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16. Abstract

The impacts of stormwater runoff from Washington State freeways on aquatic ecosystems were investigated through a series of bioassays utilizing algae, zooplankton and fish. Algae and zooplankton were adversely affected by the soluble fraction of the runoff, while suspended solids caused high mortalities of rainbow trout fry. In addition, BOD_5 values similar to those reported in the stormwater literature were measured; however, there were indications that results were influenced by toxicity to microbial populations.

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ABSTRACT

The impacts of stormwater runoff from Washington State freeways on aquatic ecosystems was investigated through a series of bioassays utilizing algae, zooplankton and fish. Algae and zooplankton were adversely affected by the soluble fraction of the runoff, while suspended solids caused high mortalities of rainbow trout fry. In addition, BOD_5 values similar to those reported in the stormwater literature were measured; however, there were indications that results were influenced by toxicity to microbial populations.

TABLE OF CONTENTS

PREFACE

The following pages list reports either completed or in process on the basis of the Highway Runoff Water Quality research results. Following the listing is an order blank to obtain a complementary copy of any report in the series.

Highway Runoff Water Quality Reports

Report No. 1. Horner, R.R. and E.B. Welch, "Effects of Velocity and Nutrient Alterations on Stream Primary Producers and Associated Organisms," November 1978.

> Velocity and nutrient studies at 12 sites in Western Washington streams indicated that 50 cm/sec is the critical average current velocity where the productive base of the food web is impacted. Swiftly flowing streams rich in nutrients should not be slowed to this value, and slowly flowing streams should not be altered to have velocities greater than this value.

Report No. 2. Horner, R.R., S.J. Burges, J.F. Ferguson, B.W. Mar, and E.B. Welch, "Highway Runoff Monitoring: The Initial Year," January 1979.

> This report covers the initial 15 months of effort to review the literature, select a prototype site, compare the performance of several automatic sampling devices, and install a prototype sampling site on I-5 north of Seattle.

Report No. 3. Clark, D.L. and B.W. Mar, "Composite Sampling of Highway Runoff: Year 2," January 1980.

> A composite sampling device was developed that can be installed at less than ten percent of the cost of automatic sampling systems currently used in Federal highway runoff studies. This device was operated for one year, side-by-side the I-5 site, to demonstrate that the composite system provides identical results to the automated system.

Report No. 4. Vause, K.H., J.F. Ferguson, and B.W. Mar, "Water Quality Impacts Associated with Leachates from Highway Woodwastes Embankments," September 1980.

> Laboratory and field studies of a woodchip fill on SR 302 demonstrated that the ultimate amounts of COD, TOC and BOD per ton of woodchips can be defined and that this material is leached exponentially by water. After a year the majority of the pollutant has been removed, suggesting that pre-treating of the woodchips prior to use in the fill can reduce the pollutant release from a fill. This chips should be protected from rainfall and groundwater intrusion to avoid the release of leachate. Release of leachate onto tidal lands can cause beach discoloration and an underground deep outfall may be required.

Report No. 5. Aye, R.C., "Criteria and Requirements for Statewide Highway Runoff Monitoring Sites," July 1979.

> Criteria for selecting statewide monitoring sites for highway runoff are established to provide representative combinations of climate, traffic, highway, land use, geographic and topographic characteristics. Using these criteria, a minimum of six sites are recommended for use in this research.

Report No. 6. Asplund, R., J.F. Ferguson, and B.W. Mar, "Characterization of Highway Runoff in Washington State," December 1980.

A total of 241 storm events were sampled at ten sites during the first full year of statewide monitoring of highway runoff. Analyses of these data indicate that more than half of the observed solids in this runoff is traced to sanding operations. The total solids loading at each site was correlated with traffic during the storm. The ratio of other pollutants to solids was linear when there were sufficient traffic generated pollutants to saturate the available solids.

Report No. 7. Mar, B.W., J.F. Ferguson, and E.B. Welch, "Year 3 - Runoff Water Quality, August 1979 - August 1980," January 1981.

This report summarizes findings detailed in Report Nos. 4 and 6 plus the yet-to-be-published work of Karen Zawlocki on trace organics in highway runoff. Several hundred compounds tentatively identified by GC-MS were grouped into nine categories, which were not mutually exclusive, by Zawlocki. Major components of these categories were petroleum products used by vehicles and incompletely combusted hydrocarbons. The concentrations of these trace organics groups were low compared to criteria proposed for protection of aquatic life.

Report No. 8. Eagon, P.D., "Views of Risk and Highway Transportation of Hazardous Materials - A Case Study in Gasoline," November 1981.

While gasoline represents one-third of all hazardous materials transported in the country by trucks, the risk associated with gas transportation, as viewed by the private sector, is small. Public perceptions of risk are much greater due to lack of knowledge on probabilities and consequences of spills. Methods to improve knowledge available to the public on gasoline spills and methods to improve estimate of environmental damages from gasoline spills are presented. Generalization of methodologies to hazardous materials in general are discussed.

Report No. 9. Zawlocki, K.R., J.F. Ferguson, and B.W. Mar, "A Survey of Trace Organics in Highway Runoff in Seattle, Washington," November 1981.

Trace organics were surveyed using gas chromotography coupled to mass spectrometry for highway runoff samples from two Seattle sites. The characterization of the organics exhibited concentrations of aliphatic, aromatic and complex oxygenated compounds. Vehicles, including exhaust emissions, were concluded to be the source of many of the organics.

Report No. 10. Wang, T.S., D.E. Spyridakis, R.R. Horner, and B.W. Mar, "Transport, Deposition and Control of Heavy Metals in Highway Runoff," January 1982.

Mass balances conducted on soils adjacent to highways indicated low mobility of metals deposited on well-vegetated surfaces. Grassy drainage channels were shown to effectively capture and retain metals (e.g. a 40 m channel removed 80 percent of the original Pb concentration). Mud or paved channels, however, demonstrated little or no ability to remove metals from runoff. Metal release studies suggested that acid precipitation could release metals bound in the soil, especially where low buffering capacity exists.

Report No. 11. Portele, G.J., B.W. Mar, R.R. Horner, and E.B. Welch, "Effects of Seattle Area Highway Stormwater Runoff On Aquatic Biota," January 1982.

The impacts of stormwater runoff from Washington State freeways on aquatic ecosystems was investigated through a series of bioassays utilizing algae, zooplankton and fish. Algae and zooplankton were adversely affected by the soluble fraction of the runoff, while suspended solids caused high mortalities of rainbow trout fry. In addition, BOD_5 values similar to those reported in the stormwater literature were measured; however, there were indications that results were influenced by toxicity to microbial populations.

Report No. 12. Chui, T.W., B.W. Mar, and R.R. Horner, "Highway Runoff in Washington State: Model Validation and Statistical Analysis," November 1981.

Results of the second year of full-time operation of nine monitoring sites in the State of Washington produced 260 observations of highway storm runoff. A predictive model was developed based on the data from two years of observation for total suspended solid loads. A high correlation was demonstrated between total suspended solids and COD, metals and nutrients. The major factor controlling pollution loads from highways in Washington State is the number of vehicles passing during each storm, not those preceding storms.

Report No. 13. Mar, B.W., J.F. Ferguson, D.E. Spyridakis, E.B. Welch, and R.R. Horner, "Year 4 - Runoff Water Quality, August 1980 - August 1981."

