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Ceriodaphnia bioassay on three types of field applied sewage sludge fertilizers /

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CERIODAPHNIA BIOASSAY ON THREE TYPES OF FIELD APPLIED SEWAGE SLUDGE FERTILIZERS

A Thesis Presented

by

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To My Mother, Father, Sister, and Brother

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ABSTRACT

CERIODAPHNIA BIOASSAY ON THREE TYPES OF FIELD APPLIED SEWAGE SLUDGE FERTILIZERS

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Ceriodaphnia dubia bioassay was used to detect the toxicity of the soil leachate of three types of commercially available sewage sludge fertilizers: Milorganite®, Terrene®, MWRA (sludge from Massachusetts Water Resource Authority); a by-product of Boston Harbor clean-up project. Evacuated ceramic lysimeters were placed in the field to collect the soil leachate. During the entire research, chemical parameters of the leachate were also investigated.

Sewage sludge application did impose an impact on Ceriodaphnia reproduction, and offered a feasible method for bio-monitoring terrestrial soil leachate.

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CHAPTER 1

INTRODUCTION/JUSTIFICATION

When federal legislation forbade the discharge of sewage sludge into receiving waters (Federal Water Pollution Control Act, 1972), large population centers concentrated in coastal zones were forced to seek alternative strategies for eliminating their waste products (Clean Water Act, 1977). The disposal of sewage sludge, however, has historically posed environmental problems (Varanka et al., 1976). All of the options commonly utilized for ridding ourselves of this material have disadvantages: Offshore dumping produces ocean pollution; appropriate landfill sites are in short supply; incineration can cause air pollution and is energy consuming. Of these tactics, offshore dumping has been the most frequently used.

Not surprising; a greater use of land application of both municipal waste water and sludge has been mandated by the Clean Water Act of 1977. This legislation has encouraged the use of terrestrial ecosystems to restore water quality at the outfall. Section 201 also stipulates, that appropriate technology should evolve advanced waste treatment techniques to recycle waste water and confine disposal of pollutants to prevent other environmental pollution. The application of municipal sewage sludge to soil-crop systems may be the most environmentally and

economically feasible method of meeting these requirements. Sludge contains valuable nutrients for agriculture: Averaging 15 Kg of nitrogen, 6 Kg of phosphorus, and 4 Kg of potassium per dry megagram (Pimentel et al., 1983). Thus, the land application of sludge is an important and growing practice in U.S.A., as indicated by a survey (U.S. EPA, 1988): Of 1008 publicly owned treatment works, accounting for over 2 million dry metric tons per day, 50% of the sludge generated is applied to soil in large scale food-chain and non-food-chain landspreading.

However, those sludges from municipalities accepting industrial effluent also contain toxic chemicals, such as polychlorinated biphenyls (PCBs), heavy metals, pesticides, phenols, a wide array of corrosive ill-defined organic chemicals, polycyclic aromatic hydrocarbons (PCAH), etc. (Furr et al., 1976; Babish et al., 1981; Mumma et al., 1984). These xenobiotics when broadcast upon the earth will enter and move through aquatic and terrestrial food webs (Neuhold et al., 1976; Hinesly et al., 1979; Chaney, 1980; Jacobs, 1981). The potential of theses substances to disrupt whole ecosystems is enormous.

In this context, remarkably, no whole animal aquatic field tests on the toxicity of sludge as fertilizer have being published. Reliance on chemical analysis alone as a quality control measure ignores the potential of additively and antagonistically interactive synergism of toxicants in

complex environments. Further, this method falsely assumes that chemical analytical techniques can replace biological assessment in estimating toxicity. To equate mere presence with biological availability seems to be a gross travesty for pollution is fundamentally a biological problem.

CHAPTER 2

OBJECTIVES

The U.S. EPA (United States Environmental Protection Agency) approved standard Ceriodaphnia dubia bioassay was implemented to assess and compare in the field the toxicity (as measured by survival, reproductive success, and life spans of the organisms) of three commercially available sewage-sludge fertilizers: Milorganite® (Milorg.) from Milwaukee, Wisconsin; Terrene® (Hag) from Hagerstown, Pennsylvania; and the product of the Massachusetts Water Resources Authority (MWRA) from Boston, Massachusetts. The values thus generated would permit assurance of the suitability for implementing the methodology to the soil leachate testing/monitoring to be used in the environmental monitoring studies, and provide the baseline for sludge quality comparisons. Both Terrene® and MWRA are pelletized sludge products and Milorganite® is non-pelletized; activated sewage sludge. Three of these sludge fertilizers are categorized as "Type I"; which makes the unregulated "nonrestricted usage" in Massachusetts legal.

CHAPTER 3

LITERATURE REVIEW

3.1 Sewage Sludge

Disposal of water treatment sludge has created a dilemma due to continuous production and a limited disposal area. Land application is a low cost alternative for disposal currently being prompted by the U.S. EPA. Accordingly, the U.S. EPA has published a process design manual regarding land application of municipal sludge (U.S. EPA, 1983), which reviews existing information, data bases and sets guidelines for projects and distribution/marketing.

Numerous studies have tested the feasibility of applying sewage sludge from waste-treatment facilities in agriculture (Hue, 1988; Dorn et al., 1986). In Seattle, WA, the feasibility of managing Douglas fir forests in the Pacific Northwest with municipal sewage sludge has been explored (Henry & Cole, 1983). Furthermore, some freshwater coagulant sludge may serve as effective liming agents in areas of acid deposition (Bugbee & Frink, 1985). Another potential benefit of water treatment sludge, particularly iron-sludge, may be an increased availability of Fe to plants grown in Fe-deficient soils because the added coagulant increases the level of plant available Fe (Lindsay, 1979).

The general application of sludge to land, however, has prompted investigation of several possible adverse impacts from sludge-borne chemicals and microbial agents. These include phytotoxicity (Hue, 1988), domestic animal toxicity (Dorn et al., 1986), threats to the public health via contamination of drinking water (Novikove et al., 1985), accumulation in edible crops (Elliot & Singer, 1988), animals, and animal products used for food (Dorn et al., 1986). In this regard, the U.S. EPA has proposed a list of 165 chemicals for analysis in municipal sludge treatment systems (U.S. EPA, 1986). Accordingly, the Association of Municipal Sewage Agencies (AMSA) and U.S. EPA have evaluated a wide range of sludge types (U.S. EPA, 1986) using the toxicity characteristic leaching procedure (TCLP) protocol and reported that some of the sludges were very close to the proposed limits and another report indicated that some of the listed target organics: Toluene, p-dichlorobenzene, chlordane, DDT metabolites, two to four-ring PAHs, pyrene, Bis-(2-ethylhexyl)phthalate, phenol, di-n-butylphthalate, 2-nitrophenol, N-nitrosodimethylamine, and Aroclor-1248, were isolated at mixture levels exceeding D&M (distribution and marketing) guidelines.

In 1988, the U.S. EPA did a analysis of the distributed and marketed (D & M) municipal sludge products from facilities in 26 cities across the U.S. and compared these evaluations with U.S. EPA proposed D&M sludge criteria and

found that copper and lead concentrations from several sites consistently exceeded the criteria for land use (U.S. EPA, 1988).

Increases in cadmium and copper concentrations resulting from sludge application have been documented for soils, vegetation, meadow voles, earthworms, birds and mammals (Chang et al., 1984; Levine et al., 1987, 1989; Boswell, 1975; Anderson & Barrett, 1982; Beyer et al., 1982). However, although the distribution of metals in sludge is well documented (Tien, 1988), comparatively little available data treating trace organics exists, for the reason that chemical matrix problems of sludge inhibit the complete survey of the latter by the technology frequently used. Nonetheless, the ecological significance of such findings hardly needs amplification: Toxic components of sludge may be translocated and incorporated into the food web via the detritus based and grazing communities. (Finlayson & MacCarthy, 1965). Culliney and Pimentel (1986) reported that ingestion of organic chemicals and heavy metals may have been the prime cause of reduced fitness in aphids grown on collards in sludge. Therefore, predators and parasites feeding on aphids and other herbivorous invertebrates would be influenced both directly and indirectly. Chlorinated hydrocarbons, including PCBs, and insecticides have been found in aquatic insect consumers (Clements & Kawatski, 1984) and predators (Cheng & Bidleman,

1977). Results of these studies suggested the existence of potentially disruptive ecological effects of chemically contaminated sludge on the functioning of ecosystems.

In 1986, U.S. EPA conducted a study and observed that several samples of leachate water from the sludge amended soil contained appreciable levels of organic mutagens, bacterial mutagenicity on the sewage sludges exhibits tremendous variation both between different sources and from a single source, and the extracts of plants also induced mutagenic responses (U.S. EPA, 1988). Although the chemical constituents of these samples were not identified, the results indicated that mutagenic chemicals can migrate below the sludge incorporation zone.

Unfortunately, the undefined complexity of the chemical matrix makes detection of a particular ion in the applied sludges was sometimes the direct cause of toxicity. Besides, the metabolic activity of earthworms possibly increased the solubility of lead and zinc in soils contaminated with heavy metals. This, in turn, may have influenced the availability of such phytotoxic heavy metals (Cu, Zn, Cd and Pb) which occurred despite the ability of earthworms to accumulate heavy metals (Hartenstein et al., 1979, 1981). The redox status of the soils receiving municipal sludge are also important factors. Where feasible alternatives exist, wet or poorly oxidized soils would be more effective for immobilizing Zn and Cd (U.S. EPA, 1986),

and increase in soil acidity usually enhances the availability of trace and toxic metals. However, scant attention has been paid only to those processes initiated by acid rain regulating trace and toxic metal availability under different pHs in sludge-amended soils.

The elemental composition of sludge varies considerably not only from one waste-water treatment plant to another, but even within each plant (Sommers, 1977). Because of this inherent variability, the U.S. EPA in 1988 proposed the data indicating the probability of site-specific problems (e.g. copper, lead and PCB) and in terms of NPW land use criteria, indicated strongly monitoring programs. However, still neither quality control nor quality assurance existed.

Thus, not only that additional research is needed to evaluate the interactions of climatic conditions and soil texture, as it affects degradation and leaching of triazine, the fate and mobility of organic pollutants in municipal sludge amended soils need to be studied, but also that short-term bioassays should be utilized to evaluate and detect the persistence of toxic chemical entity in sludge amended soils

3.2 Bioassay-Ceriodaphnia dubia

A bioassay is a test used to evaluate the relative potency of a chemical by comparing the effects of pollutants

at different concentrations on living organisms. Many of these techniques are applied to aquatic problems.

