Urban ecotoxicology:
Spatial and temporal heterogeneity of pollution

Cover illustration: Hayo Van Schreeven
Lay-out: Désirée Hoonhout
Printing: VU Huisdrukkerij

CIP-data Koninklijke Bibliotheek, Den Haag
Widianarko, Budi

Urban ecotoxicology: Spatial and temporal heterogeneity of pollution.
Budi Widianarko
Amsterdam: Vrije Universiteit, Faculty of Biology
Thesis Vrije Universiteit
ISBN 90-9010823-8

VRIJE UNIVERSITEIT

Urban ecotoxicology:
Spatial and temporal heterogeneity of pollution

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan
de Vrije Universiteit te Amsterdam,
op gezag van de rector magnificus
prof.dr. T. Sminia,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der biologie
op vrijdag 3 oktober 1997 om 15.45 uur
in het hoofdgebouw van de universiteit,
De Boelelaan 1105

door

Budi Widianarko

geboren te Semarang, Indonesië

Promotor: prof.dr. N.M. van Straalen
Copromotor: dr.ir. C.A.M. van Gestel

Prof Dr W. Admiraal of the Department of Aquatic Ecotoxicology of the University of Amsterdam is gratefully acknowledged for reviewing this thesis.

This thesis is dedicated to

My grandfather Kadri (Kwee Kiem Kie)
for his learning motivation

My parents, Heru Purnomo and Indriati
for putting forward "education" as the first priority in the family, and for their strong approval and persistent support on my decision to be one and only scientific "worker" in the family

Imevianty, Arya Pratama and Erlangga Dwitama Widianarko
for their patience and love

All of my Teachers

All of my Students

URBAN ECOTOXICOLOGY :
Spatial and Temporal Heterogeneity of Pollution

Table of Contents

CHAPTER 1

General Introduction:
Urban Pollution, A Challenge for Ecotoxicology

Urbanization can be defined as the aggregation of human populations into an area allied to subsequent perturbation of the environment (Pizl & Josens, 1995). This process usually involves serious impacts on soil and water resources. The main consequence is that urbanization increases runoff volumes and pollutant contamination (NRC, 1993; Walker, 1994).

The threats posed by urban pollution will continue to increase, since it can be predicted that the global population will increasingly be concentrated in cities. As recently as 1975, just over one third of the world's human population lived in urban areas. By 2025, the proportion will have risen to almost two third (Anonymous, 1996). Cities are the places where most of the world's resources are consumed and most of the waste is discharged (Anonymous, 1996; Loetsch, 1996). Consequently, urban pollution has become a subject of increasing interest in recent years. It has challenged science and technology to provide adequate solutions.

Many studies related to urban pollution have been done in the field of human medicine and human ecology (see e.g. Dallinger *et al.*, 1992). Another field of study which is highly relevant to the urban pollution is ecotoxicology. This newly developed scientific discipline arose from a merger between ecology and toxicology. It focuses on the fate, pathways and effects of pollutants on ecosystems. With respect to its aims, it is imperative that ecotoxicologists become involved in combating urban pollution. Unfortunately only a few ecotoxicological studies have been directed at urban pollution problems.

Development of Ecotoxicology

In the beginning, ecotoxicology was only a small branch of human toxicology; however in the last two decades there has been an exponential growth of this science, both in terms of the number of scientific publications and its application in environmental management. The explosion of interest in the well-being of the environment among politicians and the general public has ensured that ecotoxicology can stand alone as an independent scientific discipline (Depledge, 1993).

Ecotoxicology is the study of toxic effects of chemical and physical agents on living organisms, especially on populations and communities within defined ecosystems; it includes the transfer pathways of these agents and their interaction with the environment (Van Leeuwen & Hermens, 1995). Ecotoxicology is also known as the study of the three S's, i.e. the study of the toxic effects of substances on nonhuman species in complex systems. The field of interest lies beyond that of direct chemical toxicity to humans, rather, the focus is on the far more subtle effects that pollutants exert on natural biota (Forbes & Forbes, 1994).

Although ecotoxicology has become significantly differentiated from toxicology, it still has a close connection to human toxicology. There are, at least, two rationales for this connection: (1) human beings not only alter the environment but also produce and release pollutants, and (2) all changes in the environment or biota may affect, directly or indirectly, the physical, economic, or aesthetic well-being of mankind (Butler, 1984). The protection of human beings will always be an important goal in the risk assessment of chemicals. Man can be exposed through the environment directly via inhalation, soil ingestion and dermal contact, and indirectly via food products and drinking water (Van de Meent *et al.*, 1995). It is interesting to note that in recent years, the integration of human health concerns into ecotoxicological studies seems to receive more and more attention; as can be seen from the founding of societies such as the Society for Ecosystem Health and pertinent journals.

One implication of the development from toxicology into ecotoxicology is that the approach of chemical risk assessment has changed from a reactive approach (repairing damage and restoring life), to a proactive approach (enhancing, protecting and preserving the ecosystem) (Chapman, 1995). The use of ecological parameters in pollution risk assessment forms the backbone of an ecotoxicological work. Complementary to the chemical approach, degradation of environmental quality may be identified through its ecological receptor, i.e. organisms living in the exposed ecosystem (Van Straalen, 1994). The chemical approach, which relies mostly on measurements of the concentration of toxic substances in the abiotic component of the ecosystem (water, soil and air), very often produces unrealistic results, since it neglects the interaction between toxic substances and the organisms (Spellerberg, 1991; Widianarko *et al.*, 1994). A clear advantage of the ecological approach in pollution studies is that it provides a possibility to demonstrate the actual damage resulting from a pollution event. Several ecological indicator systems have been proposed for monitoring pollution. They can be based on quantitative relationships between environmental exposure and tissue concentrations of ecological specimens, or on the disturbance or inhibition of ecological processes (Dallinger *et al.*, 1992; Cotrufo *et al.*, 1995).

In its current practice, however, ecotoxicology still largely focuses on toxicological experiments in the laboratory and the fate of chemicals in the environment (Baird *et al.*, 1996; Kareiva *et al.*, 1996). The current approach leaves ecology simply as a 'seasoning' and not as 'main ingredient' (Kareiva *et al.*, 1996). Consequently, emerging theories in ecotoxicology are rooted largely in the understanding of toxic mechanisms and demonstration of effects that occur in controlled conditions when all variables are held constant (Baird *et al.*., 1996). The limited contribution of ecology to ecotoxicology may be due to the enormous diversity of ecotoxicological problems, particularly the wide variation in relative scaling of chemical persistence and distribution, the mobility and reproductive rates of affected organisms, and the stability and physical structure of habitats which the pollutant and organism co-occupy (Jepson & Sherratt, 1996).

If the aim of ecotoxicology, i.e. prediction of actual effects of chemicals in real ecosystems, is to be maintained, more ecological knowledge has to be incorporated in ecotoxicology. The incorporation of the ecological dimension will add certainty to risk predictions (Jepson & Sherrat, 1996). The use of an ecological approach implies that ecotoxicology should take the variability of ecological systems into account. The effects of any perturbation, including that of environmental contaminants, depend on the spatial scale of the event, the composition of the surrounding landscape and the dispersal rates of affected organisms (Kareiva *et al.*, 1996).

As in other areas of the world, ecotoxicology (or environmental toxicology) in South East Asia (SEA) is receiving increasing attention and showing significant growth. Confronted with the various kinds of environmental problems, many of which need immediate solution, ecotoxicology is expected to play a more concrete role, either through fundamental or applied research; however, due to the fact that this scientific discipline is still young, there is a substantial gap between development of ecotoxicology (as with other environmental sciences) and the environmental problems resulting from the rapidly increasing economic activities of the region. To fill this gap, as well as to contribute to the advancement of knowledge, research programmes adapted to the SEA context are needed. Scientists working in this region should explore the region's advantages, such as ecosystem richness, to develop research programmes which can answer both needs (Widianarko & Van Straalen, subm., Chapter 2).

The current emphasis of environmental toxicological research in SEA seems to be on single species toxicity studies and on spatial distribution of toxicants. In terms of data generation and description of the present environmental status, these approaches may be useful; but this will not be the case, if we want to proceed to the more fundamental aim of ecotoxicology, i.e. the protection of ecosystem sustainability under toxicant threats. Lacher & Goldstein (1997) argued that single-species toxicity testing may be of limited value in the tropics, because the biodiversity is extremely high. Unless a convincing argument can be made for a given species as a valuable indicator, the use of assemblage, ensemble, community or ecosystem level indices should be in favour.

A relevant question is whether the generation of locally relevant data, upon which environmental policy makers in SEA can base decisions concerning questions of immediate urgency, should follow the approaches developed in European and North American countries, or should follow from the development of its own "niche". Outlining future trends in ecotoxicology in the information age, Cairns (1995) stated that ecotoxicological information will require site-specific relevance which implies, among other things, that standardized tests must be modified into highly site-specific tests using indigenous organisms. Due to the fact that 'indigenous species' is not an easy term to define, the use of locally abundant organisms will serve the needs of site-specific ecotoxicological studies.

Ecotoxicology of Urban Pollution

Urban pollution takes many different forms including air pollution, waste water disposal, and solid and hazardous waste discharges from industrial as well as domestic activities. With respect to its contribution to global environmental problems, urban pollution can be regarded as one of the most significant environmental problems. Most of urban pollution directly contributes to the disturbance of coastal ecosystems.

Approximately 70% of the human population now lives within 60 km of an ocean coast, and this percentage is increasing (Forbes & Forbes, 1994). Only seven out of the 50 largest cities of the world are isolated from the coast. The disturbance experienced by coastal environments is clearly evident from the fact that of the 50 largest cities of the world, half are situated directly on an estuary, with another third being located further up a river from an estuary (Forbes & Forbes, 1994). Estuaries have been considered by industry to be economically valuable sites for development given the possibilities for trade, traffic and ease of pollutant discharge. From an ecological perspective, however, estuarine environments are problematic because of their pronounced tendency to trap and accumulate particle-bound pollutants such as heavy metals and many organic chemicals (see e.g. Prudente *et al.*, 1994; Fernandes *et al.*, 1994).

According to the recent data compiled by the World Resources Institute, coastal ecosystems, which are one of the richest storehouses of marine biodiversity, are threatened by development related activities along roughly half of the world's coasts (Anonymous, 1996). It is estimated that about 34 percent of the world's coasts are at high risk of degradation, while another 17 percent are at moderate risk.

Basically, urban pollution is a combination of pollution emitted from various sources in a defined spatial scale. In this case exposure assessment, that is the first phase of chemical risk assessment, is of paramount importance. Ideally, an exposure assessment would be performed using reliable and representative environmental monitoring. Such data, however, are seldomly available as the number of monitoring points, monitoring frequency and/or monitoring techniques are often inadequate. When data are available, they are liable to large variability because the emitted quantities vary from place to place (due to differences in production, processing, consumption and disposal) and because of differences in the environmental fate of a substance, which depends on environmental conditions (e.g. hydrology, soil type, temperature, etc.) and on the time elapsed since its release. Therefore, measured exposure data should only be used when it has been established that they are reliable and representative (Vermeire & Van der Zandt, 1995).

Urban pollution is characterized by the existence of two types of emission sources of pollutants, namely point and non-point sources. Accordingly three features of urban pollution should be considered, i.e., (1) the spatial distribution of pollutants in the urban ecosystem tends to be patchy or aggregated, (2) in most of the areas the exposure to pollutants occurs at relatively low doses over extended periods of time and (3) in some areas high peaks of pollutants occur for short periods of time (see e.g. Dallinger *et al.*, 1992; Croxford *et al.*, 1996; Smith *et al.*, 1996; Von Steiger *et al.*, 1996; Miles & Tome, 1997).

The patchiness of pollutants in the urban ecosystem is a logical consequence of the aggregation of the urban industrial areas and discharges produced therefrom. The chronic nature of urban pollution emitted by non-point sources, such as urban runoff and atmospheric deposition, is associated with long-term rather than acute effects. The short-term high peaks of pollutants are due to emission from point sources, i.e. industrial discharges.

The traditional assessment of ecotoxicological impacts is often based on the presumption of "balance of nature"; however, ecosystems vary in time and space, so this invalidates the assumption of equilibrium (Wiens, 1996). Spatio-temporal fluctuations of ecosystems have implications for the impact of toxic events on population survival. Population reduction from a single toxic event can be propagated through the landscape. In extreme cases, instability in metapopulation dynamics can result in regional species extinction. Therefore, the effect of a toxic event can extend beyond the area of direct impact and can last much longer than the duration of the effectiveness of the substance (Fahrig & Freemark, 1995).

Examination of the effects of toxic chemicals on a scale larger than usually considered in environmental toxicology has led to the establishment of a new approach in ecotoxicology, i.e. landscape ecotoxicology (Cairns & Niederlehner, 1996). This new aproach is characterized by the use of endpoints which make due allowance for (1) spatial distribution of toxicants, (2) interactions between physical and temporal patterns, and (3) the processes of ecological impairment, and integration of toxicity at different scales. The above characterization is logical, since actual effects of a toxic chemical on a landscape population depend on (1) the spatio-temporal configuration of the population in the landscape, and (2) the spatio-temporal distribution of toxic chemicals in the subjected landscape (Fahrig & Freemark, 1995).