This report summarizes findings presented in Report Nos. 10 - 12. Included are the results of studies aimed at improving and extending Asplund's solids loading model, increasing data on the ratios of various pollutants to TSS in the runoff, investigating the fate of heavy metals in drainage systems, and conducting bioassays on sensitive organisms exposed to highway runoff.

Report No. 14. Horner, R.R. and B.W. Mar, "Guide for Water Quality Impact Assessment of Highway Operations and Maintenance." (Draft issued Fall, 1981).

Procedures particularly applicable to Washington State have been developed to assist the highway designer in evaluating and minimizing the impacts of highway runoff on receiving waters. The guide provides computation procedures to estimate pollutant concentrations and annual loadings for three levels of analysis which depend on the watershed, the discharge system and traffic. It further provides means to judge the potential impacts of the runoff on receiving waters.

Report No. 15. Horner, R.R. and E.B. Welch, "Impacts of Channel Reconstruction in the Pilchuck River." (To be issued Winter, 1982.)

Report No. 16. Report on dissertation project during Year 5. (To be issued Summer 1982).

Report No. 17. Final report. (To be issued Summer 1982).

Please send me copies of Report No. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14

Name ______________________________

Address ______________________________

____

Phone (   ) ______________________________

Return to: B.W. Mar
Dept. of Civil Engineering
University of Washington
307 More Hall, FX-10
Seattle, WA 98195

SCOPE

The purpose of the research reported here was to examine the effects of highway stormwater runoff in the aquatic ecosystem. This evaluation included the conventional test of biochemical oxygen demand (BOD_5) plus a bioassay program to determine the response to runoff of a green alga, Selenastrum capricornutum, a zooplankter, Daphnia magna and a native salmonid, Salmo gairdneri (rainbow trout). Such a program involving three trophic levels should provide an integrated and comprehensive appraisal of the toxic effect of highway runoff water in spite of its chemical complexity and variability. Other objectives were to: (1) determine which chemical constituents were primarily responsible for any observed effects; (2) identify means of mitigating those effects; and (3) determine the extent to which the results of the study were site specific.

LITERATURE REVIEW

Although direct water pollution sources such as industrial and municipal discharges have been studied in depth, only recently have efforts been made to characterize and assess the impact of the wide variety of nonpoint sources of water pollution. An extensive literature is available regarding the characteristics and chemical composition of urban and highway runoff. Information regarding the specific effects of this nonpoint source of water pollution on aquatic biota are less abundant, however. Several investigators have pointed out that the contribution of urban and highway storm water runoff to a region's water pollution problems often exceeds that of treated municipal sewage outfalls (Shaheen, 1975; Pitt and Amy, 1973; Sartor and Boyd, 1972).

The chemical characteristics of highway runoff and subsequent mass loading inputs to receiving waters in Western Washington have been examined previously. Sylvester and DeWalle (1972) performed a preliminary appraisal of highway runoff as compared to urban runoff. Clark and Mar (1980) and Clark et al. (1981) compared the efficiency and ecological significance of utilizing composite stormwater samples as opposed to the more conventional discrete sampling systems. Asplund et al. (1980, in press) and Chui et al. (1981, in review) examined highway runoff throughout Washington State and derived a model for use in predicting pollutant loadings as a function of traffic during storms. Wang et al. (1982) have closely examined heavy metal inputs resulting from stormwater drainage as well as techniques of minimizing those inputs.

The following summary emphasizes work relating directly to highway runoff and to suspected toxicants present in highway runoff and their effects on aquatic organisms.

Biochemical Oxygen Demand of Highway Runoff

The amount of organic matter present in a wastewater and available to the aquatic community can be measured directly by the biochemical oxygen demand (BOD) test. The test exposes a natural assemblage of aquatic microbes to a water sample and measures the amount of oxygen consumed by the organisms in decomposing the organic matter within a specified time period (commonly five days).

Several authors have expressed skepticism in interpreting results of the BOD test in relation to highway runoff. Sartor and Boyd (1972) stated that toxic materials, specifically heavy metals, were often found far in excess of those concentrations known to cause substantial interference. Shaheen (1975) asserted that the classical method of determining BOD (American Public Health Association, 1975) is inadequate in dealing with highway runoff. Such inadequacy may lead to the abnormally high COD/BOD ratios observed in many highway runoff studies (Shaheen, 1974; Sartor and Boyd, 1972; Pitt and Field, 1977; Sartor and Agardy, 1974). Both Shaheen (1975) and Pitt and Field (1977) suggested additional possibilities for inhibition of microbial decomposition of stormwater runoff. The abnormally low and inconsistent BOD values may be caused by one or more of the following factors: rubber, petroleum compounds, fatty substances and plant cellulosic materials which are resistant to biological oxidation and may not be biodegradable during the normal five day period of the BOD determination.

BOD values for highway stormwater runoff as determined by the standard 5-day incubation procedure could be expected to fall anywhere in the range of 10 to 70 mg l^{-1} and would vary with duration and intensity of the storm event as well as with the traffic volume during the storm. The measured values, however, may be somewhat lower than the actual value due to the inhibition of microbial activity by one or several toxicants present in the waste.

Inorganic Contaminants in Highway Runoff

Perhaps the most thoroughly documented aspect of urban and highway runoff is the existence of inorganic pollutants, specifically the abundance of heavy metals. Although heavy metals have been proposed as the major toxicant present in runoff samples (Shaheen, 1975; Winters and Gidley, 1980), only limited attempts have been made to analyze the impact of this waste on the aquatic community. Prediction of the effects of highway runoff on aquatic biota on the basis of chemical composition is complicated and very likely misleading due to the possibilities for synergistic or antagonistic actions of the many different metal ions present within a given sample (Kaiser, 1980). Another factor contributing to the uncertainty of such predictions is the form of the metal once it reaches the receiving body. The majority of studies concerned with the metal content of highway or urban runoff present results as total metals without regard for the physical or chemical state of the metals.

Shaheen (1975) has shown that the majority of solids deposited on roadways are vehicle dependent and that 95% of this material is inorganic in nature. Less than five percent of the solids deposited originated from the vehicles themselves, however, and as Asplund _et al_. (1980, in press) pointed out, vehicles must function as a transport mechanism for pollutants as well as a source. Sartor and Boyd (1972) found that for all metals except mercury, loading intensities are highest in industrial areas and lightest in commercial areas. Metal concentrations closely follow total solids concentrations (Clark and Mar, 1980; Clark _et al_. 1981; Asplund _et al_. 1980, in press; Chui _et al_. 1981, in review). Natural transport mechanisms such as wind and rain can also deposit pollutants on the roadway (Asplund _et al_. 1980, 1981). In a study conducted in California, Wilber and Hunter (1977), found that the concentrations of heavy metals in precipitation were insignificant in relation to concentrations in water flowing off the road surface. Recent studies conducted in Western Washington contradict this, indicating that in this area bulk precipitation can contribute significant quantities of trace metals. (Larson _et al_. 1975; Dethier, 1979).

A study by Pitt and Amy (1973) concluded that, with the exception of lead, the maximum solubility of the heavy metals is associated with the larger particulates. Shaheen (1975) showed a distinct tendency for lead to be associated with fine particles, probably due to association with the very fine particulate exhaust emissions. However, Wang (1981, 1982) has found that lead at Interstate 5 in Seattle is more commonly associated with the larger particulate fraction.