Regulatory toxicity tests determine whether a toxicant meets a legal requirement, monitoring tests provide on-site information on the toxicity of effluent and receiving waters, water quality tests provide information of use in establishing water quality criteria. The evolution of standard bioassay practices to evaluate water quality received impetus from federal legislation in the early 1970s. The Toxic Substances Control Act (1976) regulates the potential ecological hazard associated with the widespread distribution of chemicals in commerce. Accordingly, The American Society for Testing and Materials (ASTM) (1976) responded to the federal mandate to assess ecological hazards and evolved standard practices for evaluating the toxicity of chemicals to aquatic organisms.

The Clean Water Act (CWA) (PL92500) (1977) is the single most important law dealing with the environmental quality of all U.S. surface waters, both marine and fresh. This act sets a national goal to restore and maintain the physical, chemical, and biological integrity of the nation's waters. Under the CWA, The U.S. EPA works with the States to monitor the quality of surface water by providing monitoring guidance and technical support. Under Section 304 of the Clean Water Act, the EPA has broad authority to develop specific chemical criteria to protect water quality and to

develop and use other assessment techniques. Furthermore, under Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), The EPA can ban, cancel, suspend, not register, or restrict the use of a pesticide or other biocide if the use poses risks of adverse effects on human health and the environment. And the testing requirements and risk assessment procedures also focus on bioassays.

In general, the U.S. EPA and the States are using three approaches to assess toxicity: Field assessments, laboratory bioassays, and chemical-specific measurements. Primarily as an economic measure, the EPA has focused its research on bioassay and chemical-specific criteria. Bioassays that measure short-term lethality and bioaccumulation are already available and have been used in the CWA and Marine Protection, Research, and Sanctuaries Act (MPRSA). For the protection of freshwater aquatic life, the U.S. EPA (U.S. EPA, 1980) has published a description of a minimum aquatic toxicity data base for a specific chemical for deriving water quality criteria.

In the field of aquatic toxicology, invertebrates have been used more widely than fish or phytoplankton in classifying natural environments (U.S. EPA, 1987; Pontasch, 1989). This may in part be attributed to a previously generated data base and the ecological role of the organisms in the food web. The ecological importance of cladocerans (e.g. Ceriodaphnia, Daphnia) in aquatic system has been

studied since 1883 (Pennak, 1989). Such organisms play a significant role in the food web and in phytoplankton and protozoan dynamics, and they have been reported to constitute from 1 to 95% by volume of fish stomach contents (Wetzel, 1975; Pennak, 1989). Aquatic invertebrates, moreover, are especially suitable for toxicity tests because of ecological and economic importance and morphological, physiological, and ecological diversity. The practical advantages to the use of aquatic invertebrates in toxicity tests include: Size (macroscopic but small enough to minimize the space and toxicant used), relatively short life cycles, and the capability of producing genetically uniform cultures. Accordingly, of the 158 aquatic species nominated by The American Public Health Association, 91 species were aquatic invertebrates (APHA, 1976).

The earliest published report utilizing aquatic invertebrates in a bioassay appears to be that of Beudant in 1816, who subjected 15 species of freshwater mollusks to various salt solutions (Beudant, 1816). Experiments with daphnids have been published in 1899 (Warren, 1899) dealing with the determination of the lethal concentration of sodium chloride. In 1916, a large number of experiments have been described for the osmotic pressure of the blood of Daphnia magna (Fritsche, 1916). About six decades ago, serious consideration started to be given to D. magna as test animals (Nauman, 1933, 1934). Since then, among all of the

invertebrates, Cladocera (e.g. daphnids and ceriodaphnids) has been one of the most widely used orders.

Almost all of the previous work were involved in determining the toxicity of various substances in terms of hours required to kill a given percentage of the testing animals. However, in 1925, Ramult determined the concentrations of sodium chloride that would inhibit the development of parthenogenetic eggs of daphnids and other cladocerans (Ramult, 1925), and the effects of altered hydrogen ion concentrations on daphnids was also noted by Strom in 1926 (Strom, 1926). Subsequently, Brown published a series of papers on the natural history of cladocerans in relation to temperature for vital activities, distribution, development, pre-adaptation and dispersal (Brown, 1929), and Breukelman in 1932, ascertained the time to stop certain bodily functions in Daphnia pulex in mercuric chloride (Breukelman, 1932).

In a sense, however, the use of daphnids in bioassay really began with the work of Einer Naumann in 1933; 1934, in a series of 17 papers on the use of Daphnia magna as a test animal: He even used Daphnia magna to test the materials for aquatic biological laboratory facilities construction, and for air analysis (Naumann, 1933).

Four basic types of tests with daphnids have been developed: Acute lethality tests are conducted over a defined time period (usually 24 to 48 hours), lifetime

chronic tests, multi-generational chronic tests to determine the possible physiological effects, and genetic tests. Chronic tests on daphnids reproduction produce the most useful results for low dose toxicity assessment (Sprague, 1976). These types of tests have been conducted in various conditions: Static; static renewal, and continuous flow.

Toxicity studies of whole sediments and elutriates with daphnids and ceriodaphnids have also been conducted in accordance with the methodology proposed by the draft ASTM. Research pertinent to responses with cladocerans were often similar to those of benthic species, both in laboratory assays and in situ communities (Burton et al., 1990). Daphnids responses in sediment assays have been related effectively to the concentration of contaminants in whole sediments, pore water, or dose dissolved from the sediment to overlying waters (Prater & Hoke, 1980; Malueg et al., 1984).

The U.S. EPA proposed standard 7-day survival, 3-brood reproduction Ceriodaphnia dubia bioassay (U.S. EPA, 1989) has been adapted to a wide range of purposes: Including acute and chronic lethality tests for complex industrial discharges as well as for pure materials. Further, being one of the more sensitive aquatic metazoans, Its data generated have been used to set water quality criteria. In this context, Ceriodaphnia has been suggested for screening toxicity tests for industries; to monitor discharges and for

measuring the efficiency of waste water treatment facilities. Further, in most comparative studies, Ceriodaphnia acute and chronic toxicity sensitivity appears to be greater than that of Daphnia (Winner, 1988; Cowgill et al., 1985). Thus, although with high water and food quality requirements (DeGraeve & Cooney, 1987), Ceriodaphnia has a principal advantage of much shorter life cycle and consequent ability to generate data rapidly.

CHAPTER 4

METHODS

The methodology was implemented in three stages: 1. Treatment application/sample collection. 2. Bioassessment of the percolated soil water. 3. Statistical analysis.

4.1 Treatment Application and Sample Collection

Applications of Milorganite®; Terrene®; MWRA sludge were randomly applied to 1 x 3 m plots with four replicates per treatment at the University of Massachusetts Turfgrass Research Facility in South Deerfield, Mass.[1] The study site contains a mixed stand of 65% Kentucky bluegrass (Poa pratensis (L.) cv. Baron) and 35% perennial ryegrass (Lolium perenne (L.) cv. Manhattan II) growing on a Hadley silt loam soil (coarse-silty, mixed, non-acid, mesic Typic Udifluvent-variant infrequently flooded). The soil had a pH of 6.0, a cation exchange capacity (CEC) of 10.8 and a organic matter content of 1.6 percent.

The applications and sampling frequencies (see Table 4.1; Table 4.2, page 23) were staggered to provide for

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[1]This research was predicated on the design of an ongoing research project funded by MWRA and awarded to Professor Richard Cooper of this department. The intent was to assess the fertilizing effects of various applications of soil amendments on the growth of Kentucky bluegrass and perennial ryegrass mixture. Selected metals and nitrate in soil water samples collected from field were monitored and it was the remaining volume of the samples that was processed with regard to toxicity.

potential differences in rates of vertical transport of sludge components and the extent of residual sludge accumulation in soil beyond the growing season. Water samples were collected approximately every two weeks or after a significant precipitation event. The samples were retrieved from an evacuated (0.33 bar) ceramic lysimeter placed at a depth of 38 cm in each plot to collect soil solution immediately below the effective root zone. The lysimeters were constructed of 6.1 cm x 3.8 cm diameter porous ceramic cups (2 bar standard) (Soil Moisture Equipment Company, Santa Barbara, CA) attached to 3.8 cm diameter x 30.5 cm PVC (poly-vinyl chloride) pipe. Sample collection and processing (bioassay) were conducted from April, 1992 to October, 1993 (see Table 4.2, page 23). This interval would provide the necessary study in time to assess the interaction among the environmental and biological factors.

4.2 Bioassessment

4.2.1 Bioassay Procedure

A culture of Ceriodaphnia dubia, obtained from Tighe & Bond Environmental Laboratory located in Westfield, MA (originally identified and established by Aquatic Research Organisms, Hampton, New Hampshire) were used in this study. Culture maintenance and the assay procedure were employed in accordance with the EPA chronic toxicity test protocol (U.S.

EPA, 1985)(see Table 4.3, page 24), except that 5 ml of testing volume was substituted for the recommended 15 ml for the tests of field samples. Use of this sample size was due to the limited quantity of sample volume available (One of a major consideration prompting adoption of C. dubia as the test species over the larger Daphnia magna and Daphnia pulex). The feasibility for this justified approach is discussed and summarized on section 4.2.3 (see page 20). In cases of sample volume that could not sustain a 7-day daily renewal test, an acute 72-hour toxicity test or 4-day daily renewal chronic toxicity test were conducted (Oris et al., 1991; Masters et al., 1991).

Mortality rates of the test animals were observed daily and recorded as acute toxicity when applicable. The survivors were observed continuously for a total of seven days or total of four days in accordance with the U.S. EPA proposed standard chronic toxicity test protocol (U.S. EPA, 1985). In cases that a 7-day test were conducted, 24-hour old neonates were used to initiate the tests, and for a four-day test, it was 72-hour old animals were used to initiate the test. During the 7-day and 4-day testing periods, the reproduction of three broods were generally observed in the controls. By comparisons of the animals brood sizes and survivals within each treatment, a chronic

toxicity database was established. During the experimental period, the testing solution were renewed daily and held in a 5 ml polyethylene beaker in which one testing animal was sustained and fed daily with the combination of a mixture of yeast-cerophyll-trout chow and single-cell algae: Selenastrum capricornutum. The hardness, alkalinity, pH and DO (dissolved oxygen) levels in the testing solution were routinely monitored.

Each of the three sewage sludge fertilizers was also mixed with the animal culture water (a moderately hard, well buffered, laboratory synthetic water (U.S. EPA, 1985) (see Table 4.4, page 25) to duplicate the concentration of solubles in the field application after an average precipitation (25 mm). The samples were prepared by first establishing a stock solution respectively (15 g sludge per liter of culture water): It was blended in a blender at high speed for 3 minutes; centrifuged for twenty minutes (12,000 G); filtered through a 0.45 uM membrane, and then diluted to 6 concentrations with laboratory synthetic water to be used for the U.S. EPA standard Ceriodaphnia dubia seven-day, daily renewal bioassay in order to establish the EC-50 (effective concentration to 50% of testing animals) values for each sludge fertilizer. This approach, affords insight not only into the toxic potential of each different sludge fertilizer but also through the interaction with soil,

physical, and biological factors. It supports quantitative field assessments into toxicity.