Heavy Metals in the Urban Environment

Heavy metals, such as lead (Pb), cadmium (Cd), copper (Cu) and zinc (Zn) are usually associated with the impact of urbanization (Pirrone & Keeler, 1996). It has been reported by many authors that children are particularly affected by urban metal emissions (see e.g. Barbash, 1984; Dallinger *et al.*, 1992; Francek *et al.*, 1994; Sutton *et al.*, 1995). Children may ingest metals in relatively large amounts due to mouthing (pica) behaviour and playing outdoors. Soil or dust particles can thus be transferred from their hands or play objects into their mouths (Van Wijnen *et al.*, 1990). This risk is particularly high in urban areas, where there is a scarcity of land. Most of the childrens playgrounds may be subjected to serious urban metal emissions. Hence, children playing in these playgrounds are exposed to a potential health risk (Wong & Mak, 1997). Some metals, specifically Pb and Hg have been associated with mental retardation in children, due to the sensitivity of the developing brain (Morselt, 1991). Other metals (Cd) accumulate in the kidney and may cause renal dysfunction. Non-essential metals, such as Cd, Cr and Ni, have also been found to be carcinogenic (Morselt, 1991 & Gurjar *et al.*, 1996).

Concentrations of heavy metals may be used as indicators of urbanization. Rapid urbanization and industrialization have resulted in the emission of contaminants, including heavy metals, in the atmosphere of urban areas (Gurjar *et al.*, 1996). Among different urban pollutants, toxic metals, such as lead, cadmium, zinc and copper, play an important role. Lead is mostly emitted by traffic exhausts, whereas cadmium is released by automobile traffic, and by the burning of refuse and fossil fuels (see e.g. Anonymous, 1993a). Despite of the fact that zinc is one of the most important building materials, it is also emitted by automobiles (Wong & Mak, 1997). Various industries, especially metal working industries, emit copper into the urban environment (Woskie *et al.*, 1996). Household activities also contribute to metal pollution, e.g. through discharges of washing products (Jenkins & Russell, 1994).

In Indonesia and other South East Asian countries, urban metal pollution has received considerable attention, especially with regard to the quality of urban rivers and food safety. The urban river is a source of drinking water, a place for sewage disposal, a mode of transportation, a recreational area, a source of food, and, more often than not, a rubbish dump (Low & Balamurugan, 1991; Djuangsih & Salim, 1994). With ever progressive urban development and population pressure, many of these rivers are increasingly polluted. The major sources of water pollution in South East Asian cities are: sewage effluent and untreated faecal matter; industrial effluent and untreated industrial wastes, domestic and industrial garbage and polluted sediment, transported from upstream areas.

The level of lead pollution in the water of one of the largest cities of Indonesia, Surabaya, is approximately 17 times the WHO threshold level (2 mg/L). In Jakarta, pollution exceeds the acceptable level over 25 times (Anonymous, 1993b). The average level of lead in Surabaya's estuaries is 34.4 mg/L, whereas in the Jakarta Bay area, lead concentrations in the water have been found to be between 50 and 200 mg/L. At one point almost 400 mg/L of lead was recorded. According to a study by the Department of Health and Department of Agriculture in 1980, the highest mercury content in the Jakarta Bay sea water had reached 350 times the permissible level of 0.5 mg/L. Meanwhile, 80% of the fish samples studied contained more than 0.5 mg/kg mercury. Closer to the shore, namely around the delta villages, the Environmental Research Institute of the Jakarta Special Territory Government found that the maximum lead level in the sea water exceeded the safety level 10,000 times; the maximum copper level exceeded the safety level 1000 times; and the mercury level 100 times (Anonymous, 1993b). Other reports showed that the lead content of vegetables grown in the urban area of Jakarta and irrigated with urban surface water, was far beyond the WHO's standard. A study of the edible kangkung (*Ipomoea aquatica*) showed lead concentrations of 29.9 μg/g and 15.5 μg/g respectively for the leaf and stem (Anonymous, 1994).

Evidence for transfer of metals from industrially contaminated areas to plants is well established (see e.g. McKenna *et al.*, 1993; Dudka *et al.*, 1996).

Although studies on heavy metals in several cities in Indonesia, including Semarang, have been carried out for years (see e.g. Darmojo *et al.*, 1985; Supriharyono *et al.*, 1989; Astuti *et al.*, 1991; Anonymous, 1993_b, 1994, 1995) , the available data and publications are very limited and patchy. A similar situation has also been reported for other parts of SEA (see e.g. Din, 1995). From two available reports (Darmojo *et al.*, 1985; Supriharyono *et al.*, 1989) it appears that lead and cadmium concentrations in estuaries of six Semarang rivers exceeded the permissible levels. These studies, however, still employed a fragmentary approach, as they focused on selected sites of the urban ecosystem.

Semarang is the fifth largest city of Indonesia and has a population of 1.2 million with an average annual growth of 1.1% during 1989 to 1993 (Anonymous, 1993_c). The main activities of this capital city of the Central Java Province are trade and industry. Semarang covers an area of 373.668 square kilometres, and is situated on the northern coast of Java. Due to its topographical characteristics, i.e. a descending altitude toward the coastal line with hundreds of water courses, from rivers to small streams, the Semarang area is subject to a continuous sedimentation. Areas of higher altitude in southern parts of the city discharge run-off water and sediment to the inner-city areas.

Results of a routine chemical monitoring (see e.g. Anonymous, 1995) showed that metal concentrations in water are not very informative for the degree of pollution. Many contaminants, which are relatively insoluble in water, are known to be adsorbed by suspended particulate organic matter which eventually settles to the bottom sediment (Reynoldson & Day, 1993). This leads to the repercussion that sediment is one of the major sources of water pollution in South East Asian cities (Low & Balamurugan, 1991).

Sediments are deposits for physical debris and sinks for a wide variety of chemicals, including heavy metals. The presence of metals in sediment will certainly have an impact on organisms living in the water body. Transfer of chemicals from sediments to organisms via pore water is now considered to be a major route of exposure for many species (Adams *et al.*, 1992). Freshwater sediments play an important role in mediating chemical exchange in the aquatic environment, namely between particulate, dissolved and biological phases (Reynoldson & Day, 1993). Furthermore, information on the status of trace metal pollution in the sediment of coastal areas is of considerable importance for public health when seafood from this area is produced for consumption (Mat & Maah, 1994; Mat *et al.*, 1994).

Information on spatial distributions of metals in sediment over the whole area will provide a full picture of the extent of the city's metal contamination. With regard to the absence of sediment quality standards for metals, which are needed for the assessment and monitoring of urban metal pollution in Indonesia, a reference value for each metal and an index indicating the degree of combined metal contamination in the sediment can be derived based on the above information. Reference values and contamination indexes of chemicals are urgently needed to provide a sound basis for environmental management (Davies, 1992). In this case, Semarang can probably be used as a model for other medium sized cities in Indonesia, since no such study has been done elsewhere before.

Among the organisms present in the aquatic environment there are many that are sensitive to changes in water quality; and due to their size and the attention paid to them, constitute excellent indicator organisms. Freshwater fish have often been adopted as the sentinel organisms for the health of the freshwater environment, because they are capable of inhabiting all zones of the aquatic habitat where the turbulence and flow rate of the water, its oxygen content and other constituents of water quality permit (Solbe, 1993).

The wild guppy (*Poecilia reticulata*), which is easily found in the urban streams of Semarang is a prospective candidate for bioindication, as well as a species for laboratory toxicity tests. *P. reticulata* is an exotic species which has adapted very well to the South East Asian urban streams, after its introduction in the 1930's for mosquito control (Chou & Lam, 1989). In Singapore, *P. reticulata* is one of the most common fishes dominating the drains, canals, reservoirs and most open-water bodies (Ng *et al.*, 1993). It is reported that this species has survived in polluted waters with free ammonia concentrations of a few hundred mg/L (Chou & Lam, 1989). Although this fish has shown its persistence in streams contaminated with a wide range of urban wastes, so far almost no ecotoxicological study has been made using this species (not including the commercially-cultured guppy).

Conventional toxicological approaches and concepts based on the premise of constant exposure of chemicals were no longer adequate to deal with time-varying exposures of chemicals, such as short-term high peaks. Therefore, recently, variable-exposure has been regarded as an attractive field of study, both experimentally (see e.g. Hodson *et al.*, 1983; Holdway *et al.*, 1994; Barry *et al.*, 1995a & 1995b) and with modelling (Mancini, 1983; Landrum *et al.*, 1991; McCarty *et al.*, 1992; Bedaux & Kooijman, 1994; Hickie *et al.*, 1995; Meyer *et al.*, 1995) studies. Most of the models proposed to deal with time-variable exposure are based on the concept of critical body residues, which integrates toxicokinetics and the effect of exposure time on toxicity (McCarty & Mackay, 1993; Hickie *et al.*, 1995). This approach is promising since some studies showed that toxicity resulting from pulse exposures is largely controlled by accumulation and elimination rates of toxicant by exposed organisms (Hickie *et al.*, 1995).

To be effectively involved in urban environmental management, ecotoxicological studies should take into account the three characteristics of urban pollution described above. Presently available urban ecotoxicological studies are fragmented in nature. There is a need to combine information on the spatial distribution of chemicals with the available toxicological information. Most of the existing studies focused on particular areas of the city, such as rivers, parks (see e.g. Venkateswarlu *et al.*, 1994; Bennet & Banerjee, 1995, Pizl & Josens, 1995) and on selected biological samples, such as human body fluid, foodstuffs, vegetation, pet animals etc (see e.g. Balba, 1991; Aschengrau *et al.*, 1994; Berny *et al.*, 1994; Smith, 1994; Cotrufo *et al.*, 1995; Surif & Chai, 1995; Coni *et al* ., 1996).

Clearly, to deal with patchiness, chronic exposure concentrations, and short-term high peaks of urban pollutants, an integrative ecotoxicological study is needed. Information on spatial distribution and ecological (i.e. chronic) impacts of the pollutants should be coupled. In addition, time scale should also be considered in the exposure assessment. The final ecological risk assessment may depend on factors such as the frequency and duration of emissions, the generation times of the organisms being considered in the effects assessment or the level of refinement of the assessment. In this thesis special attention is therefore paid to spatial and temporal variation of pollution and how this can be assessed.

Objectives of This Thesis

The aim of the research presented in this thesis is to provide a full picture of the extent of urban metal pollution in Semarang through a new approach combining spatial mapping and bioindication using a dominant freshwater fish species, *Poecilia reticulata* (Chapter 3, 4 & 5). In addition, the development of a novel risk assessment methodology for a special class of time-varying concentrations, i.e. diluted exposure concentrations, which are commonly encountered in urban environments is presented in this thesis (Chapter 6 and 7). The background information on the present status of ecotoxicology in South East Asia is reviewed, especially with respect to its scientific achievements, existing environmental problems specific for the region, and future direction of research (Chapter 2).

The spatial distribution of metals in the sediments of the greater Semarang area is identified. Based on this spatial information the background concentrations of the metals present in Semarang are estimated, and a simple tool for deriving standards for metals in the sediment is also proposed in Chapter 3 (Widianarko *et al.*, subm.a).

The association between the amounts of metal in the biota, and the concentrations in the sediment and water is evaluated. In addition, possible influences of physico-chemical properties of water and sediment on the suspected relationship are determined in Chapter 4 (Widianarko *et al.*, subm.b).

The potential use of the guppy, *P. reticulata*, as a bioindicator of urban metal pollution is assessed in Chapter 5 (Widianarko, subm.) through a comparison between guppy populations from non-polluted and metal-polluted streams. This comparison is made for several parameters related to body size and reproduction. The effects of elevated levels of metals in urban streams on fish populations and possible thresholds for ecological effects are identified, as it might be used as benchmarks in biomonitoring programmes.

A toxicokinetics-based parametric survival model for decreasing exposure concentrations of chemical is presented in Chapter 6 (Widianarko & Van Straalen, 1996). This new model relates survivorship to toxicokinetics by assuming that the hazard rate (probability of dying) is related to the concentration of the toxicant in the organism. In this model an LC_{50}-time curve incorporating degradation kinetics is introduced.

Toxicokinetics patterns of Zn in *P. reticulata* and the effect of time-decreasing Zn exposure concentrations on the survival of guppies are assessed in Chapter 7 (Widianarko *et al.*, subm.$_c$). The applicability of the toxicokinetics-based survival model, proposed in Chapter 6, is tested against data on the survival of guppies under decreasing Zn exposure concentration.

References

Adams, W.J.; R.A. Kimerle & J.W. Barnett Jr. (1992). Sediment quality and aquatic life assessment. Environ. Sci. Technol. 26: 1865-1875.

Anonymous (1993$_a$). Addressing air pollution problems from mobile sources in Metro Manila. A paper presented at the 1993 Pacific Basin Conference on Hazardous Waste. Honolulu, Hawaii. Nov. 8-12.