Effects of Inorganic Pollutants on Algae

Minimal information is available concerning the specific effects of highway runoff on the primary productivity of receiving bodies. Effects of heavy metals on plankton algae have been extensively studied However, the recent study by Winters and Gidley (1980), for the California State Department of Transportion, is one of few which has examined the effects of roadway runoff on algae. Heavy metals, particularly lead and zinc, appeared to be the factors responsible for the inhibition of algal growth.

Bartlett *et al.* (1973), utilizing a soft water medium, determined that the following concentrations of soluble metals initiated growth rate inhibition in laboratory cultures of *S. capricornutum*: copper at $\mu g\ l^{-1}$, zinc at 30 $\mu g\ l^{-1}$ and cadmium at 50 $\mu g\ l^{-1}$. Concentrations in same medium resulting in complete growth inhibition were 90 $\mu g\ l^{-1}$, 120 $\mu g\ l^{-1}$ and 80 $\mu g\ l^{-1}$, respectively.

The effects of the various heavy metals in combination with one another has been the subject of a number of investigations involving algal communities. Braek *et al.* (1976) found that the toxicity of copper and zinc ions could not be predicted from the known toxicity of the single metals. Antagonistic results were observed for copper and zinc on two species of diatoms while synergistic reactions were observed for two other diatom species. Synergistic interactions were also detected by Pietilainen (1975) while studying lead and cadmium. Cadmium was found to be more toxic than lead, and low concentrations of lead were seen to increase the toxicity of cadmium.

The most common physiological mechanism proposed for reductions in primary productivity due to metal contamination are interference with photosynthetic and/or metabolic processes, particularly enzymatic actions. Christensen *et al*. (1979) explained synergistic actions by the interrelated roles of the two metals in photosynthesis.

A substantial amount of literature has been published analyzing the resistance of planktonic algal populations to heavy metal contamination (Hollibaugh *et al*. 1980; Braek *et al*. 1976; Braek *et al*. 1980). However, Whitton (1970b) compared 37 algal populations, representing about 25 species, for resistance to metals, and concluded that there is little relationship between resistance of a species to one metal and resistance of the same species to another metal. Thus, general statements concerning resistance of a particular species to heavy metals must be treated cautiously.

Effects of Inorganic Pollutants on Zooplankton

The effect of inorganic, heavy metals on freshwater zooplankton has been examined thoroughly by Biesinger and Christensen (1972). They asserted that the toxic influence is exerted by the covalent bonding of metals at the cell surfaces. In a study reviewed by Skidmore (1964), a 64-hour LC_{50} for *Daphnia magna* exposed to zinc in soft Lake Erie water was 72 $\mu g\ l^{-1}$. Borgmann *et al*. (1980) found that in the presence of cadmium, mercury and copper, growth of freshwater copepods was reduced at considerably lower concentrations than the levels at which mortality increased.

Preliminary data from invertebrate bioassays utilizing highway runoff water from Wisconsin have been released as a part of the nationwide Envirex study (Gupta and Kreutzberger, 1981). These works reported no toxic response in a four-day exposure of *Ascellus* (an aquatic isopod), *Hexagenia* (mayfly nymph) or *Daphnia* (zooplankton) to undiluted highway runoff water. Highway runoff water did, however, exert a toxic effect on *Gammarus* (an aquatic amphipod) although no more than 40% of the observed mortality was attributed to the runoff.

Effects of Inorganic Pollutants on Fish

The effects of zinc on fish have been reviewed extensively by Skidmore (1964). Citing a study carried out in 1952, Skidmore noted that at a concentration of 40 $\mu g\ l^{-1}$ soluble zinc, no rainbow trout eggs successfully hatched. For rainbow trout fry exposed to 10 $\mu g\ l^{-1}$ soluble zinc, 46 percent survival was recorded after 28 days, while a survival rate of 98% was noted when the zinc concentration was only 3 $\mu g\ l^{-1}$. In another study reviewed by Skidmore, Lloyd (1960) observed that, for rainbow trout fingerlings, the 48-hour LC_{50} increased to 600 $\mu g\ l^{-1}$ zinc. Preliminary studies by Envirex (Gupta and Kreutzberger, 1981), utilizing acute (4 day) exposures of the fathead minnow, *Pimephales promelas*, indicated no apparent toxicity in tests with snowmelt runoff from Wisconsin Highway 15.

A review by Sprague (1969) presented toxicity curves for salmon exposed to copper and zinc. The incipient LC_{50} values determined for these two metals were approximately 50 and 600 $\mu g\ l^{-1}$ respectively. Both curves exhibited distinct lethal thresholds. Skidmore (1964) also presented evidence indicating synergistic effects on fish for both zinc and copper and zinc and nickel. Skidmore (1964) and Sprague (1969, 1970) examined some physiological effects of heavy metals on fish. Commonly mentioned was damage to gills, ranging from copious secretions of mucous which inhibited gas exchange to gill epithellium separating from gill filaments and lamellae, resulting in exposed and abraded capillaries.

Organic Contaminants in Highway Runoff

Runoff from highways has been observed to have increased concentrations of hydrocarbons, often an order of magnitude greater than runoff from urban areas (Zawlocki, 1981). Shaheen (1975) stated that the hydrocarbon fraction originates from leaks of motor vehicle lubricants and hydraulic fluids, as well as from crankcase and less volatile engine exhaust products. Many of the non-volatile organic compounds are oxygenated hydrocarbons shown to be relatively innocuous compounds in the environment (Shaheen, 1975).

The potential for toxicity of highway derived organic compounds to biological systems has been reviewed extensively by Zawlocki (1981). The majority of studies reviewed examined the carcinogenic potential of the classes of compounds found in highway runoff. Those compounds reported to occur in runoff and to have high carcinogenic potential include the polynuclear aromatic hydrocarbons (PAH) and the azaheterocyclic hydrocarbons. On the other hand, Zawlocki (1981) concluded that highly toxic compounds such as polychlorinated biphenyls (PCBs) and chlorinated aromatics were not present at detectable concentrations in the samples. Findings such as these, along with conclusions of Winters and Gidley (1980), suggest that heavy metals are the primary concern in the abatement of the detrimental effects associated with highway runoff. Due to the nature of the organic toxicants present in highway samples and the presence of low levels of pesticides and PCBs, however, the possibilities for bioaccumulation within the various trophic levels in the aquatic community must be considered.

Suspended Sediment in Highway Runoff

Studies concerning the effects of suspended sediments on fisheries resources in natural streams have produced a wide range of results. A large part of this variation is a result of inconsistencies and difficulties encountered in experimental design.

Extensive literature reviews as well as pertinent experimental data have been presented on this subject by personnel from the Fisheries Research Institute at the University of Washington. Noggle (1978) used static bioassays to determine 96-hour LC_{50} values for juvenile salmonids exposed to predominantly glacial clay sediments. Noggle estimated LC_{50} values for wild coho salmon (Oncorhynchus kisutch) to range from 0.2 g l^{-1} in August to 35.0 g l^{-1} in November.