4.2.2 Quality Assurance

According to the U.S. EPA standard Ceriodaphnia dubia testing procedure (U.S. EPA, 1985), the chronic toxicity criteria of 80% survival of the control for the testing animals during testing period had to be met. And the chronic toxicity fecundity criteria also had to be met in that 60% of the surviving Ceriodaphnia in the testing water control, at the end of 7-day testing period, completed the reproduction of three broods with an minimum total of 15 neonates per animal. Sensitivity tests were conducted monthly and immediately prior to the initiations of the experiments to assure the sensitivity of the testing animals. Sodium chloride was used as the reference toxicant in these sensitivity tests following the U.S. EPA proposed standard procedure (U.S. EPA, 1985), and the LC-50 (lethal concentration to 50% of testing animals) values were around 18% that were within laboratory acceptable criteria.

4.2.3 Justification

Because of the limited quantity of field leachate samples available, testing volume of 5 ml instead 15 ml were used to conduct the field toxicity tests. Since the feasibility of this approach could be challenged, the

following experiment was completed before initiation of the field study to ascertain the appropriateness of these tests.

Thirteen replicates in 5 ml and 15 ml culturing medium respectively were observed and compared following the U.S. EPA standard testing procedure (U.S. EPA, 1985). At the end of the testing, survival rates of both treatments were 100% and the reproduction result is listed in Table 4.5 (see page 25). During the observation period, Dissolved Oxygen (DO) and pH level were monitored regularly to be sure that both treatments were within the protocol suggested range. The result indicated successful animals' survival and completion of three-brood reproduction for the 5 ml-treatment, and the reproduction occurrences for all three broods for 5 ml-treatment did not differ from 15 ml-treatment. The average of neonates produced in 5 ml medium was slightly lower than that of in 15 ml medium, probably because of the factors of animal minimum spatial demand and of lesser feeding for the 5 ml-treatment in which animals received half of the amount of the food received by the animals in 15 ml-treatment. This feeding modification for the 5 ml-treatment was needed because of the consideration that over-feeding often would mask the testing toxicity by the chelation between feeding particles and testing ions in the testing solution.

4.3 Statistical Evaluation

The data for EC-50 generation of three sludge fertilizers was analyzed with the computer program: Toxistat 3.3 (Gulley et al., 1991), which was designed specifically for the U.S. EPA standard Ceriodaphnia dubia chronic toxicity tests usage. While treatment difference was tested using ANOVA, treatment means were compared with control using Dunnett's or Bonferroni T-test.

For field toxicity tests, computer program SAS 6.09 (SAS Institute Inc., 1989) was used for the analysis. While Two-way ANOVA was used to test the difference of treatments, means were compared with Duncan's Multiple Range Test.

Table 4.1 Sludge application rate and frequency in the experimental field.

Treatment	Rate[b] Kg/ha	Application schedule					
		1991		1992		1993	
		6-6	8-29	4-23	8-27	4-29	8-30
Milorg.	98	X	X	X	X	X	X
Hag 2N[a]	98	X	X	X	X	X	X
Hag 4N	196	X	X	X	X	X	X
Hag 6N	294	X	X	X	X	X	X
Hag 8N	392	X	X	X	X	X	X
MWRA 2N	98			X	X	X	X
MWRA 3N	147			X	X	X	X
MWRA 4N	196			X	X	X	X

[a]Pounds of N applied/1000 Ft^2.

[b]Kilograms of sludge/Hectare.

Table 4.2 Field sampling schedule.

Field Soil Water Sampling Schedule		
10-21-91	04-28-92	05-28-92
08-06-92	08-18-92	11-11-92
11-24-92	06-08-93	10-08-93

Table 4.3 Summary of test conditions for *Ceriodaphnia* chronic toxicity test.

Test type	Static renewal
Temperature (°C)	25 °C ± 2 °C
Light quality	Ambient laboratory level
Light intensity	Ambient laboratory level
Photoperiod	16 h light, 8 h dark
Test vessel size	5 ml
Test solution volume	5 ml
Renewal of test concentration	Daily
Age of test organisms	Less than 24 h[a]
Number of test organisms per chamber	1
Number of replicate chamber	1
Feeding regime	1 ml food suspension[b]
Aeration	None
Testing duration	7 days[c]
Testing water	Field soil water
Effects measured	Survival & reproduction
Dilution factor	none

[a]Except for the samples of 10-8-93, which 72-hour old animals were used.

[b]Comprised 0.5 ml algae + 0.5 ml YCT mixture.

[c]Except for the samples of 10-8-93, which was only for four days.

Table 4.4 Chemical parameters of animal culture water.

pH	8.1 - 8.2
Hardness ($CaCo_3$ mg/l)	60 - 80
Alkalinity ($CaCo_3$ mg/l)	80 - 85

Table 4.5 Neonates production comparison between 5 ml culture medium and 15 ml culture medium during seven-day period.

Replicates	Treatment	
	15 ml	5 ml
1	25	24
2	26	22
3	20	23
4	28	22
5	19	20
6	21	23
7	21	17
8	21	16
9	18	19
10	24	22
11	27	23
12	27	26
13	26	26
Mean	23.31 $\pm$ 3.43	21.77 $\pm$ 3.06

CHAPTER 5

RESULTS

Data collection during experimental period comprised eight sets of the chronic toxicity tests and one set of acute toxicity test. A two-way ANOVA of overall neonates production at the end of the experiment, comprised of eight sets of chronic toxicity test data, indicated no significant differences among all treatments at $\alpha = 0.05$ level (with p value = 0.0509). Analysis of normalized means based on these eight sets of chronic toxicity tests revealed significant differences in treatments at $\alpha = 0.05$ level: Control was significantly better than all the other sludge treatments (see Table 5.1, page 30), but no statistically significant differences among sludge treatments were observed. Analyses on each individual date indicated that only one time: August 6, 1992 with significant differences among different treatments at $\alpha = 0.05$ level, and two dates (April 28, 1992; October 8, 1993) at $\alpha = 0.1$ level.

5.1 Samples Collected on April 28, 1992

On April 28, 1992, samples were collected five days after a sludge application on April 23, 1992. No statistical difference among treatments at $\alpha = 0.05$ level were observed, but significance at $\alpha = 0.1$ level (p value = 0.0870) were noted. The mean of neonates produced for the

control was 22.5 and the MWRA 2N, MWRA 3N, MWRA 4N means were 3.25, 7.33, 5.00, respectively, lower than all the applications of Hag and Milorganite® (see Table 5.2, page 31).

5.2 Samples Collected on May 28, 1992

On May 28, 1992, about one month after a sludge application, the mean neonates reproduced in sludge treated samples were either better or close to the means of in control (see Table 5.3, page 32) indicates no detectable toxicity.

5.3 Samples Collected on August 6, 1992

On August 6, 1992, more than three months after a sludge application. Statistically significant differences among treatments were observed at $\alpha = 0.05$ level, Hag 8N had the lowest neonates production of 9.25. Except for Milorganite® and MWRA 3N, other sludge treatment means were significantly lower than control (see Table 5.4, page 33).

5.4 Samples Collected on August 18, 1992

On August 18, 1992, two weeks after a sampling (August 6, 1992) with detectable toxicity, the test indicated no significant differences among treatments. Except for three MWRA applications, all other sludge applications yielded

higher neonates production than control (see Table 5.5, page 34).

5.5 Samples Collected on October 21, 1992

On October 21, 1992, two months after sludge application on August 27,1992, Hag 8N had a neonate survival rate of 0 percent in a 72 h acute toxicity test (vs. control samples with a survival rate of 100%) (see Table 5.6, page 35).

5.6 Samples Collected on November 11, 1992

On November 11, 1992, approximately three months after a previous sludge application, no significant differences among treatments were observed. Hag 8N had the lowest mean of neonates of 7.67 (vs. control mean of 19.67). For Milorganite®, the mean of neonates were similar to the means of Hag 2N (with means of 17). MWRA 2N, MWRA 3N and MWRA 4N, with neonates of 10.50, 12.25 and 14.75, respectively, were higher than all Hag treatments except for Hag 2N (see Table 5.7, page 36).

5.7 Samples Collected on November 24, 1992

On November 24, 1992, two weeks after the previous sampling (November 11, 1992), there was no difference among treatments, the neonates production of MWRA 2N and MWRA 3N were higher than control (see Table 5.8, page 37).

5.8 Samples Collected on June 8, 1993

On June 8, 1993, one month after sludge application on April 29, 1993, no differences among treatments were observed (see Table 5.9, page 38).

5.9 Samples Collected on October 8, 1993

On October 8, 1993, a four-day chronic toxicity test with three-day old Ceriodaphnia was used due to insufficient sample volume for seven-day test. The results revealed no significant differences among treatments for neonates at α = 0.05 level, but significance were observed at α = 0.1 level. Hag 4N, Hag 8N with a neonate production means of 0.67, 1.67, vs. 10.33 for the control, were detected. The sampling was conducted one month after sludge application of August 30, 1993 (see Table 5.10, page 39).

Table 5.1. Total neonates produced per *Ceriodaphnia* during seven-day chronic toxicity test period comprised eight sampling dates.

Treatment	Replicates	Mean[1]	Mean[2]
Control	23	19.30 ± 4.64	1.00 ± 0.13[a3]
Milorganite	26	14.58 ± 8.81	0.73 ± 0.42[b]
Hag 2N	24	15.13 ± 7.15	0.78 ± 0.33[b]
Hag 4N	28	13.36 ± 9.38	0.65 ± 0.45[b]
Hag 6N	27	13.00 ± 8.81	0.68 ± 0.43[b]
Hag 8N	28	12.89 ± 9.48	0.63 ± 0.46[b]
MWRA 2N	27	11.30 ± 7.78	0.61 ± 0.40[b]
MWRA 3N	25	14.32 ± 9.36	0.72 ± 0.46[b]
MWRA 4N	26	12.62 ± 8.73	0.65 ± 0.44[b]

[1]Absolute mean, calculated by dividing the total with replicate number.

[2]Normalized mean, calculated by dividing the total of normalized mean of each individual date with total replicate number.

[3]Means with the same letter are not significantly different at the level of $\alpha = 0.05$

Table 5.2 Total neonates produced per *Ceriodaphnia* during seven-day chronic toxicity test period. Sampling date: 4-28-92.