Anonymous (1993$_b$). Measuring sediment toxins in Surabaya. Eco-Sounder. Sept. 1993.

Anonymous (1993$_c$). Kotamadya Daerah Tingkat II Semarang Dalam Angka. Kantor Statistik, Semarang.

Anonymous (1994). Timah hitam mencemari sayuran di Jakarta. KOMPAS, Nov. 1.

Anonymous (1995). Environmental Statistics of Indonesia. Central Bureau of Statistics. Jakarta.

Anonymous (1996). World Resources 1996-97, A Guide to The Global Environment: The Urban Environment. A joint publication of the WRI, UNEP, UNDP & World Bank Oxford Univ. Press. Oxford.

Aschengrau, A.; A. Beiser; D. Bellinger; D. Copenhafer & M. Weitzman (1994). The impact of soil lead abatement on urban children's blood lead level: Phase II. results from the Boston Lead-In-Soil demonstration project. Environ. Res. 67: 125-148.

Astuti; Y.; N.H. Suprapti & M. Hadi (1991). Keanekaragaman benthos sebagai bioindikator pencemaran logam Pb, Hg dan Cd di pantai utara Jawa Tengah. In R.B. Darmojo; A.G. Soemantri; R. Miranda & J. Herminanto (eds.): Bunga Rampai PIP - UNDIP II. Lembaga Penelitian Universitas Diponegoro, Semarang, pp. 5-13.

Baird, D.J.; L. Maltby; P.W. Greig-Smith & P.E.T. Douben (eds) (1996). Ecotoxicology: Ecological Dimensions. Chapman & Hall. London.

Balba, A.M.; G. El Shibiny & E.S. El Khatib (1991). Effect of lead increments on the yield and lead content of tomato plants. 57-58: 93-99.

Barbash, N.B. (1984). Physical development of infants as an indicator of the condition of the urban environment. Soviet Geography pp. 204-213.

Barry, M.J.; D.C. Logan; J.T. Ahokas & D.A. Holdway (1995a). Effects of esfenvalerate pulse-exposure on the survival and growth of larval Australian crimson-spotted rainbow fish *(Melanotaenia fluviatilis)*. Environ. Toxicol. Wat. Qual. 10: 267-274.

Barry, M.J.; D.C. Logan, R.A. Van Dam; J.T. Ahokas & D.A. Holdway (1995b). Effects of age and weight-specific respiration rate on toxicity of esfenvalerate pulse-exposure to the Australian crimson-spotted rainbow fish (*Melanotaenia fluviatilis*). Aquat. Toxicol. 32: 115-126.

Bedaux, J.J.M. & S.A.L.M. Kooijman. 1994. Statistical analysis of bioassay based on hazard modelling. Environ. Ecol. Statistics 1: 303-314.

Bennet, J.P. & M. Banerjee (1995). Air pollution vulnerability of 22 Midwestern Parks. J. Environ. Man. 44: 339-360.

Berny, P.J.; L.M. Cote & W.B. Buck (1994). Relationship between soil lead, dust lead, and blood lead concentrations in pets and their owners: Evaluation of soil lead threshold values. Environ. Res. 67: 84-97.
Butler, G.C. (1984). Developments in ecotoxicology. Ecol. Bull. 36: 9-12.
Chapman, P.M. (1995). Ecotoxicology and pollution - key issues. Invited Keynote Presentation to the International Conference on Marine Pollution and Ecotoxicology. Hong Kong, 22-25 Jan.
Cairns, J. Jr. (1995). Future trends in ecotoxicology. In J. Cairns Jr. & B.R. Niederlehner (eds.): Ecological Toxicity Testing - Scale, Complexity, and Relevance. Lewis Publ. Boca Raton. pp. 217-222.
Cairns, J. Jr. & B.R. Niederlehner (1996). Developing a field of landscape ecotoxicology. Ecol. Appl. 6: 790-796.
Chou, L.M. & T.J. Lam (1989). Introduction of exotic aquatic species in Singapore. In S.S. Silva (ed.) : Proc. of a Workshop on Introduction of Exotic Aquatic Organisms in Asia. Spec. Publ. Asian Fish Soc. 3: 91-97.
Coni, E.; A. Bocca; P. Coppoli; S. Caroli; C. Cavalluci & M.T. Marinucci (1996). Minor and trace element content in sheep and goat milk and dairy products. Food Chem. 57: 253-260.
Cotrufo, M.F.; A.V. De Santo; A. Alfani; G. Bartoli & A. De Cristofaro (1995). Effects of urban heavy metal pollution on organic matter decomposition in *Quercus ilex* L. Woods. Environ. Pollut. 89: 81-87.
Croxford, B.; A. Penn & B. Hillier (1996). Spatial distribution of urban pollution - civilizing urban traffic. Sci. Total Environ. 190: 3-9.
Dallinger, R.; B. Berger & S. Birkel (1992). Terrestrial isopods: Useful biological indicators of urban metal pollution. Oecologia 89: 32-41.
Darmojo, R.B.; A.G. Soemantri; H.R. Sunoko & R. Miranda (1985). Monitoring logam berat di perairan pantai Semarang. In R.B. Darmojo; R. Hartono; A.G. Soemantri & R. Miranda (eds): Bunga Rampai PIP - UNDIP I. Lembaga Penelitian Universitas Diponegoro, Semarang, pp. 17-22.
Davies, B.E. (1992). Trace metals in the environment, retrospect and prospect. In D.C. Adriano (ed.): Biogeography of Trace Metals in Invertebrates. Lewis Publishers. Boca Raton. pp. 383-399.
Depledge, M.H. (1993). Ecotoxicology: A science or management tool ? Ambio 22: 51-52.
Din, Z.B. (1995). Natural and anthropogenic trace-metal input into the coastal and estuarine sediments of the straits of Malacca. Bull. Environ. Contam. Toxicol. 55: 666-673.
Djuangsih, N. & H. Salim (1994). Preliminary study on the environmental pollution in the Citarum river basin, West Java, Indonesia. In B. Widianarko; K. Vink & N.M. Van Straalen (eds.): Environmental Toxicology in South East Asia. VU University Press. Amsterdam. pp. 277-288.
Dudka, S.; M. Piotrowska & H. Terelak (1996). Transfer of cadmium, lead and zinc from industrially contaminated soil to crop plants - a field study. Environ. Pollut. 94: 181-188.
Fahrig, L. & K. Freemark (1995). Landscape-scale effects of toxic events for ecological risk assessment. In J. Cairns Jr. & B.R. Niederlehner (eds.): Ecological Toxicity Testing - Scale, Complexity, and Relevance. Lewis Publ. Boca Raton. pp. 193-207.
Fernandes, H.M.; E.D. Bidone; L.H.S. Veiga & S.R. Patchineelam (1994). Heavy-metal pollution assessment in the coastal lagoons of Jacarepagua, Rio de Janeiro, Brazil. Environ. Pollut. 85: 259-264.
Forbes, V.E. & T.L. Forbes. (1994). Ecotoxicology in Theory and Practice. Chapman and Hall, London, 277 pp.
Francek, M.A.; B. Makimaa; V. Pan & J.H. Hanko (1994). Small town lead levels: A case study from the homes of pre-schoolers in Mt. Pleasant, Michigan. Environ. Pollut. 84: 159-166.
Gurjar, B.R.; M. Mohan & K.S. Sidhu (1996). Potential health risks related to carcinogens in the atmospheric environment in India. Reg. Toxicol.

Pharmacol. 24: 141-148.
Hickie, B.E.; L.S. McCarty & D.G. Dixon (1995). A residue-based toxicokinetic models for pulse-exposure toxicity in aquatic systems. Environ. Toxicol. Chem. 14: 2187-2197.
Hodson, P.V.; B.R. Blunt; U. Borgmann; C.K. Minns & S. McGraw (1983). Effect of fluctuating lead exposures on lead accumulation by rainbow trout *Salmo gairdneri*. Environ. Toxicol. Chem. 2: 225-238.
Holdway, D.A.; M.J. Barry; D.C. Logan; D. Robertson; V. Young & J.T. Ahokas (1994). Toxicity of pulse-exposed fenvalerate and esfenvalerate to larval Australian crimson-spotted fish (*Melanotaenia fluviatilis*). Aquat. Toxicol. 28: 169-187.
Jenkins, D. & L.L. Russell (1994). Heavy metals contribution of household washing products to municipal wastewater. Water Environ. Res. 66: 805-813.
Jepson, P.C. & T.N. Sherratt (1996). The dimensions of space and time in the assessment of ecotoxicological risks. In D.J. Baird; L. Maltby; P.W. Greig-Smith & P.E.T. Douben (eds): Ecotoxicology: Ecological Dimensions. Chapman & Hall. London. pp. 43-54.
Kareiva, P.; J. Stark & U. Wennergren (1996). Using demographic theory, community ecology and spatial models to illuminate ecotoxicology. In D.J. Baird; L. Maltby; P.W. Greig-Smith & P.E.T. Douben (eds): Ecotoxicology: Ecological Dimensions. Chapman & Hall. London. pp. 55-69.
Lacher, T.E. Jr. & M.I. Goldstein (1997). Tropical ecotoxicology: status and needs. Environ. Toxicol. Chem. 16: 100-111.
Landrum, P.F.; B.J. Eadie & W.R. Faust (1991). Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphiphod *Diporeia sp.* Environ. Toxicol. Chem. 10: 35-46.
Loetsch, B. (1996). The eco-city: criteria for an urban ecological sound city. In Urban Habitat: The Environment of Tomorrow. Bouwstenen 41: 97-102.
Low, K.S. & G. Balamurugan (1991). Urbanization and urban water problems in South East Asia: a case of unsustainable development. J. Env. Man. 32: 195-209.
Mancini, J.L. (1983). A method for calculating the effects on aquatic organisms, of time varying concentrations. Water Res. 17: 1335-1362.
Mat, I. & M.J. Maah (1994). Sediment trace metal concentrations from the mudflats of Kuala Juru and Kuala Muda of Malaysia. Bull. Environ. Contam. Toxicol. 53: 740-746.
Mat, I.; M.J. Maah & A. Johari (1994). Trace metals in sediments and potential availability to *Anadara granosa*. Arch. Environ. Contam. Toxicol. 27: 54-59.
McCarty, L.S.; D. Mackay; A.D. Smith; G.W. Ozburn & D.G. Dixon (1992). Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: neutral narcotic organics. Environ. Toxicol. Chem. 11: 917-930.
McCarty, L.S. and D. Mackay (1993). Enhancing ecotoxicological modelling and assessment. Environ. Sci. Technol. 27: 1719-1727.
McKenna, I.M.; R.L. Chaney & F.M. Williams (1993). The effects of cadmium and zinc interactions on the accumulation and tissue distribution of zinc and cadmium in lettuce and spinach. Environ. Pollut. 113-120.
Meyer, J.S.; D.D. Gulley; M.S. Goodrich; D.C. Szmania & A.S. Brooks (1995). Modeling toxicity due to intermittent exposure of rainbow trout and common shiners to monochloramine. Environ. Toxicol. Chem. 14: 165-175.
Miles, A.K. & M.W. Tome (1997). Spatial and temporal heterogeneity in metallic elements in industrialized aquatic bird habitats. Environ. Pollut. 95: 75-84.
Morselt, A.F.W. (1991). Environmental pollutants and diseases. Toxicology 70: 1-132.
Ng, P.K.L.; L.M. Chou; T.J. Lam (1993). The status and impact of introduced freshwater fish. Biological Conservation 64: 19-24.
NRC (1993). Managing Wastewater in Coastal Urban Areas. Nat. Acad. Press.