A more recent study by Smith (1978) examined the tolerance of juvenile chum salmon (Oncorhynchus keta) to suspended sediments. LC_{50} values determined after a 96-hour exposure period ranged from 15.8 to 54.9 g l^{-1}. Smith also referred to a study by Herbert and Richards (1961), who found that fingerling rainbow trout exposed to 90 to 110 mg

l^{-1} of suspended solids for several weeks exhibited abnormally high incidences of fin rot. Another interesting observation by Smith (1978) was the phenomenon of gut packing in exposed organisms. Smith believed that packing of the gut could lead to damage of cells required for osmoregulation or, more probably, restriction of the rate of water transport through the gut. Smith also noted an observation made by Sherk et al. (1974), who found that in the gut, entrapped particles were exposed to approximately the same acidic conditions as those used in stripping sorbed metals from particulate matter for chemical analyses. In this way insoluble metals could contribute to toxicity.

Smith utilized an application factor of 0.01 and his overall mean 96-hour LC_{50} value of 30 g l^{-1} to estimate that the maximum suspended solid concentration which would exert no sublethal effects was approximately 300 mg l^{-1}. Smith's summary of mortality data from a wide variety of studies exposing fish to suspended solids shows a range of LC_{50} values for solids of 270 to 300,000 mg l^{-1}.

The influence of sediment deposition on fish eggs and alevins has been extensively investigated. Steelhead trout eggs placed in plastic mesh bags within one mile downstream of a large clay slide on the Stillaguamish River in Washington State, failed to develop successfully (Cordone and Kelly, 1961). Cooper (1965) determined that for a silt concentration of 200 mg l^{-1} circulating through gravel at a velocity of 6.3×10^{-3} cm sec^{-1}, egg survival was 70 percent, while at a velocity of 8.2×10^{-3} cm sec^{-1} no eggs survived to hatching. These sediment concentrations are considerably lower than the acute toxicity values previously discussed and are similar to those observed in highway runoff.

Summary of the Literature

Several pollutants known to adversely affect the aquatic environment are present in highway runoff. Heavy metals may significantly alter the growth kinetics of algal populations. Soluble metals have also been shown to cause direct mortalities in zooplankton species. The variability in BOD values obtained for highway runoff may be a result of the inhibitory effects of some toxic agent, although BOD also varies with storm duration and intensity, traffic flow during storm and season of the year. Organic pollutants in highway runoff are not thought to have a significant impact on aquatic ecosystems due to their low concentrations. Fisheries resources within a receiving water environment may be affected by the following: impairment of spawning and rearing habitats by sediment deposition, direct physiological impacts of suspended sediment on fish, and acute as well as chronic toxicity from heavy metals.

EXPERIMENTAL METHODS

General Research Strategy and Design

The overall objective of the research was to relate the considerable volume of chemical information gathered in the past several years of highway runoff sampling to the biological community within the receiving water system. One of the most significant effects a pollutant can have on a biological system is to decrease the oxygen level. For this reason it was decided that a measure of potential oxygen demand was required, and the BOD test was included in the analytical scheme.

Algal assays were chosen for inclusion in this study owing to the importance of algae for sustenance of higher trophic levels within the aquatic ecosystem. The alga Selenastrum capricornutum was selected for use in this study for several reasons, the most important of which is the profusion of literature relating to the effects of a wide variety of pollutants on this species. Other justifications include its ease of culture and maintenance, along with its unicellular state and non-motile condition, making it conducive to precise enumeration. Similar reasoning pertained to the selection of Daphnia magna as a representative zooplankton species. As stated earlier, Biesinger and Christensen (1972) found D. magna representative of other abundant zooplankton in terms of sensitivity to toxic substances.

The salmonid species Salmo gairdneri (rainbow trout) was chosen for use in the study because of its economic importance in the Pacific Northwest region, both commercially and in sport fishing. Also, while algae and zooplankton are significant components of the food web and are sensitive indicators, results obtained by using more commercially and recreationally familar species, such as fish, may have more meaning to the public.

Description of the Study Area

The three sites utilized in this study have been described in detail by Clark and Mar (1980), Clark et al. (1981) and Asplund, et al. (1980, in press). Most of the bioassays used highway runoff samples collected from the composite storm sampler placed on I-5 at Northeast 158th Street in Seattle, Washington. The site consists of four 3.66 meter wide northbound lanes on a wide-radius horizontal curve of 1.1 percent upgrade, a 3.05 meter shoulder and a 3.05 meter median, resulting in a drainage area of 4,927 m^2 for the 238 meter length of pavement sampled (Clark and Mar, 1980; Clark et al. 1981). The shoulders and median are primarily of asphalt while the lanes themselves are constructed of concrete. Runoff drains from the four lanes and the median to curbing along the shoulder, through a steel culvert, calibrated H-flume and flow splitter, and subsequently into the composite sampler. Average daily traffic for the northbound lanes is approximately 50,000 vehicles.

The second site utilized in this study is also located in metropolitan Seattle, along highway SR-520. This station collects drainage water into a small composite tank from an area of 401 m^2 and consists of two 3.66 meter wide by 46 meter long westbound lanes, as well as a portion of highway on-ramp. Both the freeway lanes and 0.61 meter wide shoulder strip are composed of concrete. Average daily traffic along the westbound lanes of SR-520 is about 42,000 vehicles.

Rainfall in the Seattle area is generally of low intensity, usually less than 0.25 cm hr^{-1}. Since the average winter temperature is approximately 5°C, snowfall rarely occurs or else melts before accumulating. Both I-5 and SR-520 sites are surrounded by predominately urban/residential settings.

The third station employed in the comparative bioassay experiment is located along Interstate 90 at mile-post 41.5 between Issaquah and Snoqualmie Pass, Washington. Located on a wide radius curve of 1.2 percent grade, the site drains three 3.66 meter wide concrete lanes, along with a 3.66 meter asphalt shoulder and 1.83 meter asphalt median over a length of 43 m. Average traffic volume through the drainage area is about 7,700 vehicles per day. Located on the western slopes of the Cascade Mountain range, the I-90 site receives annual precipitation in the range of 152.4 to 254.0 mm yr^{-1}. Surrounding land use is undeveloped Western Coastal Forest.

Composite Freeway Sampling

Samples collected from the I-5 location were withdrawn from the concrete holding tank within 24 hours after a major storm event or when runoff had ceased and the pavement was dry. A subsample of highway runoff was placed in a 20 liter glass carboy that had been washed, scrubbed with hot, soapy water, acid washed (20% HCl), neutralized with a saturated solution of $NaHCO_3$, rinsed in deionized water and autoclaved. The sample was immediately transported back to the laboratory and centrifuged in polyethlene centrifuge tubes for 15 minutes at 3500 rpm in order to remove large particulates. Approximately four liters of sample were then filtered through 0.45 μm Millipore filters previously soaked in deionized water.

Biochemical Oxygen Demand Procedures

Procedure for the determination of biochemical oxygen demand followed that outlined by the American Public Health Association (1975). Nutrient enriched dilution water was utilized throughout the study, with nutrients being added on the day the tests began. BOD determinations were carried out on both unfiltered I-5 samples and Millipore filtered I-5 samples. Microbial seed used for the soluble samples was settled primary effluent obtained from the Renton Sewage Treatment Plant. Dissolved oxygen content of the dilution water and filtered and unfiltered highway samples were determined by the azide modification of the Winkler method. Samples were then incubated for five days, at which time dissolved oxygen content was again determined. In addition to samples with highway runoff water, dilution water blanks and dilution water plus seed blanks were incubated and analyzed for dissolved oxygen depletion as well.