Treatment	Replicates	Mean[1]	Mean[2]
Control	2	22.50 ± 0.71[a3]	1.00 ± 0.00
Milorganite	4	14.00 ± 10.95[abc]	0.62 ± 0.49
Hag 2N	3	19.33 ± 4.93[ab]	0.86 ± 0.22
Hag 4N	3	16.67 ± 12.10[abc]	0.74 ± 0.54
Hag 6N	4	12.25 ± 14.43[abc]	0.54 ± 0.64
Hag 8N	4	14.50 ± 4.36[abc]	0.64 ± 0.19
MWRA 2N	4	3.25 ± 2.50[c]	0.14 ± 0.19
MWRA 3N	3	7.33 ± 7.02[bc]	0.33 ± 0.31
MWRA 4N	4	5.00 ± 8.12[bc]	0.22 ± 0.36

[1]Absolute mean, calculated by dividing total with replicate number.

[2]Normalized mean, calculated by dividing absolute mean with absolute mean of control.

[3]Means with the same letter are not significantly different at the level of $\alpha = 0.10$.

Table 5.3 Total neonates produced per *Ceriodaphnia* during seven-day chronic toxicity test period. Sampling date: 5-28-92.

Treatment	Replicates	Mean[1]	Mean[2]
Control	3	19.67 ± 3.79	1.00 ± 0.19
Milorganite	3	17.00 ± 14.80	0.86 ± 0.75
Hag 2N	3	21.67 ± 1.53	1.10 ± 0.08
Hag 4N	3	23.33 ± 4.04	1.19 ± 0.21
Hag 6N	3	15.67 ± 13.58	0.80 ± 0.69
Hag 8N	4	19.00 ± 13.04	0.97 ± 0.66
MWRA 2N	4	16.50 ± 11.12	0.84 ± 0.57
MWRA 3N	4	18.25 ± 12.23	0.93 ± 0.62
MWRA 4N	3	16.67 ± 14.43	0.85 ± 0.73

[1]Absolute mean, calculated by dividing total with replicate number.

[2]Normalized mean, calculated by dividing absolute mean with absolute mean of control.

Table 5.4 Total neonates produced per *Ceriodaphnia* during seven-day chronic toxicity test period. Sampling date: 8-6-92.

Treatment	Replicates	Mean[1]	Mean[2]
Control	4	22.50 ± 4.20[a3]	1.00 ± 0.19
Milorganite	4	17.50 ± 1.73[ab]	0.78 ± 0.08
Hag 2N	4	13.25 ± 4.99[bc]	0.59 ± 0.22
Hag 4N	4	10.25 ± 4.99[bc]	0.46 ± 0.22
Hag 6N	3	13.00 ± 3.61[bc]	0.58 ± 0.16
Hag 8N	4	9.25 ± 7.50[c]	0.41 ± 0.33
MWRA 2N	4	15.00 ± 2.58[bc]	0.67 ± 0.11
MWRA 3N	4	16.00 ± 3.74[abc]	0.71 ± 0.17
MWRA 4N	3	14.33 ± 4.04[bc]	0.64 ± 0.18

[1]Absolute mean, calculated by dividing total with replicate number.

[2]Normalized mean, calculated by dividing absolute mean with absolute mean of control.

[3]Means with the same letter are not significantly different at level of $\alpha = 0.05$.

Table 5.5 Total neonates produced per *Ceriodaphnia* during seven-day chronic toxicity test period. Sampling date: 8-18-92.

Treatment	Replicates	Mean[1]	Mean[2]
Control	3	21.00 ± 2.65	1.00 ± 0.13
Milorganite	3	22.33 ± 2.08	1.06 ± 0.10
Hag 2N	1	24.00 ± 0.00	1.14 ± 0.00
Hag 4N	4	23.50 ± 2.08	1.12 ± 0.10
Hag 6N	2	23.00 ± 1.41	1.10 ± 0.07
Hag 8N	4	21.50 ± 1.73	1.02 ± 0.08
MWRA 2N	1	20.00 ± 0.00	0.95 ± 0.00
MWRA 3N	2	19.00 ± 9.90	0.90 ± 0.47
MWRA 4N	3	18.00 ± 1.73	0.86 ± 0.08

[1]Absolute mean, calculated by dividing total with replicate number.

[2]Normalized mean, calculated by dividing absolute mean with absolute mean of control.

Table 5.6 *Ceriodaphnia* survival rate after 72-hour acute toxicity test. Sampling date: 10-21-92.

Treatment	Replicate	Survival	Death	Survival%
Control	4	4	0	100
Milorganite	4	1	3	75
Hag 2N	4	1	3	75
Hag 4N	4	2	2	50
Hag 6N	4	1	3	75
Hag 8N	3	0	3	0
MWRA 2N	2	1	1	50
MWRA 3N	4	2	2	50
MWRA 4N	1	1	0	100

Table 5.7 Total neonates produced per *Ceriodaphnia* during seven-day chronic toxicity test period. Sampling date: 11-11-92.

Treatment	Replicates	Mean[1]	Mean[2]
Control	3	19.67 ± 4.16	1.00 ± 0.21
Milorganite	3	17.00 ± 7.00	0.86 ± 0.36
Hag 2N	3	17.00 ± 4.58	0.86 ± 0.23
Hag 4N	4	8.00 ± 8.20	0.41 ± 0.42
Hag 6N	4	8.50 ± 6.03	0.43 ± 0.31
Hag 8N	3	7.67 ± 6.11	0.39 ± 0.31
MWRA 2N	4	10.50 ± 5.26	0.53 ± 0.27
MWRA 3N	4	12.25 ± 11.03	0.62 ± 0.56
MWRA 4N	4	14.75 ± 6.55	0.75 ± 0.33

[1]Absolute mean, calculated by dividing total with replicate number.

[2]Normalized mean, calculated by dividing absolute mean with absolute mean of control.

Table 5.8 Total neonates produced per *Ceriodaphnia* during seven-day chronic toxicity test period. Sampling date: 11-24-92.

Treatment	Replicates	Mean[1]	Mean[2]
Control	3	19.33 ± 3.51	1.00 ± 0.18
Milorganite	4	16.00 ± 7.12	0.83 ± 0.37
Hag 2N	3	13.33 ± 11.93	0.69 ± 0.62
Hag 4N	4	13.25 ± 7.50	0.69 ± 0.39
Hag 6N	4	13.75 ± 9.22	0.71 ± 0.48
Hag 8N	2	17.00 ± 9.90	0.88 ± 0.51
MWRA 2N	2	22.00 ± 0.00	1.14 ± 0.00
MWRA 3N	3	22.00 ± 6.24	1.14 ± 0.32
MWRA 4N	3	13.33 ± 10.02	0.69 ± 0.52

[1]Absolute mean, calculated by dividing total with replicate number.

[2]Normalized mean, calculated by dividing absolute mean with absolute mean of control.

Table 5.9 Total neonates produced per *Ceriodaphnia* during seven-day chronic toxicity test period. Sampling date: 6-8-93.

Treatment	Replicates	Mean[1]	Mean[2]
Control	2	19.50 ± 2.12	1.00 ± 0.11
Milorganite	2	3.00 ± 4.24	0.15 ± 0.22
Hag 2N	3	16.67 ± 1.53	0.85 ± 0.08
Hag 4N	3	10.67 ± 9.71	0.55 ± 0.50
Hag 6N	3	17.67 ± 5.51	0.91 ± 0.28
Hag 8N	4	10.50 ± 12.56	0.54 ± 0.64
MWRA 2N	4	7.25 ± 8.62	0.37 ± 0.44
MWRA 3N	3	12.67 ± 12.06	0.65 ± 0.62
MWRA 4N	3	14.67 ± 13.05	0.75 ± 0.67

[1]Absolute mean, calculated by dividing total with replicate number.

[2]Normalized mean, calculated by dividing absolute mean with absolute mean of control.

Table 5.10 Total neonates produced per *Ceriodaphnia* during four-day chronic toxicity test period. Sampling date: 10-8-93.

Treatment	Replicates	Mean[1]	Mean[2]
Control	3	10.33 ± 0.58[a3]	1.00 ± 0.06
Milorganite	3	4.67 ± 4.16[abc]	0.45 ± 0.40
Hag 2N	4	5.50 ± 3.87[abc]	0.53 ± 0.37
Hag 4N	3	0.67 ± 0.58[c]	0.06 ± 0.06
Hag 6N	4	7.00 ± 4.24[abc]	0.68 ± 0.41
Hag 8N	3	1.67 ± 2.08[bc]	0.16 ± 0.20
MWRA 2N	4	7.75 ± 2.22[ab]	0.75 ± 0.21
MWRA 3N	2	4.00 ± 1.41[abc]	0.39 ± 0.14
MWRA 4N	3	6.00 ± 4.58[abc]	0.58 ± 0.44

[1]Absolute mean, calculated by dividing total with replicate number.

[2]Normalized mean, calculated by dividing absolute mean with absolute mean of control.

[3]Means with the same letter are not significantly different at the level of $\alpha = 0.10$.

CHAPTER 6

DISCUSSION

6.1 Bioassay Results

Analysis of normalized means of treatments comprised of eight sample collections ranging from April 28, 1992 to October 8, 1993, yielded significant difference in treatments. The control mean was significantly higher than all of the sludge treatments (see Table 5.1, page 30). Since the chemistry of the water samples collected varied with soil temperature, rainfall, frequency of sludge application, it is necessary to analyze the data with each sampling event rather than the overall impact.

Statistically, the date of August 6, 1992 showed that the control mean was significantly higher than sludge treatments (see Table 5.4, page 33). Of the eight dates of data collection, the controls of five dates (April 28, 1992; August 6, 1992; November 11, 1992; June 8, 1993; October 8, 1993) had the highest means. The means of the eight sludge treatments varied significantly among different sampling dates, while the control means remained constant during the entire research period (see Figure 6.1, page 51). Furthers, the samples collected on October 21, 1992, after a 72-hour static-daily-renewal acute toxicity test, showed that the control had a survival rate of 100 percent, and Hag 8N had 0 percent survival rate (see Table 5.6, page 35).

Analysis of the chronic toxicity test data (comprising eight sampling dates), based on original means and normalized means, indicated that the control had the overall highest means (19.30, 1.00, respectively) among all treatments. Hag 2N (15.13, 0.78) and Milorganite® (14.58, 0.73) were ranked the second and third highest means after controls. The treatment of Hag 8N (12.89, 0.63) yielded the second lowest means (see Table 5.1, page 30). Considering the means of the four different Hag's application levels, a dose-response relationship was discernible. Although the MWRA 2N generated the lowest overall mean, it was relatively close to the mean of MWRA 4N which had a mean relatively lower than MWRA 3N.

Although statistics failed to show significant differences among the various sludge treatments due to the limited replicates and non-homogeneous variance of means, the data did reveal some interesting trends. According to the nine sampling events (eight of chronic toxicity tests and one of acute toxicity), the degradation and leaching impact of applied sewage sludge in the field could be detected as early as a week to as late as three months after its application. The means of Milorganite® (14.58, 0.73) were lower than Hag 2N (15.13, 0.78), while the means of MWRA 2N, MWRA 3N and Hag 8N were the lowest three of the treatments means (see Table 5.1, page 30). It is

interesting that MWRA was applied in the field one year later than Hag and Milorganite®.