Washington DC.
Pirrone, N. & G.J. Keeler (1996). A preliminary assessment of the urban pollution in the great lakes region. Sci. Total Environ. 190: 91-98.
Pizl, V. & G. Josens (1995). Earthworm communities along a gradient of urbanization. Environ. Pollut. 90: 7-14.
Prudente, M.S.; H. Ichishashi & R. Tatsukawa (1994). Heavy metal concentrations in sediments from Manila Bay, Philippines and inflowing rivers. Environ. Pollut. 86: 83-88.
Reynoldson, T.B. & K.E. Day (1993). Freshwater sediments. In P. Calow (ed.): Handbook of Ecotoxicology. Vol. I. Blackwell Sci. Publ. Oxford-London. pp. 83-100.
Smith, C.J.; P. Hopmans & F.J. Cook (1996). Accumulation of Cr, Pb, Cu, Ni, Zn and Cd in soil following irrigation with treated urban effluent in Australia. Environ. Pollut. 94: 317-323.
Smith, S.R. (1994). Effect of soil pH on availability to crops of metals in sewage sludge-treated soils II, cadmium uptake by crops and implications for human dietary intake. Environ. Pollut. 86: 5-13.
Solbe, J.F.D.L.G. (1993). Freshwater fish. In P. Calow (ed.): Handbook of Ecotoxicology. Vol. I. Blackwell Sci. Publ. Oxford-London. pp. 66-82.
Spellerberg, I.F. (1991). Monitoring Ecological Change. Cambridge University Press. Cambridge.
Supriharyono, H.R. Sunoko & B.T. Basuki (1989). Monitoring logam berat di perairan pantai Semarang. Lembaga Penelitian Universitas Diponegoro, Semarang. (unpublished)
Surif, S. & C.Y. Chai (1995). Screening of lead exposure among workers in Selangor and Federal Territory in Malaysia. Environ. Pollut. 88: 177- 181.
Sutton, P.M.; M. Athanasoulis; P. Flessel; G. Guirguis; M. Haan; R. Schlag & L.R. Goldman (1995). Lead levels in the household environment of children in three high risk communities in California. Environ. Res. 68: 45-57.
Van De Meent, D.; J.H.M. De Bruijn; F.A.A.M. De Leeuw; A.C.M. De Nijs; D.T. Jager & T.G. Vermeire (1995). Exposure modelling. In C.J. Van Leeuwen & J.L.M. Hermens (eds.): Risk Assessment of Chemicals: An Introduction. Kluwer Acad. Publ. Dordrecht. pp. 103-145
Van Leeuwen, C.J. & J.L.M. Hermens (eds.) (1995). Risk Assessment of Chemicals: An Introduction. Kluwer Acad. Publ. Dordrecht.
Van Straalen, N.M. (1994). Biodiversity of ecotoxicological responses in animals. Neth. J. Zool. 44: 112-129.
Van Wijnen, J.H.; P. Clausing & B. Brunekreef (1990). Estimated soil ingestion by children. Environ. Res. 147-162.
Venkateswarlu, V.; P.M. Reddy & B.R. Kumar (1994). Heavy metal pollution in the rivers of Andhra Pradesh, India. J. Environ. Biol. 15(4): 275-282.
Vermeire, T. & P. Van Der Zandt (1995). Procedures of hazard and risk assessment. In C.J. Van Leeuwen & J.L.M. Hermens (eds.): Risk Assessment of Chemicals: An Introduction. Kluwer Acad. Publ. Dordrecht. pp. 293-337
Von Steiger, B,; R. Webster, R. Schulin & R. Lehmann (1996). Mapping heavy metals in polluted soil by disjunctive kriging. Environ. Pollut. 94: 205-215.
Walker, K.P. (1994). Urban runoff and combined sewer overflows. Water Environ. Res. 66: 305-309.
Widianarko, B.; K.H. Timotius & K. Vink (1994). Ecotoxicological approaches to environmental standards, with special reference to soil quality. In B. Widianarko, K. Vink & N.M. van Straalen (eds): Environmental Toxicology in South East Asia. VU University Press. Amsterdam. pp. 7-18.
Widianarko, B. & N.M. Van Straalen (1996). Toxicokinetics-based survival analysis in bioassays using nonpersistent chemicals. Environ. Toxicol. Chem. 15: 402-406.
Widianarko, B. & N.M. Van Straalen. Scientific research on environmental toxicology in South East Asia: a view of its position in the international arena. (To appear in the Australasian Journal of Ecotoxicology)

Widianarko, B.; R.A. Verweij; C.A.M. Van Gestel & N.M. Van Straalen. Spatial distribution of trace metals in sediments from urban streams of Semarang, Central Java, Indonesia. [submitted$_a$]

Widianarko, B.; C.A.M. Van Gestel; R.A. Verweij & N.M. Van Straalen. Associations between trace metals in sediment, water and guppy, *Poecilia reticulata* (Peters) from urban streams of Semarang, Indonesia. [submitted$_b$]

Widianarko, B. Heavy metals in urban streams of Semarang: bioindication potential of body size and reproductive parameters of the guppy (*Poecilia reticulata*). [Submitted]

Widianarko, B.; F.X.S. Kuntoro; C.A.M. Van Gestel & N.M. Van Straalen. Toxicokinetics and toxicity of zinc decreasing-exposure in field guppy, *Poecilia reticulata* (Peters). [Submitted$_c$]

Wiens, J.A. (1996). Coping with variability in environmental impact assessment. In D.J. Baird; L. Maltby; P.W. Greig-Smith & P.E.T. Douben (eds): Ecotoxicology: Ecological Dimensions. Chapman & Hall. London. pp. 55-69.

Wong, J.W.C. & N.K. Mak (1997). Heavy metal pollution in children playgrounds in Hong Kong and its health implications. Environ. Technol. 18: 109-115.

Woskie, S.R.; M.A. Virji; D. Kriebel; S.R. Sama; D. Eberiel; D.K. Milton; S.K. Hammond & R. Moureeraso (1996). Exposure assessment for a field investigation of the acute respiratory effects of metalworking fluids. 1. Summary of findings. Am. Indust. Hyg. Assoc. J. 57: 1154-1162.

CHAPTER 2

Scientific Research on Environmental Toxicology in South East Asia: A View of Its Position in the International Arena

with Nico M. van Straalen

ABSTRACT

Environmental toxicology in South East Asia, as other areas of the world is receiving increasing attention and shows significant growth. Three questions which are more or less specific to the area: (1). the participation of South-East Asian (SEA) scientists in international networks and scientific journals, (2). the environmental problems specific for the region, and (3). the use of indigenous species for toxicity testing are renewed in this paper. Based on a survey of seven journals, it can tentatively be concluded that SEA scientists are under-represented in the international general journals on environmental toxicology. Their works are mostly focused on studies that rely on well established methods, i.e. single species toxicity test and spatial distribution of toxicants. A great variation in the number of articles published from different countries is also observed. Confronted with various kinds of environmental problems, many of which need immediate solution, environmental toxicology is expected to play a more concrete role, either through fundamental research or applied research. To contribute to environmental problem solving in the region, and to increase the contribution to the scientific knowledge, SEA scientists utilize the comparative advantage of the region, i.e. its ecological richness.

1. Introduction

Environmental toxicology is receiving increasing attention in the South-East Asian region. This situation is not different from other parts of the world, but the conditions under which ecotoxicology is developing in South East Asia (SEA) are different from elsewhere. As a science, there is no reason why environmental toxicology should be different because of geographic distinctions. In this age of electronic communication, few barriers exist that prohibit the dissemination of scientific information. Experience teaches, however, that exchange of information is limited.

Independent activities already exist in each country of the region, both in the field of teaching and in research programmes. Unfortunately, in terms of scientific networks, environmental toxicologists in SEA have limited forum to interact with their colleagues from the same region. Until recently, no society and no scientific journal on environmental toxicology have been established in the region (note: only recently has an embryo of the Asia-Pacific branch of SETAC been formed).

The lack of a network could lead to the loss of opportunities to address regional issues in a coordinated manner. Moreover, lack of scientific communication between environmental toxicologists in the region may lead to absence of peer review, repetition and duplication of efforts.

Environmental toxicology has to face various pollution problems which need immediate solutions, but this scientific discipline is still young. This leads to a substantial gap between the development of environmental toxicology (as with other environmental sciences) and the environmental problems resulting from the rapidly increasing economic activities. This gap will certainly contribute to shaping the scientific orientation of institutes that work in the region.

Research programmes on environmental toxicology, that exist al-present in SEA, cover a wide area of interests and approaches. Research programmes adapted to the SEA context are needed to contribute better to the solution of environmental problems in the region as well as to the advancement of knowledge. Scientists working in this region should explore the region's advantages, such as ecosystem richness, to develop research programmes which can answer both needs.

A review of three issues that we consider to be critical for present day environmental toxicology in South-East Asia is presented in this paper: The three issues are: (1) the participation of SEA scientists in international networks, (2) environmental problems needing immediate solution, and (3) the direction of research including the need for using indigenous species and ecosystems.

2. International Participation

The developers of environmental toxicology in SEA deserve credit for their rapid acquisition of knowledge developed in more advanced regions. Environmental toxicology which has experienced rapid development in the past two decades, was recognised by scientists in SEA shortly after its inception in the US and Europe. In Indonesia, for example pioneering in this field can be traced back to the early 70's (Djuangsih, 1993).

Interest in environmental toxicology has been growing in SEA in the last few years. Most likely, this trend will be maintained in the years to come. To date, environmental toxicology has been introduced into many curricula, either in under-graduate or post-graduate courses. Moreover, research programmes in environmental toxicology have also been implemented in many SEA institutes and universities.

The presence of international organisations, such as NETTLAP (Network of Environmental Training at Tertiary Level in Asia Pacific), an educational unit of UNEP, is of considerable importance for the region. NETTLAP has organised several workshops and symposia on environmental education and training at the tertiary level, which also cover environmental toxicology (Hay & Thom, 1993). Access to NETTLAP is facilitated by the appointment of a focal point for each member country.

Although research activities in this field have spread over the countries of SEA, a network of scientists working in this field barely exists, even at a national level, there is often no network. In some cases, however, scientific meetings have been organised at the national level (see e.g. Soemardi & Notosoedarmo, 1987).

Initiatives, such as a conference on environmental toxicology in SEA at Salatiga, Indonesia in 1992, have proven to be a good forum to identify the activities and potentials of networking at the regional level (Widianarko *et al.*, 1994). The continuation of such activities with a regular frequency appears to be difficult to realise. The lack of a network may put the progress of ecotoxicology in SEA at risk, because achievements obtained by one researcher will not directly benefit a colleague working at another place.

A survey of the following seven relevant international journals was conducted to obtain an indication of the present international participation of SEA environmental toxicologists: Aquatic Toxicology, Environmental Toxicology and Chemistry, Environmental Pollution, Archives of Environmental Contamination and Toxicology, Chemosphere, Ecotoxicology and Environmental Safety, and Bulletin of Environmental Contamination and Toxicology. These journals were selected because they cover a range of environmental aspects and cover a range of impact ratings. All volumes appearing in the years 1993 through 1995 were screened for the affiliation of the first author and articles reporting on work conducted in SEA countries were classified regarding the topic of research.

During 1993 to 1995, contribution of SEA scientists in these journals amounted to 36 out of 4154 articles, or about 0.8%. Results of the survey are shown in Table 1, 2 and 3. With only one exception (Bulletin of Environmental Contamination and Toxicology) the contribution of SEA scientists in all of the journals was below 1%. The level of contribution is not correlated with the impact rates of the journals, although most South East Asian contributions may be found in the Bulletin of Environmental Contamination and Toxicology which has the lowest impact rate among the journals covered in this study.

In the last three years (1993, 1994 and 1995), the contribution of SEA scientists to these journals has addressed a wide variety of areas, which can be categorised into nine areas, i.e. (1) spatial distribution of toxicants (Abdullah *et al.*, 1994; Mat & Maah, 1994; Mat *et al.*, 1994; Prudente *et al.*, 1994; Tan &Vijayaletchumy, 1994; Din, 1995; Maah *et al.*, 1995; Tan, 1995), (2) toxicity studies (Curvin-Aralar &Aralar, 1993; Din & Abu, 1993; Vogt & Quinito, 1994; Curvin-Aralar &Aralar, 1995; Kim Oanh & Bengtsson, 1995; Law, 1995; Low & Sin, 1995; Shazili, 1995; Vink *et al.*, 1995), (3) environmental health (Mokhtar *et al.*, 1994; Ong *et al.*, 1993; Surif & Chai, 1995), (4) biodegradation (Sahid & Wei, 1993; Sahid & Teoh, 1994), (5) microbial toxicology (Sahid & Zaabar, 1993; Sahid & Yap, 1994), (6) toxicants in food (Ibrahim, 1993; Mat, 1994), (7) waste treatment (Low *et al.*, 1995; Millamena, 1994), (8) field toxicity studies (Calumpang *et al.*, 1995; Kim-Oanh *et al.*, 1995), and (9) atmospheric pollution (Eong, 1993; Murdiyarso, 1993; Snidvongs, 1993; Soepadmo, 1993; Soegiarto, 1993; Husin *et al.*, 1995).