Algal Bioassay Procedures

Algal bioassays employed the methodology proposed by Miller *et al.* (1978a, 1978b) with some modifications. Analyses for orthophosphorus (Strickland and Parsons, 1972), nitrate and nitrite-nitrogen (U.S. Environmental Protection Agency (1979), and pH (Metrohm/Brinkman model pH-104 meter) were performed prior to each bioassay. Initial nutrient concentrations and pH values of both test water and dilution

Table 1: Storm data and water quality characteristics of highway samples used in algal bioassays.

Experiment Number	Storm No.	Date of Collection	Sampling Site	Measured Rainfall (in.)	Vehicles During Storm	Initial pH	Ortho-P ($\mu g\ l^{-1}$)	$NO_3^-+NO_2^-$-N ($\mu g\ l^{-1}$)	Total P ($\mu g\ l^{-1}$)	Total N ($\mu g\ l^{-1}$)
1	169	1-19-81	I-5	0.450	40,650	6.8	7.0	839	220	-
		1-12-81	L. Washington	-	-	7.2	2.0	295	-	-
2	172	2-13-81	I-5	2.360	112,950	6.8	15.5	608	43	847
		1-12-81	L. Washington	-	-	7.2	2.0	275	-	-
3	176	3-16-81	I-5	0.690	51,150	6.7	3.0	895	29	1079
		3-16-81	L. Washington	-	-	7.1	11.0	353	21	-
4	180	4-6-81	I-5	0.340	12,250	6.6	3.3	405	-	419
	48	4-11-81	SR-520			6.5	3.0	691		
	42	4-17-81	I-90	0.250	1,868	6.7	6.7	116		
	-	4-24-81	L. Washington		-	6.9	1.0	248		

water are given in Table 1 for the four algal bioassays, along with total phosphorus and total Kjeldahl nitrogen determinations for the storm samples. Total phosphorus values were determined by the persulfate digestion method (American Public Health Association, 1975). Total Kjeldahl nitrogen was analyzed by the semi-micro Kjeldahl digestion-Selenium method (Baummer, 1972). Aliquots were also taken for metals and analyzed by either atomic absorption or by an Inductively Coupled Plasma (ICP) spectrophotometer.

The inhibitory potential of the highway runoff water was determined by adding enough soluble phosphorus, nitrogen and micronutrients to both the stormwater samples and Lake Washington dilution water to achieve an equivalent potential for growth in each. The N:P ratio was adjusted to at least 12.5:1. Assuming that phosphorus is the limiting nutrient (Welch, 1980), the maximum standing crop of algae which should be achieved in each test flask, can be estimated from the P available (Miller _et_ _al_. 1978b). A micronutrient solution was added in the proportion of 1 ml of solution to 999 ml of test water.

Test flasks utilized in the study were 125 ml Erlenmeyer flasks fitted with foam rubber stoppers. Flasks had been previously cleaned and autoclaved according to the methods of Miller _et_ _al_. (1978). Nutrient-enriched Lake Washington water and highway runoff water were mixed to a final volume of 40 ml in the following proportions for the first three experiments: 1) 100 percent lake water; 2) 75 percent lake water and 25 percent highway water; 3) 50 percent lake water, 50 percent highway water, 4) 25 percent lake water and 75 percent highway water; and 5) 100 percent highway water. Three replicates were prepared for each of the five dilutions. The test alga *Selenastrum capricornutum* was obtained from the Environmental Protection Agency, Corvallis, Oregon. Stock algal cultures were maintained in a synthetic nutrient medium as specified by Miller *et al*. (1978b).

Test flasks were placed on a shaker table and incubated at 24 ± 2°C in a controlled temperature room under cool white fluorescent lighting of 3800 ± 380 lux. Experiments were conducted for a period of 14 days. Biomass was determined indirectly by an electronic particle counter (Coulter Counter, Model ZBI) capable of counting the cell concentration of the sample with an error of ± 3 percent or less (Sheldon and Parsons, 1967). The counter was used in conjunction with a mean cell volume computer (Coulter Channelyzer) for determination of the mean algal cell volume. Measurements of biomass were made daily for the first seven days of the test and every other day for the remainder of the exposure period.

The mean and standard deviation of the three replicates was calculated for each dilution on the fourteenth day of the test, or on the day having the maximum biomass. A one way analysis of variance was performed for each experiment to determine whether or not significant differences existed between sets of replicates within a test (Zar, 1974). If heterogeneity was detected between sets of replicates, the Student-Newman-Keuls multiple range test was used to determine where the significant differences occurred.

IC_{50} (the concentration which inhibits algal growth by 50%) determinations were carried out on the basis of the method outlined by Joubert (1980). After the percentage inhibition resulting from the various dilutions as compared to controls was determined, it was plotted on probability paper versus concentration of highway runoff as percent volume in the sample. The statistical method of Litchfield and Wilcoxon (1949) was then used to assure that the resulting straight line was representative of the values obtained. The confidence limits for the IC_{50} at a confidence level of 95 percent were also obtained in this manner.

Daphnia Bioassay Procedures

Stock cultures of *Daphnia magna*, used in the testing sequence, were obtained from University of Washington College of Fisheries personnel. Cultures were maintained in wide-mouth, 7.6 liter glass jars and fed a mixture of *S. capricornutum* cells and a slurry of Fleishmann's dry yeast in deionized water.

Dilutions of lake water and highway runoff were prepared in the following proportions: 1) 100 percent lake water, 2) 50 percent lake water, 50 percent highway runoff and 3) 100 percent highway runoff water. For the first experiment ten *D. magna* were added to each of the test containers; however, for the last two experiments twenty organisms were added to each test flask with no apparent effect in the control replicates. With the aid of a set of small standard brine shrimp sieves, efforts were made to select the smallest daphnids possible. It was felt that such discrimination would select those individuals that were youngest and healthiest and therefore the most apt to survive the exposure period.

Incubation periods for the three experiments varied from 48 to 96 hours depending upon the response of the organisms. The end point for the test was complete immobilization or death of the test organisms.

Trout Bioassay Procedures

Rainbow trout used in this portion of the study were obtained from the hatchery at the University of Washington's College of Fisheries. All fish for this series of bioassays were obtained from the same egg lot so that variation in genetic composition of test organisms was minimized. This procedure also allowed accurate determinations of the age of the fish (± 48 hours).

The first experiment was carried out employing Millipore-filtered highway runoff and dilution water to test the response of fish to soluble pollutants. This test involved yolk sac stage fry obtained from the hatchery, all of which had been hatched within the past 48 hours (G. Yokoyoma, personal communication, 4-15-81). The experiment was carried out in a constant temperature water bath at 12°C ± 1°, with fish being placed in one liter beakers covered with a strip of Parafilm. The initial water volume was 1000 ml; but, due to the activity of the aeration system, this volume was often reduced by 20 percent by the end of the 96 hour exposure period. Four dilutions were prepared in the following proportions: 1) 100 percent lake water; 2) 70 percent lake water and 30 percent highway sample; 3) 30 percent lake water and 70 percent highway; and 4) 100 percent highway runoff water. Dilutions were prepared in triplicate. The test containers were allowed to acclimate in the water bath for a minimum of eight hours prior to the introduction of test organisms. Following acclimation, 20 trout were placed in each container.