Considering the fact that the treatments detected toxicity albeit inconsistently, suggests the possible downward migration of the toxicant and thus potential contamination to the groundwater. Among those factors that could synergize the toxicity of the applied sludges are: Hardness, alkalinity, pH, nitrate, metals, and organic pollutants.

6.2 Toxicology

Variance of hardness could confound the toxicity evaluated. Generally, toxicity decreases with increasing hardness. The samples of August 6, 1992; August 18, 1992; November 11, 1992; November 24, 1992 (n= 103) yielded a hardness ranging from 48 to 140, with a mean of 84 mg/l as $CaCo_3$. The number of neonates reproduced was slightly correlated with hardness (see Figure 6.2, page 52). According to Cowgill and Milazzo (1990, 1991), Ceriodaphnia reproduction is chronically sensitive to hardness. The EC-50 (effective concentration for 50% of the population) investigated was 38 mg $CaCo_3$/l with a NOEL (no observable effect level) of 72 mg $CaCo_3$/l. Animals showed sign of stress only while being exposed to the hardness of under 9 mg $CaCo_3$/l and above 650 mg $CaCo_3$/l.

The hardness levels of the field samples were well within the ideal Ceriodaphnia testing range and also close to the hardness of the culturing water (80 - 85 mg $CaCo_3$/l) in which animals were raised pre-experiments (see Table 6.1, page 53). Thus it is unlikely that hardness is a contributing factor to toxicity. Based on the data of August 6,1992, the control hardness ranged from 65 to 104 (with average neonates reproduction of 22.5) while the Hag 8N ranged from 53 to 114 mg $CaCo_3$/l (with average neonates reproduction of 9.25) (see Table 6.2, page 54). Such similar hardness ranges but difference of means indicated that the difference of reproduction was not caused by the factor of hardness.

Ceriodaphnia was reported (Cowgill & Milazzo, 1991) to be more sensitive to alkalinity than hardness. Based on the data of October 8, 1993, an alkalinity ranged from 29 to 34 mg $CaCo_3$/l (see Table 6.3, page 55), according to Cowgill and Milazzo, should not impose a measurable impact on Ceriodaphnia reproduction within this low range.

Analysis of pH based on the samples of May 28, 1992; August 6, 1992; November 11, 1992; November 24, 1992 and June 8, 1992 (n = 114) revealed a rang of 6.33 to 8.41, with mean of 7.49 (see Table 6.4, page 56). There was no correlation between the pH and the number of neonates reproduced (see Figure 6.3, page 57). According to the study by Belanger and Cherry (1990), Ceriodaphnia

reproduction and mortality would not be impaired between pH range of 6.14-8.99 regardless of pH acclimation history.

Taub and Dollar (1964) reported that Daphnia were sensitive to nitrate. Therefore, among treatments, correlation between average reproduction and average nitrate concentration were analyzed. Based on the data of April 28, 1992; August 6, 1992; August 18, 1992; November 11, 1992, the outcome didn't indicate any correlation (see Figure 6.4, page 58). However, on August 6, 1992, Hag 8N and Hag 6N had the highest nitrate concentrations (4 mg/l; 4.2 mg/l respectively) (see Table 6.2, page 54) together with significant lower reproduction than most of the other treatments. However, on April 28, 1992, MWRA 2N; 3N; 4N had the lowest NO_3 concentrations and also lowest reproduction (Table 5.2; Table 6.5, page 31; 59).

Nitrate is a convenient indicator for nitrogen available to plants but it only comprises a very small portion of the total nitrogen in the soil. Unlike urea, sewage sludge contains nitrogen mostly in organic form (Sommers, 1977) which must be mineralized into inorganic ammonium before being utilized by plants. In the soil nitrogen cycle, it is through the microbial nitrification process that ammonia converts to nitrite and nitrate. When the nitrification process is impeded, ammonia may accumulate. Total ammonia is the measurement of NH_3 (un-ionized) and NH_4^+ (ionized) combined and a pH increase from

7 to 8 at the temperature of 25°C results in a roughly tenfold increase of the concentration of NH_3 (Thurston et al., 1978). Of the nitrogen species, un-ionized ammonia is the most toxic form, nitrite secondly and nitrate is the least. Gersich and Hopkins (1986) reported a LC50-48h (Lethal concentration to 50% of the population at the end of 48 hours) of 2.94 mg NH_3 mg-N and a MATC (Maximum acceptable toxicant concentration) of 0.60 mg/l NH_3-N to Daphnia magna. Anthonisen et al. (1976) reported that un-ionized ammonia at concentrations of 0.1 - 1 mg/l NH_3-N inhibited nitrobactors. This impeded the conversion of nitrite to nitrate resulting in the accumulation of nitrite and ammonia.

Furthermore, the toxicity of ammonia is affected synergistically by the presence of other pollutants. Brown et al. (1969) tested a mixture of three pollutants: ammonia, zinc and phenol and observed the toxicity of the mixture was greater than the sum of the individual toxicity of each of these three pollutants. The synergistic effect of the compound formed by ammonia and chlorine was also reported (Kaniewska-Prus, 1982). There is evidence that a combination of ammonia and copper, zinc, nitrate, are additive, except when ammonia to nitrate ratios are very low (Herbert & Vandyke, 1964; Ministry of Technology, U.K., 1962; Rubin & Elmaraghy, 1977). The fact of the elevated nitrate concentration and elevated pH, occurred with the lowest Ceriodaphnia reproduction on August 6, 1992 suggested

that these factors may be operative (see Table 6.2, page 54). There are, as well, several organic compounds which occur commonly in industrial waste (thus possibly in sewage sludge) which inhibit the nitrification process (Hockenbury & Grady, 1977). Thus although lacking the data of nitrite and ammonia in this study (due to insufficient volume of samples), it is reasonable taking into consideration that these two nitrogen forms possibly play roles in detected toxicity. Especially when also taking the factor of rainfall into account, of these nine sampling dates, August 6, 1992 had a 26 mm rainfall six days before sampling and it might have had generated a reduced environment and more solubles pollutants and nitrate.

It was reported that the chronic effects of metal interactions may be additive for Daphnids reproduction and mortality (Spehar & Fiandt, 1986; Biesinger et al., 1986). Therefore, the toxicant quotient approach to evaluate the joint toxicity of the metals mixture was used in this study.

This approach is based on the rationale that the joint toxicity of metals to aquatic organisms can be predicted assuming the simple addition of the proportional contribution of each toxicant. Selection of these six metals was due to the fact that these metals are commonly found together as mixture in sewage and industrial wastes (U.S. EPA, 1980).

The Toxic quotient was first calculated by dividing the metal concentration found in the sample with Ceriodaphnia chronic MATC if available or otherwise the chronic EC-50 of Daphnia magna (Spehar & Fiandt, 1986; Kszos et al., 1992; Khangarot & Ray, 1989). Thus the measured concentration of cadmium in testing samples is divided by 2.2 ug/ml; chromium by 63 ug/ml; copper by 45 ug/ml; lead by 52 ug/ml; nickel by 15 ug/ml; zinc by 560 ug/ml (see Table A.1..9, page 64..72). This was followed by summing each individual metal quotient in each treatment (see Table 6.6, page 60). It is this value that used to evaluate the mixture of six metals interaction to animal reproduction. Examining the date of August 6, 1992 alone (see Table 6.2, page 54), control had the highest toxic quotient (3.18) and highest reproduction, which also occurred similarly on the date of May 28, 1992; November 24, 1992 (see Table 6.6, page 60). The result suggested that these metals were either not completely biologically available or simply posed no impact due to very trace amount (total toxic quotient ranges 0.4 to 12.6) and antagonism.

Lima et al. (1984) reported that the presence of food decreases the toxicity of metals to Daphanids. Especially with low metal concentration, food presence might under-evaluate the toxicity by chelating with metal ions (U.S. EPA, 1985). Anderson and Weber (1975) also suggested discrepancies of joint action of metals in organisms due to

water quality characteristics. Generally, low pH, high hardness, alkalinity and organic content decreases the toxicity of metals by decreasing bio-availability (Oikari et al., 1992; Belanger & Cherry, 1990; Stackhouse & Benson, 1989).

Sewage sludge fertilizer potentially has high amounts of metals (see Table 6.7, page 61), but in reality, the bioavailability of metals applied to is limited by: Water solubility, exchangeability, adsorbtion, chelation and precipitation as metal oxides. Besides, sewage metals applied in field have been reported to be concentrated by earthworm (Hartenstein et al., 1981). Furthermore, Mackintosh (1993) reported that maximal metal leaching from Hag 8N was similar to control (non-fertilized plots) throughout year of 1991 and 1992. This study indicated that for sludge application, the toxicity did not result from metals leaching.

The U.S. EPA conducted an investigation (1988) that detected PAHs, DDT metabolites, phenol, chlordane, PCB, Aroclor 1248 in distribution/marketing municipal sludge samples. Also mutagenic chemical migration below the sludge incorporation zone was found (U.S. EPA, 1988). Low-molecular-weight PAHs such as naphthalene and phenanthrene have higher aqueous solubilities and are less lipophilic than the high molecular weight compounds. They can serve as sole carbon sources for soil microbes, where the multi-

ringed species may be degraded in the environment by slower co-oxidation processes (Keck et al., 1989). There is a broad inverse relationship between the rate of biodegradation and the number of benzene rings (Bossert & Cerniglia, 1987). This is largely a function of changes in the aqueous solubility, bio-availability, and structural stability of PAHs through the compound group. Field-based studies have indicated that a residual fraction composed of the high-molecular weight PAHs remains in the soil many years subsequent to sludge application (Wild et al, 1990). PAHs in sewage sludge are likely to be adsorbed onto sludge particulates, thus limiting bio-availability. It is also well documented that sludge additions enhance soil adsorption capacity. PAHs may be more susceptible to degradation because sludge application to soil causes a period of enhanced microbial activity due to the addition of an available substrate and essential nutrients.

Munoz and Tarazona (1993) using Daphnia for PAHs toxicity tests indicated that toxicity of PAHs increases with increasing molecular size until the 4-5-ring molecules are reached. Studies on the effects of mixtures revealed that some organic compounds may even be more than additive on a chronic basis.

The difference between sludge treatments and control is presented in this study, however, the correlation between toxicity to Ceriodaphnia and chemical concentrations in the

leachate is lacking. It might have been due to not sampling and testing the soil water samples over a sufficient number of dates or that the target chemical measurements were not the cause. Also the toxicity of a specific chemical may be changed by the factors of the presence of chemicals complexing ligands, or physical adsorption that alter the biological availability.