It can be seen from the research areas listed above that the environmental toxicological works in SEA are mostly concentrated on studies that rely on well established methods, i.e. toxicity studies (single species) and spatial distribution

Table 1. Number of articles by authors from South East Asia, India, Japan, China and other Asian countries in seven international journals with different impact rates (ISI, 1993) during 1993-1995. The figures between brackets express the percentages in terms of the total number of articles published by the journals

Journal	Impact Rate	South East Asia	India	Japan	China	Other Asia Countries	TOTAL
Aquatic Toxicology	1.564	1 (0.56)	1 (0.56)	0 (0.00)	0 (0.00)	2 (1.12)	179
Environmental Toxicology and Chemistry	1.553	1 (0.23)	1 (0.23)	5 (1.15)	1 (0.23)	4 (0.92)	435
Environmental Pollution	1.302	4 (0.50)	25 (3.10)	20 (2.48)	7 (0.87)	10 (1.24)	806
Archives of Environmental Contamination and Toxicology	1.252	2 (0.44)	2 (0.44)	24 (5.28)	1 (0.22)	4 (0.88)	456
Chemosphere	0.877	7 (0.59)	16 (1.35)	96 (8.09)	17 (1.43)	23 (1.94)	1187
Ecotoxicology and Environmental Safety	0.870	0 (0.00)	13 (5.22)	2 (0.80)	0 (0.00)	3 (1.20)	249
Bulletin of Environmental Contamination and Toxicology	0.580	21 (2.49)	109 (12.94)	49 (5.82)	11 (1.31)	30 (3.56)	842

Table 2. South East Asian articles in seven international journals (1993-1995): breakdown by country and area of interest

AREA OF INTEREST	COUNTRY					
	Malaysia	Philippines	Singapore	Indonesia	Thailand	TOTAL
Spatial Distribution of Toxicants	7	1	0	0	0	8
Toxicity Studies (Single Species)	3	3	1	1	1	9
Environmental Health (Human Toxicology)	2	0	1	0	0	3
Biodegradation	2	0	0	0	0	2
Microbial Toxicology	2	0	0	0	0	2
Toxicants in Foodstuffs (Food Safety)	2	0	0	0	0	2
Waste Treatment (Remediation)	1	1	0	0	0	2
Field Toxicity Studies	0	1	0	0	1	2
Atmospheric Pollution	2	0	0	3	1	6
	21 (58.3%)	6 (16.7%)	2 (5.6%)	4 (11.1%)	3 (8.3%)	36 (100%)

Table 3. Chemical substances and ecosystem types in South East Asian articles in seven international journals

Substance	Type of ecosystem				TOTAL
	Urban/Industry	Freshwater	Coastal/Marine	Terrestrial/Agricultural	
Heavy Metals	4	4	7	0	15 (41.7%)
Pesticides	0	1	0	6	7 (19.4%)
Non-pesticide Organic Polutants	3	4	1	0	8 (22.2%)
Atmospheric Gases	0	0	3	3	6 (16.7%)
	7 (19.4%)	9 (25.0%)	11 (30.6%)	9 (25.0%)	36 (100%)

of toxicants. It should be noted that a reasonable number of articles on atmospheric pollution are listed in Table 2, but these are mostly review papers derived from a conference which was published as a special edition of the journal Chemosphere.

In terms of substances & type of ecosystem SEA scientists have mostly studied heavy metals (41.7%) and coastal/marine ecosystems (30.6%). It can be seen from Table 3, that heavy metal studies are followed by those on non-pesticide organic pollutants (22.2%), pesticides (19.4%) and atmospheric gases (16.7%). Among the type of ecosystems studied, both freshwater and terrestrial/ agricultural ecosystems received a reasonable attention (i.e. 25%). The least studied ecosystem was the urban/industry ecosystem (19.4%).

The results are, of course, by no means free from sampling bias as this survey was limited to only seven journals. For example, pesticide studies in paddy field ecosystems seem to be under-represented, although this area of research is one of the most elaborated topics in the region (see Abdullah *et al.*, 1997), as is illustrated by publications in other journals from a research group at the University of the Philippines (Tejada *et al.*, 1993; Varca & Magallona, 1994; Bajet & Tejada, 1995). This may indicate that SEA scientists tend to publish in specialized and local journals, rather than in general international journals.

In terms of the number of articles published there is a great variation between countries (Table 2). Most of the contributions come from Malaysia (58.3%), followed respectively by Philippines (16.7%), Indonesia (11.1%), Thailand (8.3%) and Singapore (5.6%). It is not the aim of this paper to compare the progress between countries in the region, since the present survey is limited to seven journals and three years; but, at least, we can indicate that there is a difference in the involvement of the scientists of each country in the international development of this field.

Overall, the participation of South East Asian scientists in the international arena of environmental toxicology must be considered as small. A possible explanation for this limited participation is that some works are not widely published. Some good results may be found in Masters and PhD theses at local universities. Since there is no obligation for PhD students in the region to publish their work in international scientific journals, the impetus for a wider dissemination is often absent.

Other means of publication, such as national/regional scientific journals, conference proceedings, consultancy reports and interim publications, have also contributed to the limited participation of SEA in international journals. International publication often leads to no reward and is often an insignificant factor for career progression within universities or research institutes. We can expect that in the years to come, the situation will change, since governments are beginning to provide support for scientific research and publications.

3. Environmental Problems Needing Solution

The accelerating industrial and agricultural development coupled with the rapidly increasing populations in South East Asian countries during recent years exert considerable pressure on ecosystems. Agricultural and industrial development is associated with an increasing use of toxic substances in SEA (Lacher & Goldstein, 1997). With regard to pesticides, for example, more than 90% of the global end-user market in pesticides for rice production is located in Asia (Mabbet, 1991 cited by Abdullah *et al.*, 1997). The combination of three factors: agriculture, industry and population, has exposed SEA ecosystems to a diverse range of contaminants from industry and agriculture, and to a high degree of urban expansion (or landscape transformation).

It is also evident that in all countries of SEA industrial development is encouraged, and this leads to foreign investment, mostly in the form of industrial relocation. There is an indication that this relocation is not driven simply by classical factors such as low labour costs, but also by the less stringent environmental regulations in the region.

Expansion of the industrial sectors has resulted in an ever increasing generation of hazardous wastes. Most industries with a major contribution to the region's economy, such as textile, pulp and metal finishing industries, have discharged and continue to discharge considerable quantities of hazardous waste (Kim Oanh & Bengtsson, 1995; Kusnoputranto, 1993; Goeltenboth, 1994; Ismail *et al.*, 1994; Sarmani & Madjid, 1994). It was estimated that in JABOTABEK (Jakarta and its satellite cities, Indonesia) 2,000,000 tons of industrial waste were produced in 1990, 2,250,000 tons in 1995, and 2,500,000 tons will be produced in the year 2000. Approximately 50% of this amount is considered to be hazardous waste, which needs specific handling and treatments (Kusnoputranto, 1993). In Peninsular Malaysia, 220,000 m^3 toxic and hazardous waste is produced annually, of which approximately 44% can be attributed to the metal finishing industries (Din, 1995). Studies on these industrial pollutants have shown that significant impacts on receiving ecosystems (Goeltenboth, 1994; Ismail *et al.*, 1994; Mena, 1993) and human environmental safety are evident (Kusnoputranto, 1993; Sarmani & Madjid, 1994).

Another aspect related to economic development is an increase in living standards, the consumption patterns of the people in SEA are changing rapidly. This triggers the presence of previously unknown environmental pollution problems, such as exposure to volatile organic solvents in correction fluids and other modern consumer materials (see e.g. Ong *et al.*, 1993).

An extensive use of agrochemicals cannot be avoided given the intensive agricultural practices in SEA. In 1985, the pesticide market for the ASEAN countries was about US$ 498 millions (Magallona, 1994). During 1980 to 1985, Indonesia and the Philippines posted the highest growth rates in pesticide usage, i.e. 30% and 16%, respectively. The use of pesticides in SEA is mostly associated with rice production (Magallona, 1994).

Three environmental issues related to pesticides identified in SEA are residues, human poisoning and ecological impacts of these compounds. Studies on pesticide residues in SEA can be found for as early as 1971 (Gorbach *et al.*, 1971). This classical investigation is rather exceptional, since the researchers were from the company which produced the pesticide. They investigated the residue of Thiodan in an aquatic ecosystem, following an extensive campaign of treatment on rice fields, in East Java, Indonesia. Pesticide poisoning due to acute occupational exposure is a latent problem of large magnitude in SEA (see e.g. Tantiyaswasdakul, 1993). This problem results from malpractice in pesticide handling and application.

Overuse of pesticides in rice fields has led to the development of resistance and pest resurgence. In 1986, following a massive devastation of the rice crop in Indonesia by the brown planthopper, a presidential decree was issued to ban 57 of the 61 most commonly used pesticides (Sunoko, 1993). This was for the first time that pesticides have been banned for ecological reasons rather than to protect human health (Christopher, 1988). This presidential decree included the adoption of integrated pest management (IPM), which has resulted in a marked reduction in pesticide use in Indonesia (Sunoko, 1995). In terms of the total number of formulations and active ingredients, the country has to face more new chemicals (Tjahjadi, 1993). This, of course, will only increase the pressure on the environment.

Since the 1980s, SEA has been experiencing a more rapid environmental transformation than any other Third World country in the humid tropics, surpassed perhaps only by Brazil (Low & Balamurugan, 1991). The major element of this transformation is the conversion of forest to agricultural land and also from agricultural land to urban area. One of the most important driving forces behind this transformation is the rapid growth of the population.

It is estimated that by the year 2000, there will be at least 15 cities of over 1 million people in SEA. Three of the largest cities, Bangkok, Jakarta and Manila are expected to grow into megacities, each consisting of over 10 million inhabitants (Low & Balamurugan, 1991). These major cities hold the keys to economic development of the countries. All major economic activities, whether commercial, financial or industrial, are expected to be located in these cities and their immediate hinterlands. Unless waste treatment facilities grow at the same rate, a marked increase in environmental stress may be expected in the vicinities of these megacities.

Occurrence of heavy metals in the surface water in SEA has been reported by several authors (see e.g. Low & Balamurugan, 1991; Anonymous, 1993a; Djuangsih, 1993; Djuangsih & Salim, 1994; Anonymous, 1994). Over-concentration of industries in the suburb areas of the cities is the major contributor to this problem. The lead concentration in surface water in two major cities, Jakarta and Surabaya, was reported to be up to 25 and 17 fold of the WHO threshold level respectively (Anonymous, 1993a). A recent study revealed that the lead content of vegetables grown in the urban area of Jakarta and irrigated with urban surface water is far beyond the WHO's standard (Anonymous, 1994).

A study of water pollution in the Citarum river basin, West Java, gives an excellent example of industrial discharge combined with agricultural and domestic wastes in the upstream area which has put the catchment area at risk (Djuangsih, 1993; Djuangsih & Salim, 1994). A study at the Laguna Lake Basin, the Philippines revealed a similar evidence of combined contamination (Mena, 1993).

Transportation also contributes to the urban pollution problems. Population growth in the major cities of SEA has been accompanied by a marked increase in the number of vehicles in the area. It is reported that of the 700,000 vehicles in the Metro Manila in 1993, 75% are petrol fuelled and 25% are diesel fuelled (Anonymous, 1993b). Petrol vehicles, fuelled by leaded petrol and not equipped with pollution control facilities emit significant quantities of lead, carbon monoxide, nitrogen oxides and hydrocarbons. An observed impact of this sort of emission is the occurrence of heavy metal residues in vegetables cultivated adjacent to highways (Luwihana, 1994).

Another environmental problem induced by urban expansion is the discharge of domestic and human waste. Although this waste may contain some potentially toxic components, the main problem is due to the fact that dumpsites serve as food and breeding grounds for disease vectors. Furthermore, the process of poorly planned human settlement in urban areas of SEA, especially in coastal areas, involves potential ecological disturbances to coastal ecosystems (Navarro, 1995).

Confronted with the various kinds of environmental problems, which all need an immediate solution, the knowledge of environmental toxicologists, and also environmental scientists in general, should be of significant importance. Unfortunately, although the availability of accurate and up-to-date data is critical for the success of the environmental management regime, there is a lack of an environmental database in SEA. According to a recent study (Allen, 1993), data problems exist with respect to both quantity and quality. It is often the case that environmental information is presented without a proper context. With regard to this lack of data, environmental toxicologists in SEA are challenged to generate data relevant to environmental impact assessments.

4. Future Directions of Research

Facing the region's environmental problems and the wish to contribute more to the international scientific community, development of environmental toxicology in SEA is faced with a dilemma. Should it be directed to novel or fundamental research, which will contribute to the general advancement of knowledge or to applied research, or should it concentrate on the exploitation of available knowledge and techniques developed elsewhere to tackle the local, national or regional problems?

As mentioned in the previous section, the current emphasis of environmental toxicological research in SEA seems to be on single species toxicity studies and on spatial distribution of toxicants. In terms of data generation and description of the present environmental status, these approaches may be useful; but this will not be the case if we want to proceed to the more fundamental aim of environmental toxicology, i.e. the protection of ecosystem sustainability under toxicant threats.

A relevant question is whether the generation of locally relevant data, upon which environmental policy in SEA can base decisions concerning questions of immediate urgency, should follow the approaches developed in European and North American Countries, or should follow from the development of its own "niche".

In the case of pesticides, an argument often heard is that if a product has been registered for use under European or North American laws, an evaluation of the environmental risks has already been made and additional data on species indigenous to tropical countries would be redundant. Some of the species used in toxicity testing in Europe and North America actually originate from the tropics (e.g. Tilapia). Therefore one may argue that it is not necessary for each country in SEA to test the same chemical with its own species. Several countries in SEA have implemented pesticide registration procedures based on these considerations.

It can, however, be misleading to rely only on toxicity data derived from "generic" species for particular ecosystems, (see e.g. Cao, 1993; Widianarko, 1993). There is presently insufficient scientific information that would support the claim that indigenous tropical species have the same range of sensitivities as temperate species. To avoid both false positives and false negatives in the evaluation of toxicants in the tropics, testing under tropical conditions, using indigenous species in addition to data already available from temperate regions, is advisable. There are some important arguments that support the use indigenous species in testing chemicals, particularly pesticides.

