effecting mobility of bioavailable metals (As (III), Cd, Ni, Pb and Hg).

4. PROCEDURES AND EXPERIMENTAL DESIGN

We will conduct field studies to investigate the impact of fertilizer applications on total metals and bioavailable metals at agriculturally relevant sites. The sampling sites are designed to quantitate current persistent, bioaccumulative metals concentrations prior and post fertilizer application. The proposed sites include: NWREC, MES, CBREC and KARES. Land use history is known for all agricultural sites currently proposed. Field sampling for all locations will be recorded using GPS. See attached spreadsheet for a listing of the 44 field sites. Samples will be taken a minimum of 10 m from the edge of a field to minimize road effects, utility poles, etc. A 2-in bucket auger will be used to collect soil samples from the surface to 20 cm at each location (excluding organic debris). At each site, three samples approximately 2-10 m apart will be taken along a transect. Each will be composite of 3 samples taken along a 1-2 m line. Three samples of ca. 500 g will be collected, one for metal analyses described here, one for standard soil fertility (chemical characterization) analyses and one for archiving. The archived sample will be labeled, frozen, and maintained in a GLP facility freezer. Total recoverable metals (As, Cd, Ni, Pb) will be determined via US EPA method 3050B (digestion) and either US EPA method 6010B (ICPAES) or 6020 (ICPMS) if lower detection limits are needed. Mercury digestions will follow a modified US EPA method 7472/7471A. These methods provide adequate sensitivity and interferences and other analytical challenges have been previously defined[11,12]. Each year, at each site (5 sites- and for each application a total of 12 field sites), chemical characterization of the soils will be performed via standard agronomy methods (e.g. pH, NO_3, NH_4, K, P, SO_4, B, organic matter, cation exchange capacity)[13]. Within each site several 1 m deep samples will be collected for metal analyses. Samplers will be used to collect runoff waters and sediments, these will be composited depending on the amount of runoff, dissolved metals in the waters and total recoverable metals in the runoff sediments will be determined, see table for a complete listing of the sampling schedule. Water samples high in arsenic will be analyzed for arsenic (III), the toxic arsenic species, by anodic stripping voltametry, US EPA Method 7063. DGT will be deployed along transects at the same time as soil sampling and each year a time dependence study will be setup to estimate K_d's (both conventional and with DGT).

Quality Assurance and Quality Control Statement:

Our facility is a full **GLPS** (good laboratory practices standard) program and has successfully completed five **GLPS** audits within the last 18 months, in compliance with FIFRA, 40 CFR Part 160 and TSCA, CFR part 792. As a GLP facility, we have in place a standard operating procedures (SOP) manual and a quality assurance program plan[14], both have withstood several external audits. We will adhere to the guidelines of our QA plan. A sampling of *some* of our QC goals as applicable to individual projects include: 10% of our field deployments will be duplicates, 3-5 point calibration and correlation coefficient of >0.98, fortification studies, certified reference materials, continuing analysis of blanks run at 10% of total samples, and formal method detection limit study according to EPA standard procedures (40 CFR 136). The full QA plan was attached in the original proposal (not included here).

5. IMPLEMENTATION PLAN

Sites at North Willamette, Columbia Basin (Pendleton), Malheur and Klamath will be established summer/fall of 2003. Analytical methods are already established in our laboratory[14]. Sampling will take place beginning in 2003. Chemical analyses will begin shortly thereafter. Baseline data will be collected

August/September of 2003. All chemical and data analyses for the 2003 season will be completed by spring 2004. Post fertilizer samples will be pulled at each station, at Pendleton (Columbia Basin) and Malheur this will occur in the fall, at North Willamette and Klamath this will occur each spring. Replication will occur in from 2004- 2006, see attached spreadsheets. Annual reports for sampling, chemical analysis, and data analysis will be completed by summer of each subsequent year. A final report for the study will be completed by July 2007. About 850 samples will be collected *each* year for elemental analyses (As, Cd, Pb, Ni & Hg) and As (III) as needed.

Each soil sample listed in the table represents a composite of 3 field samples. Sample collection will occur post-fertilizer application, and at harvest. As feasible, samples will be pulled at the same interval (days post application) at all sites so comparison will be relevant. Rain/weather data will be collected via www. Plant tissue will be collect at time of harvest at each research station, at NWREC, CBRES, and MES wheat will be grown all three years, and we will analyze wheat kernels on a dry weight bases. Klamath will grow either wheat or potato depending on normal crop rotations at the station. The human edible portion (tuber, wheat kernel) will be analyzed each year. Total samples listed in the tables are *approximate since in some years, at some sites, surface waters and ground waters may not be available*. TBD = to be determined. Soils will be pulled (one from each paired field) each yr for extended soil chemical characterization
The proposed project described here will ultimately:

1. provide data and analysis that will develop a better understanding of background levels of Cd, Pb, Hg, Ni, As, & As(III) in agriculturally relevant Oregon soils following fertilizer applications
2. provide data that will be more relevant to toxicity (bioavailability)
3. develop a better understanding of the rate of change of metals in soils under defined agricultural practices
4. allow environmental risk associated with fertilizer applications to be more accurately evaluated
5. provide both the seasonal and magnitude of contributions of bioavailable metals to surface waters
6. further establish the use of DGT probes for use in fieldwork to relate bioavailable metals & K_d's
7. provide insight into water quality variability from fertilizer applications

The research proposed here will provide significant data from conventional analytical techniques at defined paired Oregon agricultural sites, typical agronomic practices and known fertilizer application rates. The data will provide the bases for answering questions of what are the impacts and risk from metals from fertilizer applications on Oregon soils. In addition, the research proposed here will provide significant insight into the bioavailability of metals at these paired sites and provide the bases for answering the questions of what are the biological and ecological risks of fertilizer applications on Oregon soils. This research proposed here provides a unique approach to establish links with conventional approaches combined with powerful insight into biologically relevant techniques.

Project Coordination with other Researchers

Dr. Gwen Johnson, Portland State University, and her research group will be accompanying our group during the site setup and baseline/background data collection August 2003. Sites will be established and samples collected by both research groups. Subsequent sampling will most likely occur together, either during post application or harvest each year, although Dr. Johnson may pull samples occasionally at other times during the study. We will assist her staff as needed during field retrievals.

6. PERSONNEL: attached in original proposal

7. BUDGET (8/03-12/06)

Funds Requested	What will be purchased/accomplished?
$212,968	Field deployment & retrieval of soil, waters, and passive sampling devices. Analytical extraction, identification, confirmation and quantization of metal contaminants in soils, surface waters, runoff waters and sediments, plant tissue and DGT, and data analysis, final report generation
$9,609	Deployment and retrieval of samples at 4 OSU agricultural research stations
$80,378	DGT devices, filters, acids for extraction and clean-up of samples, ICPAES/MS consumables, samples bottles for storage and transport
Total $302,955	

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