Throughout the exposure period, the beakers were checked at least once every ten hours. Once every 24 hours temperature and dissolved oxygen content of the samples were measured by means of a Yellow Springs YSI Model 57 Dissolved Oxygen/Temperature meter. The number of dead fish in each of the flasks was counted and recorded. Dead fish were then removed with the aid of a small net to prevent the spread of disease. Total suspended solids were measured in all test and dilution water samples according to the procedure outlined by the American Public Health Association (1975).

Based on the results of the initial experiment, the second and third tests were conducted with unfiltered highway samples and Lake Washington water. Trout used in the second test were about 23 days old. The third experiment was essentially a repeat of the second with the inclusion of an additional comparison. Triplicate dilutions of 50 and 100% unfiltered highway runoff samples were prepared as previously described. Another sample from the same storm event was obtained by allowing the highway runoff, which by-passed the composite sampling tank, to run approximately 60 m down a grass channel adjacent to the highway right-of-way.

EXPERIMENTAL RESULTS

BOD_5 Results

Biochemical oxygen demand of highway runoff from I-5 in Seattle for both unfiltered and filtered water samples is presented in Tables 2 and 3. The possibility of toxic responses in BOD samples suggested by Shaheen (1975) and Sartor and Boyd (1972) was explored. Statistical comparison of means obtained in unfiltered samples resulted in a significant difference between dilutions in only one sample (storm number 182). On the basis of these findings it does not appear as though BOD determinations were influenced by toxicants present in the samples. The overall means of the 12 BOD_5 values obtained with five and ten percent dilutions were 15 mg l^{-1} (range 5 to 25 mg l^{-1}) and 14 mg l^{-1} (range 4 to 21 mg l^{-1}), respectively.

Statistical analyses of filtered samples revealed three storms where BOD of five percent dilutions were significantly higher than ten percent values. The overall mean of the five percent BOD_5 values is 21 mg l^{-1} (range 12 to 27 mg l^{-1}) while the mean of the ten percent values is 14 mg l^{-1} (range 7 to 22 mg l^{-1}). Thus, there are indications of a toxic response but not a uniform tendency in that direction. The values from filtered samples are somewhat higher than those observed in unfiltered samples; and apparently are a result of the large microbial population introduced in the filtered samples by seeding.

BOD values obtained in this study agree with the range reported in the literature (Asplund *et al*. 1980, 1981; Shaheen, 1975; Sartor and Boyd, 1972). In particular, results correspond well with those

Table 2: BOD_5 of unfiltered samples of highway runoff from Interstate-5 in Seattle, Washington.

Storm No.	Date	Mean BOD_5 of 5% Dilution (mg l^{-1})	Range (mg l^{-1})	Mean BOD_5 of 10% Dilution (mg l^{-1})	Range (mg l^{-1})	Vehicles During Storm	Storm Duration (hours)
151	10-13-80	11	9 - 14	16	13 - 18	13,200	7
152	10-21-80	19	19 - 19	19	17 - 20	11,200	6
153	10-25-80	16	15 - 16	19	19 - 20	29,600	6
162	12-1-80	17	16 - 18	8	7 - 10	65,750	34
169	1-19-81	17	16 - 17	12	9 - 14	40,650	18
172	2-13-81	16	12 - 19	13	13 - 14	112,950	80
176	3-16-81	15	11 - 19	15	13 - 16	51,150	25
180	4-6-81	5	5 - 7	4	4 - 4	12.250	9
182	4-24-81	16	16 - 17	9**	9 - 10	17,350	9
183	4-29-81	7	6 - 8	6	5 - 7	36,200	15
185	5-8-81	21	20 - 22	21	17 - 23	14,650	10
190	6-4-81	25	24 - 26	21	18 - 24	18,650	8

**Significant difference in BOD_5 compared to 5% dilution value ($P=0.05$).

Table 3: BOD_5 of Filtered Samples of Highway Runoff from I-5 in Seattle.

Storm No.	Date	BOD_5 of 5% Dilution	Range	BOD_5 of 10% Dilution	Range	Ratio of 5% Dilution / 10% Dilution
162	12-1-80	27	-	18**	18 - 19	1.5
169	1-19-81	24	-	22	20 - 24	1.1
172	2-13-81	25	6 - 44	17	14 - 20	1.5
180	4-6-81	20	16 - 26	9**	8 - 9	2.2
182	4-24-81	12	8 - 14	7	6 - 9	1.7
183	4-29-81	14	12 - 17	10	9 - 10	1.4
185	5-8-81	27	25 - 29	12**	11 - 12	2.3
190	6-4-81	22	20 - 24	17	17 - 18	1.2

**Significant difference in BOD_5 compared to 5% dilution value (P=0.05).

presented by Asplund et al. (1980,1981) for highways with greater than 15,000 vehicles per day. BOD values from highways with this traffic level are typically lower than those from roads with lower volumes.

Response of Selenastrum capricornutum to Highway Runoff

Results of the four algal bioassays conducted as a part of this study are shown in Tables 4 and 5. Results of the first three bioassays (Table 4) reveal the consistent pattern of reduced algal biomass as the proportion of highway runoff from I-5 is increased, in spite of the fact that soluble phosphorus and nitrogen levels were equilized in both highway runoff and Lake Washington dilution waters. Additionally, the data suggest an inverse association between IC_{50} and vehicles traveling during the storm.

Results of the 3-site comparison (Table 5) indicate no apparent toxicity in either SR-520 or I-90 samples. The toxic effect at the I-90 site was anticipated to be relatively low on the basis of the lower traffic volumes observed there. Average traffic volumes at the I-5 and SR-520 sites do not differ greatly, however. Subsequent metal analyses on the acid extractable fractions of storms from each site revealed a marked difference in zinc concentrations (see Table 6). I-5 was the only site where zinc levels exceeded the detection limit, the maximum zinc level being 0.317 mg l^{-1}. Lead concentrations were consistently below the detectable limit (Wang, 1981). In light of the normally high concentrations of total lead observed in the samples, this finding indicates that a very high proportion of the lead was associated with particulate matter.

Table 4: 14-day maximum *S. capricornutum* standing crops of I-5 runoff-Lake Washington water dilutions and corresponding IC_{50} determinations.

Storm No.	Date of Storm	Vehicles During Storm	Percent of Storm Water Used[a]	Dry Weight (mg l^{-1})	Standard Deviation	IC_{50} Value (%)	95% Confidence Limits
			0 (Control)	29.47	1.54		
			25	8.18**	2.39		
169	1-19-81	40,650	50	2.44**	1.19	13.5	3.0 - 60.8
			75	1.44**	1.23		
			100	0.34**	0.11		
			0 (Control)	22.17	0.60		
			25	16.87**	2.42		
172	2-13-81	112,950	50	6.30**	0.98	44.5	41.1 - 48.2
			75	7.73**	3.36		
			100	3.45**	0.24		
			0 (Control)	29.21	2.07		
			25	27.60	1.04		
176	3-16-81	51,150	50	13.36**	1.54	50.2	30.0 - 84.0
			75	6.11**	2.79		
			100	1.77**	0.34		
			0 (Control)	25.36	2.09		
			50	24.48	1.88		
180	4-16-81	12,250	75	14.94**	1.18	72.5	52.8 - 99.6
			100	1.89**	0.92		

[a]Both storm and dilution samples filtered with 0.45 μm Millipore filter.