(1) Even if a product has been registered in Europe and North America, the information required on ecotoxicity is often very limited. For the aquatic environment the information usually does not go further than acute toxicity to Daphnia and fish, and inhibition of algal growth. For soil, the data are even more limited. It can be argued that the basis for risk assessment can be improved greatly by extending the range of test species and undertaking tests using regionally relevant test conditions.

(2) It is likely that there are tropical species that are more sensitive to pesticides than species from temperate countries, given the great biodiversity of animal and plant groups in the tropics. When it is assumed that sensitivities of species follow a bell-shaped statistical distribution, with very sensitive and very tolerant species being relatively rare, the probability of finding a very sensitive species increases with the total number of species in the community (Kooijman, 1987).

(3) The same taxonomic group may have a different spatial distribution in tropical ecosystems compared to temperate ecosystems. Groups of animals that would not be exposed to pesticides in temperate ecosystems may be exposed in tropical ecosystems if their niches are different.

(4) The conditions under which organisms are exposed to environmental chemicals are often totally different between tropical and temperate ecosystems. The physico-chemical environment, including temperature, humidity and seasonal changes, influences both the fate of a chemical, its bioavailability, and the sensitivity of the organisms.

Regarding possible competitive advantages of South East Asia in environmental toxicology, scientists from this region may wish to explore the wealth of tropical ecosystems, which can be expected to react differently towards toxicants, with those of other climatic regimes. Rainfall, temperature, sunlight, and microorganisms in a tropical ecosystem provide a different degree of pesticide degradation and loss (Magallona, 1989). So, degradation of agrochemicals will be one of the prospective areas of study. Taking into account this phenomenon, we can develop methods which are contextual, in the sense that answers relevant to questions of the tropical ecosystem are addressed, and at the same time a contribution is made to general existing knowledge (see e.g. Widianarko & Van Straalen, 1996).

Another prospective research topic for SEA is field toxicity studies in species-rich communities. These type of studies can contribute to the development of knowledge on the flux of toxicants in complex ecosystems. Fundamental knowledge on the functioning of food chains and community effects of chemicals is the subject of recent scientific debates. Intricate interactions of species have been studied since years, but only recently has it been suggested that the overall food-web structures of communities result from the interaction process in a more or less predictable way (Pim *et al.*, 1991). The resilience of communities and therefore their ability to recover from toxicant exposure is tightly related to food-web structure. Tropical and temperate systems may have different food web topologies and consequently differ in their response to toxicants (Van der Valk, 1995).

Community approaches can be applied to the study effects in different types of ecosystems which are typical for the SEA region, such as rice field ecosystems, urban ecosystems, rain forest ecosystems, dry land (rain-fed) agroecosystems, etc (see e.g. Calumpang *et al.*, 1995; Vink, 1994). Recently, a promising result has been shown with the application of a food-web approach in the assessment of pesticide impacts in the Philippines rice field ecosystem (Cohen *et al.*, 1994).

5. Concluding Remarks

As do their colleagues in other regions, environmental toxicologists in SEA have to direct their work towards two objectives, namely to provide reliable answers to the environmental problems of their region, and to increase their contribution to the scientific knowledge. To be able to accomplish these objectives simultaneously, they have to utilize the comparative advantage of the region, i.e. its ecological richness.

Over-emphasis on single species toxicity studies and spatial distribution of toxicants will potentially result in inefficient use of resources. The application of such limited data is often difficult, because ecological complexity is not taken into account. If SEA scientists study the species-rich communities available to them, there is a great prospect for the development of a regionally focused environmental toxicology.

6. Acknowledgements

This work was supported by the Center for Development Cooperation of the Faculty of Biology (COB), Vrije Universiteit Amsterdam and the Universitas Katolik Soegijapranata, at Semarang, Indonesia. The authors would like to thank Dr. Chris Hickey for the invitation to present a paper at the Tropical Toxicology session of the 2nd SETAC World Congress, Vancouver from which this article was developed.

7. References

Abdullah, A.R.; C.M. Bajet; M.A. Matin; D.D. Nhan & A.H. Sulaiman (1997). Ecotoxicology of pesticides in the tropical paddy field ecosystem. Environ. Toxicol. Chem. 16: 59-70.

Abdullah, A.R.; N.M. Tahir & L.K. Wei (1994). Hydrocarbons in sea water and sediment from the west coast of Peninsular Malaysia. Bull. Environ. Contam. Toxicol. 53: 618-626.

Allen, J. (1993). Oranges in the apple cart: gathering environmental data in Asia. Asian J. Env. Man. 1: 7-19.

Anonymous (1993a). Some major urban environmental hazards in Indonesia. Eco-Sounder 9: 2-6.

Anonymous (1993b). Addressing air pollution problems from mobile sources in Metro Manila. Proceedings the 1993 Pacific Basin Conf. on Hazardous Wastes, Honolulu. Hawaii, USA, 8-12 Nov.

Anonymous (1994). Lead contaminates vegetables in Jakarta (Timah hitam mencemari sayuran di Jakarta). KOMPAS, 1 Nov

Bajet, C. & A.W. Tejada (1995). Pesticide residues in the Philippines: analytical perspectives. Trends Anal. Chem. 14: 430-434.

Calumpang, S.M.F.; M.J.B. Medina; A.W. Tejada & J.R. Medina (1995). Environmental impact of two molluscicides: Niclosamide and metaldehyde in a rice paddy ecosystem. Bull. Environ. Contam. Toxicol. 55: 494-501.

Cao, H.F. (1993). Management and assessment of ecological toxicity of chemicals in China. In J.E. Hay & N.G. Thom (eds.): Contributions to the management of toxic chemicals in the Asia-Pacific region and report of the first NETTLAP resources development workshop for education and training at tertiary level in toxic chemicals and hazardous waste management, Bangkok, Thailand, 28-30 Sept., NETTLAP Publication No. 5. pp. 141-153.

Christopher, J. (1988). Nature helps Indonesia to cut its pesticide bills. New Straits Times, 14 July.

Cohen, J.E.; K. Schoenly; K.L. Heong; H. Justo; G. Arida; A.T. Barrion & J.A. Litsinger (1994). A food web approach to evaluating the effect of insecticide spraying on insect pest population dynamics in a Philippines irrigated rice ecosystem. J. Appl. Ecol. 31: 747-763.

Curvin-Aralar, M.L.A. & F.V. Aralar (1993). Effects of long-term exposure to a mixture of cadmium, zinc, and inorganic mercury on 2 strains of Tilapia, *Oreochromis niloticus* and *Oreochromis aureus*. Bull. Environ. Contam. Toxicol. 50: 891-897.

Curvin-Aralar, M.L.A. & F.V. Aralar (1995). Resistance to a heavy metal mixture in *Oreochromis niloticus* progenies from parents chronically exposed to the same metals. Chemosphere 30: 953-963.

Din, Z.B. (1995). Natural and anthropogenic trace-metal input into the coastal and estuarine sediments of the Straits of Malacca. Bull. Environ. Contam. Toxicol. 55: 666-673.

Din, Z.B. & A.B. Abu (1993). Toxicity of produced water from crude oil terminals to *Photobacterium phosphoreum*, *Chaetoceros sp.* and *Donax faba*. Bull. Environ. Contam. Toxicol. 50: 413-416.

Djuangsih, N. (1993). Understanding the state of river basin management from an environmental toxicology perspective: an example from water pollution at Citarum river basin, West Java, Indonesia. Sci. Total Environ. (supplement), pp. 283-292.

Djuangsih, N. & H. Salim (1994). Preliminary study on the environmental pollution in the Citarum river basin, West Java, Indonesia. In Widianarko, B.; K. Vink & N.M. Van Straalen (eds.): Environmental Toxicology in South East Asia, VU University Press, Amsterdam. pp. 277-288.

Eong, O.J. (1993). Mangroves - a carbon source and sink. Chemosphere 27: 1097-1107.

Goeltenboth, F. (1994). The impact of a textile factory in Salatiga on the Ledok river system, Central Java. In Widianarko, B.; K. Vink & N.M. Van Straalen (eds.): Environmental Toxicology in South East Asia, VU University Press, Amsterdam. pp. 301-307.

Gorbach, S.; R. Haarring; W. Knauf & H.J. Werner (1971). Residue analyses in the water system of East Java (River Brantas, Ponds, Sea-Water) after continued large-scale application of THIODAN in rice. Bull. Environ. Contam. Toxicol. 6: 40-47.

Hay, J.E. & N.G. Thom (eds.) (1993). Contributions to the management of toxic chemicals in the Asia-Pacific region and report of the first NETTLAP resources development workshop for education and training at tertiary level in toxic chemicals and hazardous waste management. Bangkok, Thailand, 28-30 Sept., NETTLAP Publication No. 5.

Husin, Y.A.; D. Murdiyarso; M.A.K. Khalil; R.A. Rasmussen; M.J. Shearer; S. Sabiham; A. Sunar & H. Adijuwana (1995). Methane flux from Indonesian wetland rice: the effects of water management and rice variety. Chemosphere 31: 3153-3180.

Ibrahim, N. (1993). Trace element analysis of the traditional medicine, Jamu. Bull. Environ. Contam. Toxicol. 51: 199-202.

Institute for Scientific Information (1993). Science Citation Index, Journal Citation Reports. Philadelphia.

Ismail, A.; H. Wahidah & J.H. Ali (1994). Heavy metal contamination in Malaysian rice field snails. In Widianarko, B.; K. Vink & N.M. Van Straalen (eds): Environmental Toxicology in South East Asia, VU University Press, Amsterdam. pp. 181-184.

Kim Oanh, Ng. T. & B.E. Bengtsson (1995). Toxicity to Microtox, micro-algae and duckweed of effluents from the Bai Pang paper company (BAPACO), a Vietnamese bleached kraft pulp and paper mill. Environ. Pollut. 90: 391-399.

Kim Oanh, N.T.; B.E. Bengtsson; L. Reutergardh; P.A. Bergqvist; P.A. Hyinning & M. Remberger (1995). Levels of contaminants in effluent, sediment, and biota from Bai Bang, a bleached kraft pulp and paper mill in Vietnam. Arch. Environ. Contam. Toxicol. 29: 506-516.

Kooijman, S.A.L.M. (1987). A safety factor for LC50 values allowing for differences in sensitivity among species. Water Res. 21: 269-276.

Kusnoputranto, H. 1993. Institutional capacities for hazardous waste management in Indonesia. In J.E. Hay & N.G. Thom (eds.): Contributions to the management of toxic chemicals in the Asia-Pacific region and report of the first NETTLAP resources development workshop for education and training at tertiary level in toxic chemicals and hazardous waste management, Bangkok, Thailand, 28-30 Sept., NETTLAP Publication No. 5. pp. 109-119.

Lacher, T.E. Jr. & M.I. Goldstein (1997). Tropical ecotoxicology: Status and Needs. Environ. Toxicol. Chem. 16: 100-111.

Law, A.T. (1995). Toxicity study of the oil dispersant Corexit 9527 on *Macrobrachium rosenbergii* (de Man) egg hatchability by using a flow-through bioassay technique. Environ. Pollut. 88: 341-343.

Low, K.S. & G. Balamurugan (1991). Urbanization and urban water problems in South East Asia: a case of unsustainable development. J. Env. Man. 32: 195-209.

Low, K.S.; C.K. Lee & S.T. Owwee (1995). Removal of chromium (III) from aqueous solution using chrome sludge. Bull. Environ. Contam. Toxicol. 55: 270-275.

Low, K.W. & Y.M. Sin (1995). Effects of mercuric chloride on chemiluminescent response of phagocytes and tissue lysosyme activity in Tilapia, *Oreochromis aureus*. Bull. Environ. Contam. Toxicol. 54: 302-308.

Luwihana, S. 1994. Lead content of vegetables grown in fields adjacent to highways in Java, Indonesia. In Widianarko, B.; K. Vink & N.M. Van Straalen (eds.): Environmental Toxicology in South East Asia, VU University Press, Amsterdam. pp. 185-190.

Maah, H.J.; I. Mat & A. Johari (1994). Trace metals in sediments and potential availability to Anadara granosa . Arch. Environ. Contam. Toxicol. 27: 54-59.

Maah, H.J. ; I. Mat & A. Johari (1995). Geochemical partitioning of trace metals in the potential culture-bed of the marine bivalve, *Anadara granosa*. Bull. Environ. Contam. Toxicol. 54: 191-197.

Magalona, E.D. (1989). Effects of insecticides in rice ecosystems in South East Asia. In P. Bourdeau; J.A. Haines; W. Klein & C.R. Krishna Murti (eds.): Ecotoxicology and Climate. SCOPE. John Wiley & Sons Ltd. New York. pp. 265-297.

Magalona, E.D. (1994). Impact of pesticides on tropical ecosystems, with emphasis on rice production in the ASEAN countries. In Widianarko, B.; K. Vink & N.M. Van Straalen (eds.): Environmental Toxicology in South East Asia, VU University Press, Amsterdam. pp. 19-32.