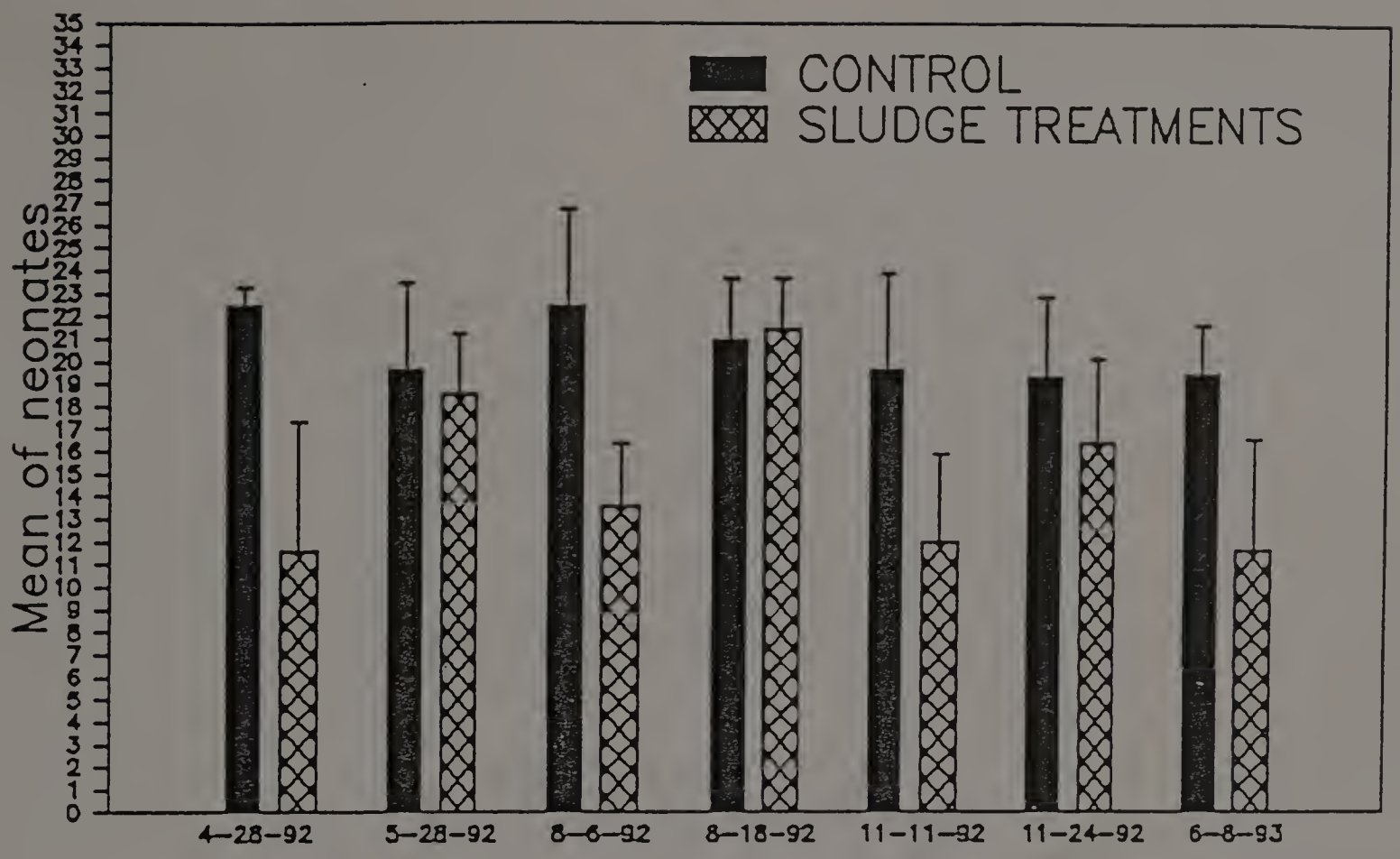

Figure 6.1 Neonates comparison between control and sludge treatments.

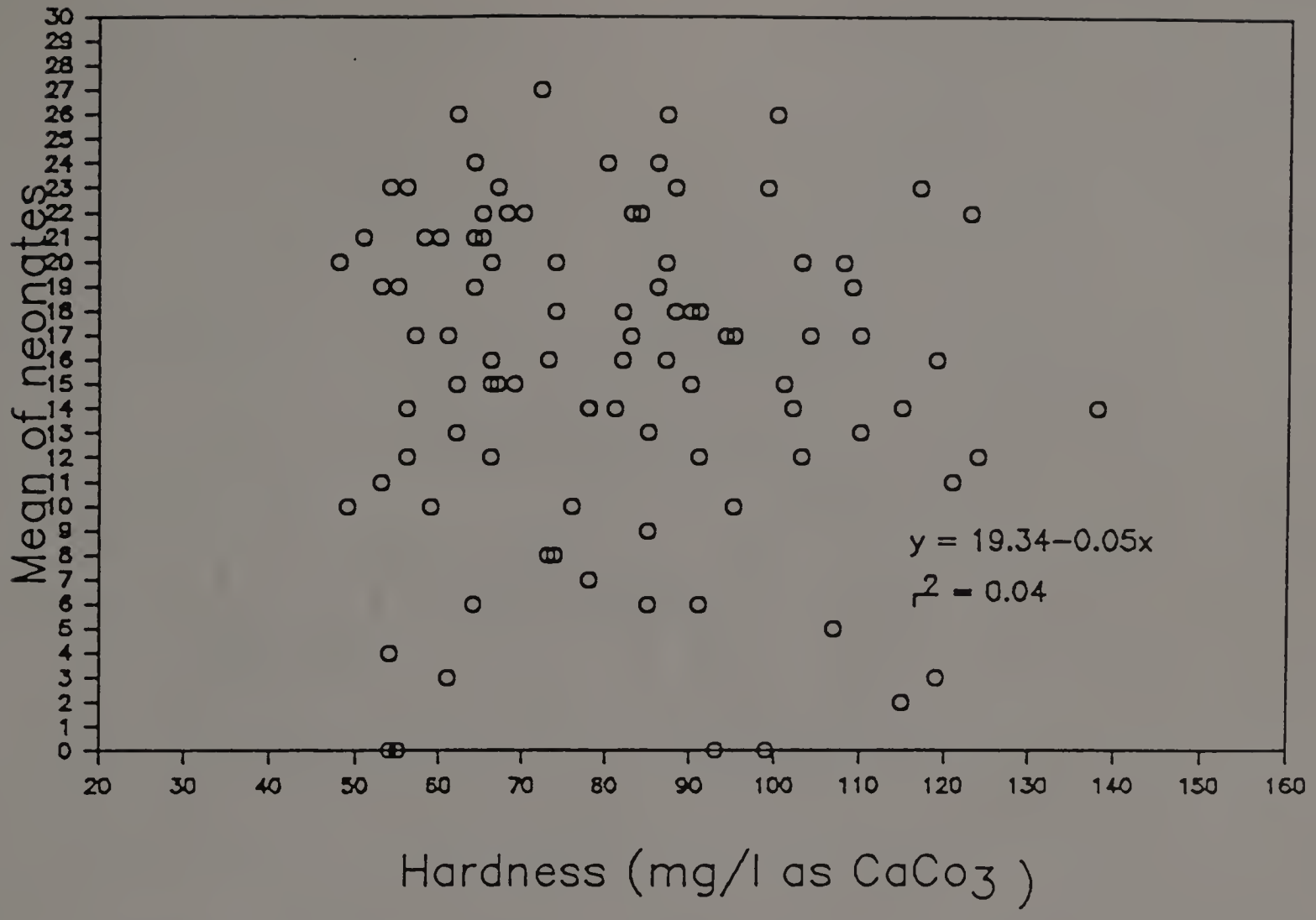

Figure 6.2 Correlation between reproduction and hardness.

Table 6.1 Hardness (mg/l as $CaCo_3$) of field treatments for selected dates.

Treatment	Sampling dates			
	8-6-92	8-18-92	11-11-92	11-24-92
Control	80	86	62	63
Milorg.	82	101	72	82
Hag 2N	78	-	63	68
Hag 4N	87	61	67	63
Hag 6N	90	-	130	79
Hag 8N	96	98	85	73
MWRA 2N	100	87	79	87
MWRA 3N	121	102	80	82
MWRA 4N	82	74	74	80

Table 6.2 Chemical parameters for the samples of date: 8-6-92.

Treatment	Neonates	Hardness $CaCo_3$ mg/l	pH	Nitrate mg/l	Metal[a] quotient
Control	22.5	80	8.19	0.1	3.18
Milorg.	17.5	82	8.22	0.8	1.62
Hag 2N	13.25	78	8.31	0.8	0.89
Hag 4N	10.25	87	8.25	0.7	2.51
Hag 6N	13	91	8.22	4.2	0.99
Hag 8N	9.25	96	8.27	4.0	0.99
MWRA 2N	15	100	8.33	0.4	0.79
MWRA 3N	16	121	8.32	0.44	0.71
MWRA 4N	14.33	82	8.33	0.95	0.68

[a]From Table 6.6.

Table 6.3 Chemical parameters for the samples of date: 10-8-93.

Treatment	Neonates	Hardness	Alkalinity	Conduc-tivity	pH
Control	10.33[a]	60[b]	52[c]	200[d]	7.82
Milorg.	4.67	78	34	145	7.90
Hag 2N	5.50	71	44	157	7.67
Hag 4N	0.67	98	35	217	7.68
Hag 6N	7.00	177	29	375	7.40
Hag 8N	1.67	99	34	200	7.70
MWRA 2N	7.75	84	62	210	7.89
MWRA 3N	4.00	129	55	320	7.74
MWRA 4N	6.00	99	52	230	7.68

[a]Results of four-date chronic toxicity test.

[b]mg/l as $CaCo_3$.

[c]mg/l as $CaCo_3$.

[d]siemens (mhos).

Table 6.4 pH of field treatments for selected dates.