**Significant difference in dry weight as compared to the control (P=0.05).

Table 5: 14-day maximum *S. capricornutum* standing crops for three-site comparison of algal toxicity of highway runoff water and corresponding IC_{50} values.

Site of Sample	Storm No.	Date of Storm	Average Daily Traffic	Percent of Storm Water Used[a]	Dry Weight (mg l^{-1})	Standard Deviation	IC_{50} Value (%)	Confidence Limits
Lake Washington	-	-	-	Control	25.36	2.09	-	-
I-5	180	4-16-81	50,000	50	24.48	1.88	72.5	52.8 - 99.6
				75	14.94**	1.18		
				100	1.89**	0.92		
SR-520	48	4-11-81	42,000	50	27.78	4.25	*	
				75	21.78	1.70		
				100	27.74	2.77		
I-90	42	4-17-81	7,700	50	25.68	0.46	*	
				75	23.50	2.16		
				100	23.91	3.67		

*Not applicable since no significant difference was detected in comparison to controls.

**Significant difference in dry weight as compared to the control (P=0.05).

[a]Both storm and dilution samples filtered with 0.45 μm Millipore filter.

Table 6: Metal concentrations for storm events used in bioassays from three-sites in Western Washington and corresponding IC_{50} values.

Site - Storm	IC_{50} Value (percent)	Metal concentration: extracted from soluble fraction (mg l^{-1}) Cu	Pb	Zn	Metal concentration: extracted from total fraction (mg l^{-1}) Cu	Pb	Zn
SR-520 - 48	*	.006	BDL	BDL			
SR-520 - 49	*	.004	BDL	BDL			
I-90 - 49	*	BDL	BDL	BDL			
I-5 - 169	13.5						
I-5 - 172	44.5						
I-5 - 176	50.2			.138	.040	.541	.309
I-5 - 180	72.5			.096	.045	.411	.382
I-5 - 185	*	.024	BDL	.232			
I-5 - 188	*	.013	BDL	.317			

*Not applicable.

BDL - Below detectable limit

Differences in copper concentrations between soluble fractions of the samples are also noteworthy. Soluble copper in the I-5 samples was three to six times higher than values determined for the SR-520 site in spite of the similarities in traffic flow.

Response of Daphnia magna to Highway Runoff

The response of *D. magna* to freeway runoff water at two dilutions is presented in Tables 7-9. Experiment 1 was conducted to investigate differences in impacts between soluble and unfiltered fractions of highway runoff. Table 7 indicates that very little difference existed.

The second experiment, summarized in Table 8, compared toxicity of runoff at I-5 and SR-520 sites. Although the 100% SR-520 sample did adversely affect the zooplankton (43% mortality), the effects were not nearly as great as what occurred with the I-5 samples.

The third experiment was performed to investigate a duplicate of the three-site comparison as had previously been done with *S. capricornutum*. Results were similar to those observed with the algal exposure, in that survival in both SR-520 and I-90 site samples actually exceeded that of the controls (Millipore-filtered Lake Washington water), while survival was much reduced in stormwaters drained from the I-5 road surface. It is notable that low survival rates occurred with I-5 samples representing both relatively high and low within storm traffic volumes.

Table 7: *Daphnia magna* Experiment 1. Bioassay conducted 5-11-81 to 5-15-81 comparing effects of filtered and unfiltered samples.

Date of Collection	Sample Site	Storm No./ Treatment	Vehicles During Storm	96 Hour Survival (%) Controls	50%	100%
5-10-81	I-5	185/Soluble	14,650	90	0	0
5-10-81	I-5	185/Unfiltered	14,650	80	10	6.7

Table 8: *Daphnia magna* Experiment 2. Bioassay conducted 5-24-81 to 5-27-81 comparing I-5 and SR-520 toxic response to filtered stormwater samples.

Date of Collection	Sample Site	Storm No.	Vehicles During Storm	72 Hour Survival (%) Controls	50%	100%
5-18-81	SR-520	49	N/A	100	96.7	56.7
5-21-81	I-5	188	62,200	100	13.3	0

Table 9: *Daphnia magna* Experiment 3. Bioassay conducted 6-1-81 to 6-3-81 comparing I-5, SR-520 and I-90 toxic responses to filtered stormwater samples.

Date of Collection	Sample Site	Storm No.	Vehicles During Storm	48 Hour Survivial (%) Controls	50%	100%
4-17-81	I-90	42	4,980	96.7	100	100
4-11-81	SR-520	48	N/A	96.7	98.7	100
5-10-81	I-5	185	14,650	96.7	68.3	16.7

N/A - Not available

Response of Rainbow Trout to Highway Runoff

Results of tests incorporating filtered and unfiltered samples are summarized in Table 10. The experiment performed with filtered water showed no toxic effect on the young trout. When unfiltered dilution and stormwaters were used, the impact on the alevin trout was markedly increased. These results indicate that mortalities were due to the presence of suspended solids, or the pollutants associated with the particulates.

The third test was designed to examine the proposed management option of allowing stormwater runoff to travel along grass channels prior to entering any receiving water. The test involved comparing results of bioassays conducted on stormwater directly off the road surface with those using a composited sample from the end of the grass channel. As can be seen in Table 10, the water which traveled through the grass channel was considerably less toxic than the water collected directly off the road surface. Total suspended solids concentration for the untreated sample was 97 mg l^{-1}, while the TSS level in the end-of-channel sample was reduced to 24 mg l^{-1}. Flow diversion through the channel resulted in 61 and 77 percent removal of acid extractable zinc and lead, respectively.

Table 10: Summary of bioassays exposing rainbow trout (*Salmo gairdneri*) to I-5 stormwater runoff under various conditions.

Date of Collection	Sample Site	Storm No.	Vehicles During Storm	Dates of Exposure	Sample Treatment Prior to Exposure	96 hour Survival of Exposures (%) Control	30%	50%	70%	100%
4-16-81	I-5	180	12,250	4-15 to 4-19	L. Washington dilution and storm sample 0.45 Millipore filtered	100	100	-	93.3	93.3
5-10-81	I-5	185	14,650	5-11 to 5-19	Used unfiltered L. Washington dilution and unfiltered storm sample	100	-	30	-	0
5-21-81	I-5	188	62,200	5-21 to 5-25	Same treatment as above, stormwater taken directly off the roadway	100	-	60	-	0
5-21-81	I-5	188	62,200	5-21 to 5-25	Same storm event as above, stormwater allowed to run 60 m through a grassy ditch adjacent to right-of-way	100	-	100	-	93.3

DISCUSSION

General statements concerning the effects of highway runoff on aquatic organisms or the relative contribution of a specific pollutant to these effects, are difficult to make and should be treated cautiously. The proportions of the various pollutants and their actual concentrations change seasonally as well as from storm to storm, so that an infinite spectrum of possibilities for interaction exist. The possibilities for bioaccumulation of soluble storm water-derived pollutants, both within an aquatic community and throughout the food web, have yet to be examined. Although the particulate component of highway runoff and those pollutants associated with it may be expected to settle out of the water column fairly rapidly, the soluble metals and trace organics will remain in the system until they are degraded or removed by biological or physical processes.

Table 11 compares the range of concentrations of total metals found in I-5 runoff with the range of values found to be detrimental to organisms at three trophic levels. The table shows considerable overlap between quantities observed in runoff and those levels known to endanger test organisms. The results of this study suggest that these metals are present in a biologically available form at harmful levels in some highway runoff samples.