Mat, I. (1994). Arsenic and trace metals in commercially important bivalves, *Anadara granosa* and *Paphia undulata*. Bull. Environ. Contam. Toxicol. 52: 833-839.

Mat, I. & M.J. Maah (1994). Sediment trace metal concentrations from the mudflats of Kuala Juru and Kuala Muda of Malaysia. Bull. Environ. Contam. Toxicol. 53: 740-746.

Mena, M.M. (1993). Toxic chemicals and hazardous waste management case study for the Philippines: toxic chemicals and hazardous waste management for the laguna lake area. In J.E. Hay & N.G. Thom (eds.): Contributions to the management of toxic chemicals in the Asia-Pacific region and report of the first NETTLAP resources development workshop for education and training at tertiary level in toxic chemicals and hazardous waste management, Bangkok, Thailand, 28-30 Sept., NETTLAP Publication No. 5. pp. 249-256.

Millamena, O.M. (1994). Effect of detention time on aerobic waste stabilization pond performance in South East Asia. Bull. Environ. Contam. Toxicol. 52: 856-863.

Mokhtar, M.B.; A.B. Awaluddin; C.W. Fong & W.M.B.H.M. Woojdy (1994). Lead in blood and hair of population near an operational and a proposed area for copper mining, Malaysia. Bull. Environ. Contam. Toxicol. 52: 149-

154.
Murdiyarso, D. (1993). Policy options to reduce CO release resulting from deforestation and biomass burning in Indonesia. Chemosphere 27: 1109-1120.
Navarro, R.G. (1995). Improving sanitation in coastal communities. A paper presented at the International Conference on Urban Habitat, Delft, The Netherlands, 15-16 Feb.
Ong, C.N.; D. Koh; S.C. Foo; P.W. Kok & T.C. Aw (1993). Volatile organic solvents in correction fluids - Identification and potential hazards. Bull. Environ. Contam. Toxicol 50: 787-793.
Pim, S.L.; J.H. Lawton & J.E. Cohen (1991). Food web patterns and their consequences. Nature 350: 669-674.
Prudente, M.S.; H. Ichihashi & R. Tatsukawa (1994). Heavy metal concentrations in sediments from Manila Bay, Philippines and inflowing rivers. Environ. Pollut. 86: 83-88.
Sahid, I.B. & S.S. Teoh (1994). Persistence of terbutylazine in soils. Bull. Environ. Contam. Toxicol. 52: 226-230.
Sahid, I.B. & C.C. Wei (1993). Degradation of two acetanitrile herbicides in a tropical soil. Bull. Environ. Contam. Toxicol. 50: 24-28.
Sahid, I.B. & W.R.W. Zaabar (1993). Effects of thiram and terbuthylazine on cellulose decomposition in two soils. Bull. Environ. Contam. Toxicol. 51: 605-611.
Sahid, I.B. & M.Y. Yap (1994). Effects of two acetanilide herbicides on microbial populations and their cellulolytic activities. Bull. Environ. Contam. Toxicol. 52: 61-68.
Sarmani, S. & A.A. Madjid (1994). Dietary intake of toxic trace elements from seafood consumption in Malaysia. In B. Widianarko; K. Vink & N.M. Van Straalen (eds.): Environmental Toxicology in South East Asia, VU University Press, Amsterdam. pp. 209-214.
Shazili, N.A.M. (1995). Effects of salinity and pre-exposure on acute cadmium toxicity to seabass, Lates calcarifer. Bull. Environ. Contam. Toxicol. 54: 22-28.
Soegiarto, A. (1993). The Southeast Asian marine aquatic environment, the carbon cycle and the need for regional cooperative research. Chemosphere 27: 1121-1126.
Soemardi, A.W. & S. Notosoedarmo (eds.) (1987). Proceedings, National Conference on Soil Ecology and Ecotoxicology, UKSW, Salatiga, Indonesia, 27-28 Nov.
Soepadmo, E. (1993). Tropical rain forests as carbon sinks. Chemosphere 27: 1025-1039.
Snidvongs, A. (1993). Sedimentary calcium carbonate dissolution in the gulf of Thailand and its roles as a carbon dioxide sink. Chemosphere 27: 1083-1095.
Sunoko, H.R. (1993). Pesticide use in Indonesia: regulation and practices. Majalah Penelitian UNDIP 7: 70-78.
Surif, S. & C.Y. Chai (1995). Screening of lead exposure among workers in Selangor and Federal Territory in Malaysia. Environ. Pollut. 88: 177-181.
Tan, G.H. (1995). Residue levels of phthalate esters in water and sediment samples from the Klang River basin. Bull. Environ. Contam. Toxicol. 54: 171-176.
Tan, G.H. & K. Vijayaletchumy (1994). Organochlorine pesticide residue levels in peninsular Malaysian rivers. Bull. Environ. Contam. Toxicol. 53: 351-356.
Tantiyaswasdakul, P.S. (1993). Problem assessment and alternative options for hazardous pesticide control in Thailand. Proceedings, the 1993 Pacific Basin Conf. on Hazardous Wastes, Honolulu. Hawaii, USA, 8-12 Nov.
Tjahjadi, R.V. (1993). Pesticides and its problems (Pestisida dan permasalahannya). Pesticide Action Network, Jakarta.
Tejada, A.W.; L.M. Varca; P. Ocampo; C.M. Bajet & E.D. Magallona (1993). Fate and residues of pesticides in rice production. Int. J. Pest Man. 39: 281-287.

Van der Valk, H. (1995). Communities in hot arid ecosystems - potential implication for insecticide risk assessment. Abstract, SETAC Europe Congress 1995, Copenhagen, Denmark.

Varca, L.M. & E.D. Magallona (1994). Dissipation and degradation of DDT and DDE in Philippine soil under field conditions. J. Environ. Sci. Health 29: 25-35.

Vink, K. (1994). A laboratory method to test side-effects of pesticides on tropical litter decomposition. In Widianarko, B.; K. Vink & N.M. Van Straalen (eds.): Environmental Toxicology in South East Asia, VU University Press, Amsterdam. pp. 223-234.

Vink, K.; L. Dewi; J. Bedaux, A. Tompot; M. Hermans & N.M. Van Straalen (1995). The importance of the exposure route when testing the toxicity of pesticides to saprotrophic isopods. Environ. Toxicol. Chem. 14: 1225-1232.

Vogt, G. & E.T. Quinitio (1994). Accumulation and excretion of metal granules in the prawn, *Penaeus monodon*, exposed to water-borne copper, lead, iron and calcium. Aquat. Toxicol. 28: 223-241.

Widianarko, B. (1993). In search of relevant parameters for pesticide risk evaluation in Indonesia: A study on a non-target soil animal. Proceedings, the 1993 Pacific Basin Conf. on Hazardous Wastes, Honolulu, Hawaii, USA, 8-12 Nov.

Widianarko, B. & N.M. Van Straalen (1996). Toxicokinetics-based survival analysis in bioassays using nonpersistent chemicals. Environ. Toxicol. Chem. 15: 402-406.

Widianarko, B.; K. Vink & N.M. Van Straalen (eds.) (1994). Environmental Toxicology in South East Asia. VU University Press, Amsterdam.

CHAPTER 3

Spatial Distribution of Trace Metals in Sediments from Urban Streams of Semarang, Central Java, Indonesia

With Rudo A. Verweij, Cornelis A.M. Van Gestel and Nico M. Van Straalen

ABSTRACT

Elevated environmental concentrations of metals are usually associated with the impact of urbanization. The present study is focused on metal contamination in urban sediments. A field survey was carried out to determine the distribution of four metals, i.e. cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn) in the coastal urban area of Semarang, Central Java, Indonesia. Sediment samples were collected from 101 grids of 2 km x 2 km. To map the spatial distribution of these metals, concentrations of each metal were plotted against the corresponding grid coordinate. Cd was below the detection limit (< 0.03 µg/g) in all samples, whereas concentrations of Pb, Zn and Cu fell into a wide range. Frequency distributions of Pb, Zn and Cu concentrations showed a similar pattern, in which the major proportion of the sites had a low metal concentration. Some sites, however, had extremely high metal concentrations, Zn up to 1257 µg/g, Pb up to 2666 µg/g and Cu up to 448 µg/g. The data were used to define background concentrations for sediments in coastal zones of Indonesia ("reference values"). The proposed reference values are 25.6 µg/g, 132.2 µg/g and 40.7 µg/g respectively for Pb, Zn and Cu. The degree of metal contamination of each individual site was classified according to the calculated value of a combined pollution index, W. Four categories of the degree of metal contamination were proposed, i.e. unpolluted, slightly polluted, polluted and heavily polluted. Based on this classification, from the 101 sites investigated in the greater Semarang area, 51 are unpolluted; 36 slightly polluted; 9 polluted and 5 heavily polluted.

1. Introduction

Urbanization is generally defined as aggregation of human populations in a limited area with a subsequent perturbation of the local environment. This process usually involves serious impacts on soil, as well as on water resources. Elevated concentrations of heavy metals, such as lead (Pb), cadmium (Cd), copper (Cu) and zinc (Zn) are usually associated with the impact of urbanization. Therefore, it is common to use concentrations of these metals as indicators of urbanization.

Urban metal pollution has become a subject of increasing interest in human medicine, ecology and ecotoxicology (Dallinger *et al.*, 1992, Kratz, 1996). Several authors have reported evidence for the accumulation and effects of heavy metals emitted from urban activities on animals and plants (see e.g. Berger and Dallinger, 1993; Depledge et al., 1993; Cotrufo et al., 1995; Mackey & Hodgkinson, 1995, Pizl & Josens, 1995).

In addition to sewage effluent, untreated faecal matter, industrial effluent, untreated industrial wastes, domestic and industrial garbages, sediment is one of the major sources of water pollution in South East Asian cities (Low & Balamurugan, 1991). Sediments are deposits for physical debris and sinks for a wide variety of chemicals, including heavy metals. The concern associated with metal contamination in sediments is that many commercial species and organisms in the aquatic food chain spend a major portion of their life cycle in or on sediments. This provides a pathway for these metals to be consumed by higher organisms. Direct transfer of chemicals from sediments to organisms is now considered to be a major route of exposure for many species (Adams *et al.*, 1992). Information on the status of trace metal pollution in the sediment of coastal areas is of considerable importance in the interest of public health when seafood from this areas is available for consumption (Mat & Maah, 1994).

Although studies on heavy metals in several cities in Indonesia, including Semarang, have been carried out for years (see e.g. Darmojo *et al.*, 1985; Supriharyono *et al.*, 1989; Astuti *et al.*, 1991; Anonymous, 1993_a, 1994_a), the available data and publications are very limited and patchy. A similar situation has also been reported in other parts of South East Asia (see e.g. Din, 1995).

Information on the spatial distribution of metals through out the city will provide a full picture of the extent of the city's metal contamination. Furthermore, the absence of sediment quality standards for metals hampers the assessment and monitoring of urban metal pollution in Indonesia. Data on the spatial distribution of metals in non-polluted and polluted sediments may allow the establishment of a reference value of each metal and an index indicating the degree of combined metal contamination in the sediment can be derived based on the above information.

The present study is the first which maps the spatial distribution of metals through out a city, not just a limited set of sampling sites as demonstrated by most metal studies conducted in the region. The objectives of the present study were: (1) to identify the spatial distribution of metals in the sediments of the greater Semarang area, (2) to estimate the background concentrations of the metals present in Semarang, and (3) to provide a simple tool for deriving standards for metals in the sediment.

2. Materials and Methods

2.1. Study Area

Semarang is the fifth largest city of Indonesia and has a population of 1.2 million with an average annual growth of 1.1% during 1989 to 1993 (Anonymous, 1993_b). The main activities of this capital city of the Central Java Province are trade and industry. Semarang covers an area of 373.668 square kilometres, and is situated on the northern coast of Java. Due to its topographical characteristics, i.e. a descending altitude toward the coastal line, and hundreds of water courses, from rivers to small streams, the Semarang area is subject to continuous sedimentation. Areas of higher altitude in southern parts of the city discharge run-off water and sediment to the inner-city areas. These flows, combined with sea water invasion, due to the up-levelling of coastal land induced by the reclamation of the coastal area, has resulted in a high prevalence of flooding in the city.

2.2. Sampling

Sampling was conducted from 21 April to 19 August 1995. Based on the topographical map of Semarang the whole area of the city was subdivided into 101 grids of 2 km x 2 km, samples of sediment (5 replicates) and water (3 replicates) were collected for each grid, while five fish (guppy, *Poecilia reticulata*) samples were taken wherever guppies were present.

A systematic sampling programme was performed from west to east, starting from the coastal area. To reduce systematic errors, due to the difference in sampling times, we jumped one grid to the next grid, and the grid in between was sampled in the next round of sampling, from east to west.