Treatment	Sampling dates				
	5-28-92	8-6-92	11-11-92	11-24-92	6-8-92
Control	7.53	8.19	7.00	7.85	6.61
Milorg.	7.53	8.22	7.66	7.85	6.63
Hag 2N	7.53	8.31	-	7.86	6.69
Hag 4N	7.51	8.25	7.01	7.72	6.60
Hag 6N	7.53	8.22	-	7.47	6.66
Hag 8N	7.59	8.27	-	7.72	6.77
MWRA 2N	7.44	8.33	6.89	7.69	6.70
MWRA 3N	7.50	8.32	7.27	7.82	6.62
MWRA 4N	7.55	8.33	6.92	7.74	6.61

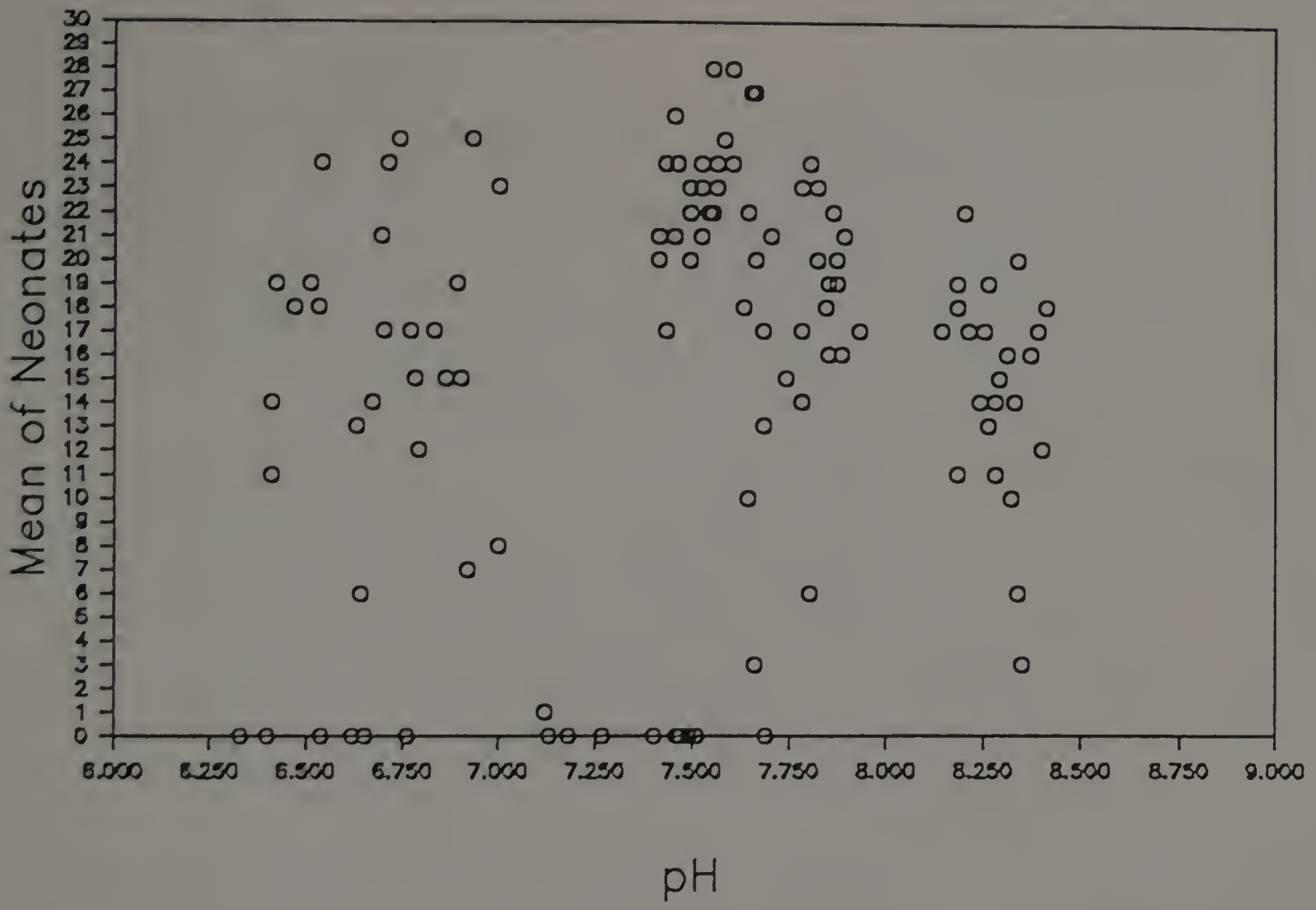

Figure 6.3 Correlation between reproduction and pH.

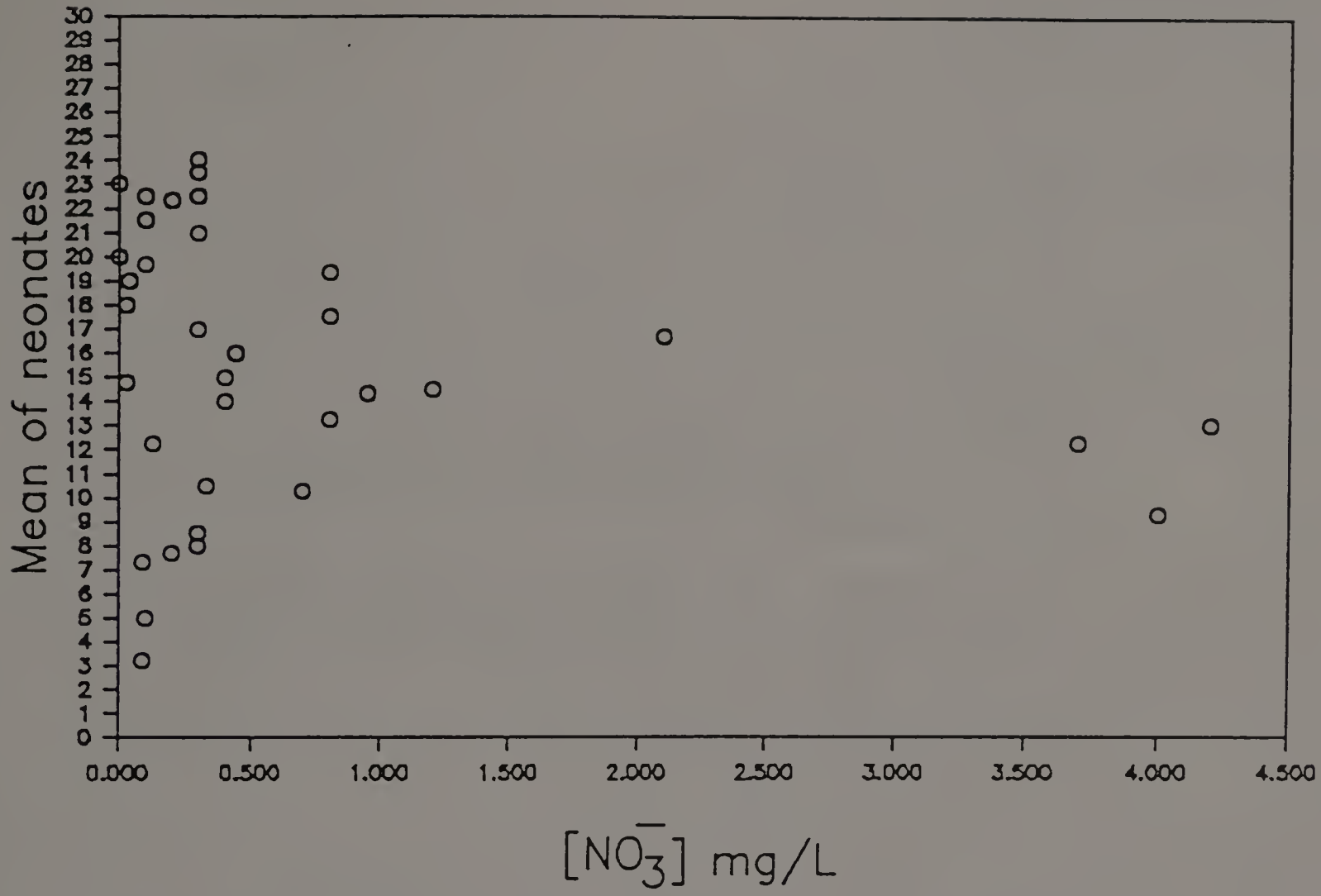

Figure 6.4 Correlation between reproduction and nitrate.

Table 6.5 Nitrate concentration (mg/l) of field treatments for selected dates.

Treatment	Sampling dates			
	4-28-92	8-6-92	8-18-92	11-11-92
Control	0.30	0.10	0.30	0.10
Milorg.	0.40	0.80	0.20	0.30
Hag 2N	0.80	0.80	0.30	0.30
Hag 4N	2.10	0.70	0.30	0.30
Hag 6N	3.70	4.20	0.00	-
Hag 8N	1.20	4.00	0.10	0.20
MWRA 2N	0.09	0.40	0.00	0.33
MWRA 3N	0.09	0.40	0.04	0.13
MWRA 4N	0.10	0.95	0.03	0.03

Table 6.6 Metals quotients summary for field treatments of three dates.

Date	Treatment	Toxic quotient						Total
		Cd	Cr	Cu	Ni	Pb	Zn	
5-28-92	Control	0.14	0.00	0.16	0.00	1.92	0.11	2.33
	Milorganite	2.27	0.06	0.22	0.67	2.50	0.14	5.86
	Hag 2N	2.27	0.13	0.44	0.00	2.69	0.14	5.67
	Hag 4N	3.18	0.21	0.44	1.31	4.81	0.11	10.06
	Hag 6N	2.70	0.21	0.32	1.33	4.42	0.16	9.14
	Hag 8N	1.80	0.11	0.67	1.33	4.23	0.14	8.28
	MWRA 2N	4.09	0.37	0.44	1.33	3.27	0.23	9.73
	MWRA 3N	4.50	0.43	0.89	2.67	4.23	0.14	12.86
	MWRA 4N	2.70	0.32	0.89	2.00	6.54	0.11	12.56
8-6-92	Control	2.27	0.02	0.44	0.00	0.38	0.07	3.18
	Milorganite	0.00	0.08	0.44	0.00	0.96	0.14	1.62
	Hag 2N	0.00	0.00	0.44	0.00	0.38	0.07	0.89
	Hag 4N	1.36	0.03	0.67	0.00	0.38	0.07	2.51
	Hag 6N	0.00	0.00	0.67	0.00	0.19	0.13	0.99
	Hag 8N	0.00	0.00	0.67	0.00	0.19	0.13	0.99
	MWRA 2N	0.00	0.08	0.22	0.00	0.38	0.11	0.79
	MWRA 3N	0.00	0.03	0.44	0.00	0.19	0.05	0.71
	MWRA 4N	0.00	0.03	0.22	0.00	0.38	0.05	0.68
11-24-92	Control	0.45	0.00	0.22	0.00	0.00	0.14	0.81
	Milorganite	0.00	0.05	0.22	0.00	0.00	0.13	0.40
	Hag 2N	1.80	0.03	0.44	0.19	0.00	0.07	2.53
	Hag 4N	1.80	0.03	0.44	0.00	0.19	0.18	2.64
	Hag 6N	2.70	0.02	0.22	0.00	0.19	0.30	3.43
	Hag 8N	0.00	0.06	0.44	0.00	0.19	0.16	0.85

Continued, next page

Table 6.6 (continued)

Date	Treatment	Toxic quotient						Total
		Cd	Cr	Cu	Ni	Pb	Zn	
11-24-92	MWRA 2N	0.00	0.00	0.44	0.00	0.00	0.58	1.02
	MWRA 3N	4.09	0.13	0.67	0.67	1.15	0.13	6.17
	MWRA 4N	1.82	0.06	0.89	0.67	1.15	0.22	4.81

Table 6.7 Selected metals content in three field applied sewage sludge fertilizer.