Table 11. Range of total metals observed in runoff at Interstate-5 site as compared to LC_{50} ranges presented in literature.

Metal	Range of Concentrations[a] Observed at I-5 (mg l^{-1})	LC_{50} Values Presented in Literature[b] (mg l^{-1}) [Test organism]
Pb	0.10 - 5.50	1.20 - 542. [rainbow trout] 0.45 - 1.90 [daphnia] 0.50 - 1.00 [algae]
Cu	BDL - 0.50	0.02 - 0.89 [rainbow trout] 0.01 - 0.50 [daphnia] 0.006- 8.00 [algae]
Zn	BDL - 2.50	0.28 - 7.21 [rainbow trout] 0.10 - 0.66 [daphnia] 0.10 - 1.20 [algae]

[a]Values obtained from Clark (1980) and Asplund (1980)

[b]Values obtained from U.S. Environmental Protection Agency (1980a, 1980b, 1980c).

BLD -- below detectable limit.

Algal bioassays conducted using Selenastrum capricornutum to assess the potential toxicity of runoff from I-5 exhibited a definite trend toward reduced growth as the ratio of highway sample to dilution water increased. Stormwater enriched with nutrients to levels equivalent to controls consistently demonstrated greater than 85 percent inhibition of maximum algal biomass. Comparison of the I-5 sampling location to a site with comparable traffic (SR-520 in central Seattle) indicated significant differences in toxic response between the two sites. Higher concentrations of soluble zinc and copper in I-5 runoff may be responsible for the observed toxic response.

Levels of soluble zinc in stormwater collected from I-5 are noteably higher than any other heavy metal analyzed. Shaheen (1975) observed that soluble zinc levels in runoff were generally higher than soluble lead, in spite of the fact that materials deposited on roadways contained approximately eight times more lead than zinc. In addition, Shaheen observed that during a storm characterized by a second peak in TSS levels (from an increased rate of rainfall), lead concentrations went up accordingly while zinc levels continued to decrease, presumably because much deposited zinc had already been removed in solution prior to the second flush of roadway materials.

After confirming that soluble zinc levels at the I-5 site were higher than those at SR-520, street sweepings were collected from both sites to explore the possibility that dryfall deposition rates or variations in substances ground off road surfaces could account for the difference. Results showed no significant difference in total zinc content of the sweepings at the two sites (1.548 mg g^{-1} at I-5 and 1.725 mg g^{-1} at SR-520).

The most likely explanation for the difference in soluble zinc at the two sites is different concentrations in precipitation. Findings of Larson et al. (1975), where zinc levels of up to 600 $\mu g\ l^{-1}$ were observed in rainfall in the vicinity of the I-5 site, could account for the soluble metal levels detected in runoff.

Rainbow trout exposed to Millipore filtered stormwater showed no harmful effects in a 4-day exposure. Bioassays conducted with unfiltered samples resulted in significant mortalities in both 50 and 100 percent dilutions. It is logical to conclude that either the particulates present in the samples or the pollutants associated with the solids were responsible for these deaths. Total suspended solids concentrations for the tests ranged from 35 to 97 mg l^{-1}.

The suspended solids levels observed for highway runoff are considerably lower than values observed to directly harm fish, all in the parts per thousand range. However, none of the studies reviewed incorporated fish at life stages as early as those tested here. The very young fry used in this study would possess proportionately smaller buccal cavities and gill openings than more mature fish, thus enabling suspended particulates to clog mouth parts and interfere with respiratory activities more readily. Wang et al. (1982) have shown that 92 percent by weight of particulates in highway runoff are greater than 20 μm in diameter. Large particulates of this sort would enhance the potential for obstructing the mouth parts and respiratory processes of rainbow trout sac fry. It is also possible that the young, hatchery reared fish had not acclimated to high suspended sediments or that the hatchery population of fry included more sensitive individuals than a wild population. Noggle (1978) noted that wild coho salmon, which had been exposed to (and survived) high suspended sediment concentrations during a large storm prior to their collection for the study, were significantly more tolerant of sediment than were hatchery reared coho salmon.

Smith (1978) asserted that increases in the angularity (referring to the frequency of sharp edges on particles) decreases tolerance to sediments. Electron micrographs prepared by Noggle (1978) showed that, of five sediment types studied, a roadbed sample was clearly the most angular. Noggle hypothesized that the grinding action of vehicle tires caused the angularity.

Both Noggle (1978) and Smith (1978) presented evidence to support the contention that fish exposed to suspended sediments suffer from hypoxia. Smith found that fish on the verge of death exhibited reactions characteristic of respiratory stress; gulping of air, loss of equilibrium and floating on the side with occassional attempts to swim. The latter two symptoms were often noted for fish in 50 and 100 percent samples of unfiltered highway runoff water.

Smith (1978) also considered the possibility that inorganic toxins influenced suspended sediment tolerances, noting especially the synergistic effects of copper and zinc. Even insoluble metals could contribute to toxicity if exposed to acidic conditions, which the environment of the gut or gill area may provide. Skidmore (1964) proposed that metals enter fish through both the skin and gills. Lloyd (1960) hypothesized that zinc acts specifically on the gills of fish due to the large volume of water passing that region. On the basis of these findings, the following mechanism may be postulated: high rates of respiration result in fish being stressed by the presence of suspended particulates; this stress leads to higher volumes of water passing over the gill and further reductions in pH in the region of the gills; under these conditions metals are more readily desorbed from particulates and can be taken up by the fish.

Bioassay studies performed on highway runoff from the I-5 site in North Seattle revealed mortalities to both algae and zooplankton that were not observed from runoff sampled at other Western Washington highway sites. The presence of heavy metal contaminants in concentrations similar to literature LC_{50} values were also found in the I-5 samples. Further studies are underway to resolve the source of these heavy metal contaminants, since preliminary tests suggest that they may not be entirely traffic related.

Since particulates and pollutants associated with particulates were observed to be effectively removed by vegetated areas, provisions should be made to establish or maintain vegetated buffer zones between the highways and the receiving waters. If dilution alone is used to reduce the impact of highway runoff on the receiving waters, a dilution of 80 percent (four parts dilution/one part runoff) is needed to avoid oxygen debt. To protect biota from heavy metals in cases where traffic flow exceeds 10,000 vehicles per day, however, a dilution of 100/1 is required to provide the level of dilution recommended by the U.S. Environmental Protection Agency (1976). Further bioassay studies are being conducted to evaluate the impact of runoff at low traffic volume Eastern Washington sites.

On the basis of the research results several recommendations pertinent to highway drainage design can be advanced. First, any means of removing particulate and soluble substances which may subsequently be oxidized in the aquatic environment should be considered. To avoid oxygen debt in receiving systems, highway runoff should be diluted by at least 80 percent.

The application factor of 0.01, recommended by the Environmental Protection Agency (1976) for determining safe levels of heavy metal pollutants, should be considered for highway runoff at those sites characterized by traffic flows exceeding 10,000 vehicles per day. That is, inputs of highway runoff to a receiving body should not exceed one percent of the system's total volume.

When feasible during the construction or renovation of roadways, provision should be made for establishing vegetated buffer strips adjacent to the highway. If buffer strips are already in existence, it is suggested that they be maintained in grass to maximize the efficiency of pollutant removal.

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