Element[a]	Milorganite® ppm	Terrene® ppm	MWRA-Boston ppm
Cadmium	5	1	6
Chromium	3216	25	83
Copper	222	289	691
Lead	144	39	277
Nickel	38	8	37
Zinc	448	348	917

[a]Results of ICP analysis.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

Some conclusions and recommendations could be drawn from this research regarding the impact of commercial sewage sludge fertilizer application for field turf management.

7.1 Conclusions

1. The overall impact of sewage sludge fertilizer application to the soil did impose an impact on the reproduction results of Ceriodaphnia dubia.
2. Ceriodaphnia dubia standard bioassay for aquatic systems offers a feasible method for bio-monitoring terrestrial soil leachate.

7.2 Recommendations

1. Long-term and frequent samples collections should be conducted to complete the data base.
2. Besides metals, organic components should be analyzed targets.

APPENDIX

METAL TOXIC QUOTIENTS CALCULATIONS

Table A.1 Six metals concentrations: Calculated toxic quotients for controls of three sampling dates.

Chemicals	Sampling date	Concentration (ppb)	Chronic[a] value (ppb)	Toxic[b] quotient
Cadmium	5-28-92	0.3	2.2	0.14
	8-6-92	5		2.27
	11-24-92	1		0.45
Chromium	5-28-92	0	63	0.00
	8-6-92	1		0.02
	11-24-92	0		0.00
Copper	5-28-92	10	45	0.16
	8-6-92	20		0.44
	11-24-92	10		0.22
Lead	5-28-92	100	52	1.92
	8-6-92	20		0.38
	11-24-92	0		0.00
Nickel	5-28-92	0	15	0.00
	8-6-92	0		0.00
	11-24-92	0		0.00
Zinc	5-28-92	60	560	0.11
	8-6-92	40		0.07
	11-24-92	80		0.14

[a]Chronic values, otherwise lowest observed effect concentrations from literature.

[b]Toxic quotient = $\frac{\text{Concentration}}{\text{Chronic value}}$

Table A.2 Six metals concentrations: Calculated toxic quotients for Milorganite of three sampling dates.

Chemicals	Sampling date	Concentration (ppb)	Chronic[a] value (ppb)	Toxic[b] quotient
Cadmium	5-28-92	5	2.2	2.27
	8-6-92	0		0.00
	11-24-92	0		0.00
Chromium	5-28-92	4	63	0.06
	8-6-92	5		0.08
	11-24-92	3		0.05
Copper	5-28-92	10	45	0.22
	8-6-92	20		0.44
	11-24-92	10		0.22
Lead	5-28-92	130	52	2.50
	8-6-92	50		0.96
	11-24-92	0		0.00
Nickel	5-28-92	10	15	0.67
	8-6-92	0		0.00
	11-24-92	0		0.00
Zinc	5-28-92	80	560	0.14
	8-6-92	80		0.14
	11-24-92	70		0.13

[a]Chronic values, otherwise lowest observed effect concentrations from literature.

[b]Toxic quotient = $\frac{\text{Concentration}}{\text{Chronic value}}$

Table A.3 Six metals concentrations: Calculated toxic quotients for Hag 2N of three sampling dates.

Chemicals	Sampling date	Concentration (ppb)	Chronic[a] value (ppb)	Toxic[b] quotient
Cadmium	5-28-92	5	2.2	2.27
	8-6-92	0		0.00
	11-24-92	4		1.80
Chromium	5-28-92	8	63	0.13
	8-6-92	0		0.00
	11-24-92	2		0.03
Copper	5-28-92	20	45	0.44
	8-6-92	20		0.44
	11-24-92	20		0.44
Lead	5-28-92	140	52	2.69
	8-6-92	20		0.38
	11-24-92	10		0.19
Nickel	5-28-92	0	15	0.00
	8-6-92	0		0.00
	11-24-92	0		0.00
Zinc	5-28-92	80	560	0.14
	8-6-92	40		0.07
	11-24-92	40		0.07

[a]Chronic values, otherwise lowest observed effect concentrations from literature.

[b]Toxic quotient = $\frac{\text{Concentration}}{\text{Chronic value}}$

Table A.4 Six metals concentrations: Calculated toxic quotients for Hag 4N of three sampling dates.

Chemicals	Sampling date	Concentration (ppb)	Chronic[a] value (ppb)	Toxic[b] quotient
Cadmium	5-28-92	7	2.2	3.18
	8-6-92	3		1.36
	11-24-92	4		1.80
Chromium	5-28-92	13	63	0.21
	8-6-92	2		0.03
	11-24-92	2		0.03
Copper	5-28-92	20	45	0.44
	8-6-92	30		0.67
	11-24-92	20		0.44
Lead	5-28-92	250	52	4.81
	8-6-92	20		0.38
	11-24-92	10		0.19
Nickel	5-28-92	20	15	1.33
	8-6-92	0		0.00
	11-24-92	0		0.00
Zinc	5-28-92	60	560	0.11
	8-6-92	40		0.07
	11-24-92	100		0.18

[a]Chronic values, otherwise lowest observed effect concentrations from literature.

[b]Toxic quotient = $\frac{\text{Concentration}}{\text{Chronic value}}$

Table A.5 Six metals concentrations: Calculated toxic quotients for Hag 6N of three sampling dates.

Chemicals	Sampling date	Concentration (ppb)	Chronic[a] value (ppb)	Toxic[b] quotient
Cadmium	5-28-92	6	2.2	2.7
	8-6-92	0		0.00
	11-24-92	6		2.7
Chromium	5-28-92	13	63	0.21
	8-6-92	0		0.00
	11-24-92	1		0.02
Copper	5-28-92	20	45	0.32
	8-6-92	30		0.67
	11-24-92	10		0.22
Lead	5-28-92	230	52	4.42
	8-6-92	10		0.19
	11-24-92	10		0.19
Nickel	5-28-92	20	15	1.33
	8-6-92	0		0.00
	11-24-92	0		0.00
Zinc	5-28-92	90	560	0.16
	8-6-92	70		0.13
	11-24-92	170		0.30

[a]Chronic values, otherwise lowest observed effect concentrations from literature.

[b]Toxic quotient = $\frac{\text{Concentration}}{\text{Chronic value}}$

Table A.6 Six metals concentrations: Calculated toxic quotients for Hag 8N of three sampling dates.

Chemicals	Sampling date	Concentration (ppb)	Chronic[a] value (ppb)	Toxic[b] quotient
Cadmium	5-28-92	4	2.2	1.8
	8-6-92	0		0.00
	11-24-92	0		0.00
Chromium	5-28-92	7	63	0.11
	8-6-92	0		0.00
	11-24-92	4		0.06
Copper	5-28-92	30	45	0.67
	8-6-92	30		0.67
	11-24-92	20		0.44
Lead	5-28-92	220	52	4.23
	8-6-92	10		0.19
	11-24-92	10		0.19
Nickel	5-28-92	20	15	1.33
	8-6-92	0		0.00
	11-24-92	0		0.00
Zinc	5-28-92	80	560	0.14
	8-6-92	70		0.13
	11-24-92	90		0.16

[a]Chronic values, otherwise lowest observed effect concentrations from literature.

[b]Toxic quotient = $\frac{\text{Concentration}}{\text{Chronic value}}$

Table A.7 Six metals concentrations: Calculated toxic quotients for MWRA 2N of three sampling dates.

Chemicals	Sampling date	Concentration (ppb)	Chronic[a] value (ppb)	Toxic[b] quotient
Cadmium	5-28-92	9	2.2	4.09
	8-6-92	0		0.00
	11-24-92	0		0.00
Chromium	5-28-92	23	63	0.37
	8-6-92	5		0.08
	11-24-92	0		0.00
Copper	5-28-92	20	45	0.44
	8-6-92	10		0.22
	11-24-92	20		0.44
Lead	5-28-92	170	52	3.27
	8-6-92	20		0.38
	11-24-92	0		0.00
Nickel	5-28-92	20	15	1.33
	8-6-92	0		0.00
	11-24-92	0		0.00
Zinc	5-28-92	130	560	0.23
	8-6-92	60		0.11
	11-24-92	80		0.58

[a]Chronic values, otherwise lowest observed effect concentrations from literature.

[b]Toxic quotient = $\frac{\text{Concentration}}{\text{Chronic value}}$

Table A.8 Six metals concentrations: Calculated toxic quotients for MWRA 3N of three sampling dates.

Chemicals	Sampling date	Concentration (ppb)	Chronic[a] value (ppb)	Toxic[b] quotient
Cadmium	5-28-92	10	2.2	4.50
	8-6-92	0		0.00
	11-24-92	9		4.09
Chromium	5-28-92	27	63	0.43
	8-6-92	2		0.03
	11-24-92	8		0.13
Copper	5-28-92	40	45	0.89
	8-6-92	20		0.44
	11-24-92	30		0.67
Lead	5-28-92	220	52	4.23
	8-6-92	10		0.19
	11-24-92	60		1.15
Nickel	5-28-92	40	15	2.67
	8-6-92	0		0.00
	11-24-92	10		0.67
Zinc	5-28-92	80	560	0.14
	8-6-92	30		0.05
	11-24-92	70		0.13

[a]Chronic values, otherwise lowest observed effect concentrations from literature.

[b]Toxic quotient = $\frac{\text{Concentration}}{\text{Chronic value}}$

Table A.9 Six metals concentrations: Calculated toxic quotients for MWRA 4N of three sampling dates.

Chemicals	Sampling date	Concentration (ppb)	Chronic[a] value (ppb)	Toxic[b] quotient
Cadmium	5-28-92	6	2.2	2.70
	8-6-92	0		0.00
	11-24-92	4		1.82
Chromium	5-28-92	20	63	0.32
	8-6-92	2		0.03
	11-24-92	4		0.06
Copper	5-28-92	40	45	0.89
	8-6-92	10		0.22
	11-24-92	40		0.89
Lead	5-28-92	340	52	6.54
	8-6-92	20		0.38
	11-24-92	60		1.15
Nickel	5-28-92	30	15	2.00
	8-6-92	0		0.00
	11-24-92	10		0.67
Zinc	5-28-92	60	560	0.11
	8-6-92	30		0.05
	11-24-92	120		0.22

[a]Chronic values, otherwise lowest observed effect concentrations from literature.

[b]Toxic quotient = $\frac{\text{Concentration}}{\text{Chronic value}}$

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