Only sediment samples will be considered for the purpose of the present paper. Data on metals in fish will be published elsewhere. Sediment samples were taken from the 5 cm upper layer of surface sediment. Each sample unit was approximately 1 kg and transported to the laboratory in a plastic bag. Upon arrival at the laboratory the pH (H_2O) and dry matter content of the fresh sediment were measured.

Prior to the metal analyses 10 g of oven dried sediment from each sample were prepared, ground and passed through a 1-mm mesh sieve. The sediment powder was then stored dry and transported to Amsterdam in a polyethylene bag.

2.3. Metal Analyses

Metal analyses were carried out at the laboratory of the Department of Ecology and Ecotoxicology, Vrije Universiteit Amsterdam. A portion of 1 g oven dried sediment was digested using 6 ml of a mixture of nitric acid, chloric acid and demineralised water (4 : 1 : 1 v/v) in a microwave oven (CEM MDS 81D). Following the digestion, 10 ml of demineralised water was added to rinse the digestion tube. The complete solution was collected in a perspex tube and was ready for metal analyses. Concentrations of Pb, Zn and Cu were determined using a flame Atomic Absorption Spectrophotometer (Perkin Elmer 1100 B). As Cd concentrations appeared to be below the detection limit of this method, an attempt to determine Cd concentrations was made using a graphite furnace Atomic Absorption Spectrophotometer. Certified reference material (calcareous loam, BCR Reference Material no. 141) was routinely digested and analysed to maintain quality control.

2.4. Data Analysis

Data on the concentrations of Zn, Pb and Cu in the sediment were evaluated using the STATISTICA software package for Windows, Release 4.5, StatSoft, Inc. (1993). Normalization of the data set for each metal using a logarithmic transformation was carried out to satisfy the assumption of constant variance and normality. The Kolmogorov-Smirnov test for normality was used to detect the normality of each data set (Sokal & Rohlf, 1995 and Zar, 1984). Distribution of normalized data of each metal was then presented in a frequency table. Furthermore, the median and the 95th percentile were determined for each data set.

3. Results

Cd concentrations were below the detection limit (0.03 µg/g) at all sites, whereas concentrations of Pb, Zn and Cu fall into a wide range (Table 1). Some sites have extremely high metal concentrations, i.e. Zn up to 1257 µg/g, Pb up to 2666 µg/g and Cu up to 448 µg/g. Frequency distributions of Pb, Zn and Cu concentrations showed similar patterns, in which the major proportion of the sites had a low metal concentration (see Table 2). Results of the normality tests showed that only Cu concentrations satisfied the assumption of normality after a logarithmic transformation. The other two distributions were more peaked than normal (leptokurtic).

Table 1. Summary of metal concentrations in sediments (in µg/g) of the Semarang area.

Metal	Number of sites	Minimum	Maximum	Percentiles 50th (median)	95th
Zn	101	53.7	1257	105	585
Pb	101	5.2	2666	18.2	344
Cu	101	12.3	448	41.6	71.1

From Figures 1, 2 and 3, it appears that the spatial distribution is as follows : (i) the highest Zn concentrations are in the coastal grids, this could be an effect of sedimentation; (ii) the highest Pb concentrations are clustered in the center of the city, possibly due to effects of traffic; (iii) there are two hot spots, i.e. one site in the north-east with extremely high Cu (but normal Zn and Pb), and one site in the south-east with high Zn and Pb (but normal Cu). These are probably due to localized industrial contaminations. Seventy five percent of the sites have

Table 2. Frequency distribution of lead, zinc and copper concentrations (in µg/g, log-transformed data) in sediments from the Semarang area.

Class Interval	Frequency		
	Lead	Zinc	Copper
I	5	7	1
II	29	29	4
III	40	29	23
IV	12	12	48
V	7	10	22
VI	2	4	3
VII	1	4	0
VIII	3	3	0
IX	1	1	0
X	1	2	1

Lead: I = $0.57 \leq X < 0.87$, II = $0.87 \leq X < 1.17$, III = $1.17 \leq X < 1.47$, IV = $1.47 \leq X < 1.77$, V = $1.77 \leq X < 2.07$, VI = $2.07 \leq X < 2.37$, VII = $2.37 \leq X < 2.67$, VIII = $2.67 \leq X < 2.97$, IX = $2.97 \leq X < 3.27$, X = $3.27 \leq X < 3.57$

Zinc: I = $1.65 \leq X < 1.81$, II = $1.81 \leq X < 1.97$, III = $1.97 \leq X < 2.13$, IV = $2.13 \leq X < 2.28$, V = $2.28 \leq X < 2.43$, VI = $2.43 \leq X < 2.58$, VII = $2.58 \leq X < 2.73$, VIII = $2.73 \leq X < 2.88$, IX = $2.88 \leq X < 3.03$, X = $3.03 \leq X < 3.18$

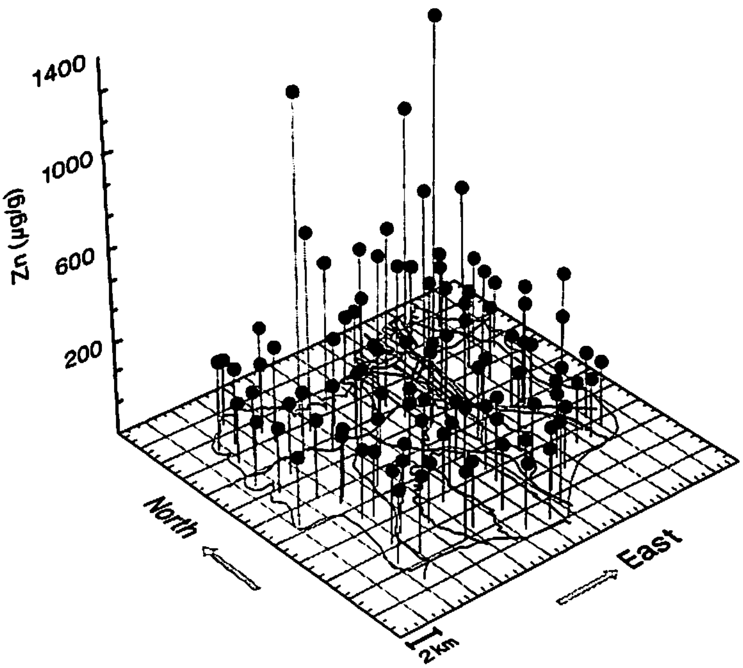
Copper: I = $1.00 \leq X < 1.17$, II = $1.17 \leq X < 1.34$, III = $1.34 \leq X < 1.51$, IV = $1.51 \leq X < 1.68$, V = $1.68 \leq X < 1.85$, VI = $1.85 \leq X < 2.02$, VII = $2.02 \leq X < 2.19$, VIII = $2.19 \leq X < 2.36$, IX = $2.36 \leq X < 2.53$, X = $2.53 \leq X < 2.70$

low metal concentrations, i.e. Pb < 30.0 µg/g, Zn < 172 µg/g and Cu < 49.3 µg/g. Due to the above facts, it seems justified to derive the average background concentrations of Cu, Zn and Pb.

The frequency distribution of metal concentrations were markedly skewed, even on a logarithmic scale, due to the presence of peak concentrations at polluted sites. When the data for polluted sites were omitted (using the 95th percentile as a cut-off level), the remaining data had a symmetric distribution on a logarithmic scale. The geometric mean of these data was considered to represent the "reference value" (Table 3). The proposed reference values are 25.6 µg/g, 132 µg/g and 40.7 µg/g, respectively for Pb, Zn and Cu.

The concentration of the three metals was combined into one pollution index. Metal concentrations from each site were divided by the corresponding background concentration ("reference value"). This produced a number that indicates by which factor the background concentration is exceeded at a certain site. The factors for each of the three metals were then multiplied and the logarithm of the product was taken. The same result will be obtained if first the logarithm of each metal concentration is taken, subtracted by the logarithm of the background concentration and the result summed for the three metals. We called this index W (Eq. 1).

Figure 1. Spatial Distribution of Zinc in Sediments from Urban Streams of Semarang



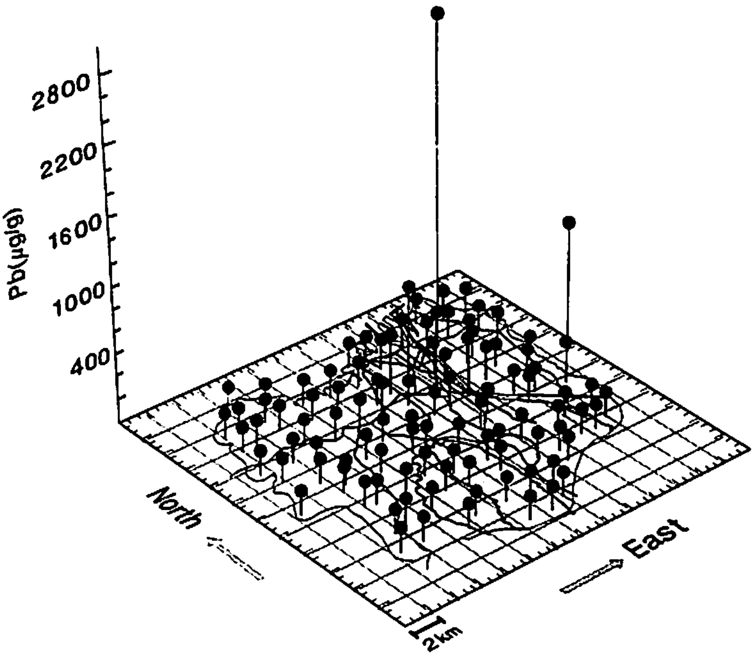
$$W = \log \left(\prod_{i=1}^{n} C_i / C_{0i} \right) \quad (1)$$

where C_i = concentration of metal i
C_{0i} = background concentration of metal i
n = number of metals

Spatial distributions of the metals Zn, Pb and Cu and W-values are presented in a three-dimensional scatterplot. The X and Y axes represent the distance (in kilometer) to the city center from west to east and from north to south respectively. The Z axis represents the corresponding concentration of each metal and the W-value.

The degree of metal contamination of each individual site can be classified according to the calculated value of the combined contamination index, W (Eq. 1). It is proposed that the degree of metal contamination is classified into four categories, i.e. unpolluted, slightly polluted, polluted and heavily polluted. A site is classified as unpolluted if its combined contamination index is less than

Figure 2. Spatial Distribution of Lead in Sediments
from Urban Streams of Semarang



or equal to zero (W ≤ 0). In that case the average metal concentration is equal to, or lower than the reference value. A site is classified as slightly polluted if its combined contamination index is larger than zero and less than or equal to one (0 < W ≤ 1). A site is classified as polluted if its combined contamination index is larger than one and less than or equal to two (1 < W ≤ 2). A site is classified as heavily polluted if its combined contamination index is larger than two (W > 2).

Since the antilog of W indicates by which factor the background concentration is exceeded, it implies that the metal contamination at unpolluted sites is less than or equal to the background concentration, whereas at slightly polluted and polluted sites the metal contamination is greater than 1 to 10 and greater than 10 to 100 times the background concentration, respectively. At heavily polluted sites metal contamination is more than 100 times the background concentration. It has to be considered that W measures the degree of pollution of all metals jointly, so if, for example, three metals are a factor of two above the reference value, antilog W equals 8. Based on this classification, from the 101 sites investigated in the greater Semarang area, 51 sites are unpolluted, 36 sites are polluted, 9 are polluted and 5 are heavily polluted (Figure 4).

Figure 3. Spatial Distribution of Copper in Sediments from Urban Streams of Semarang

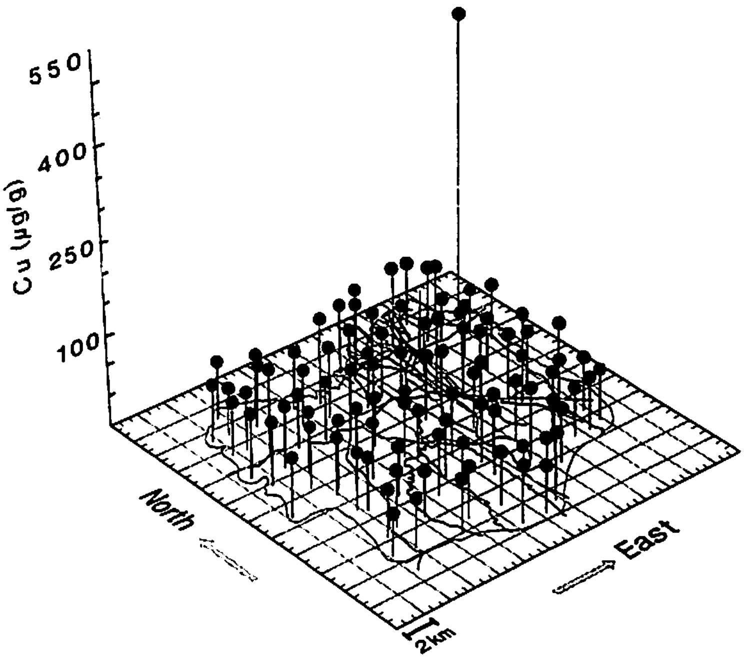
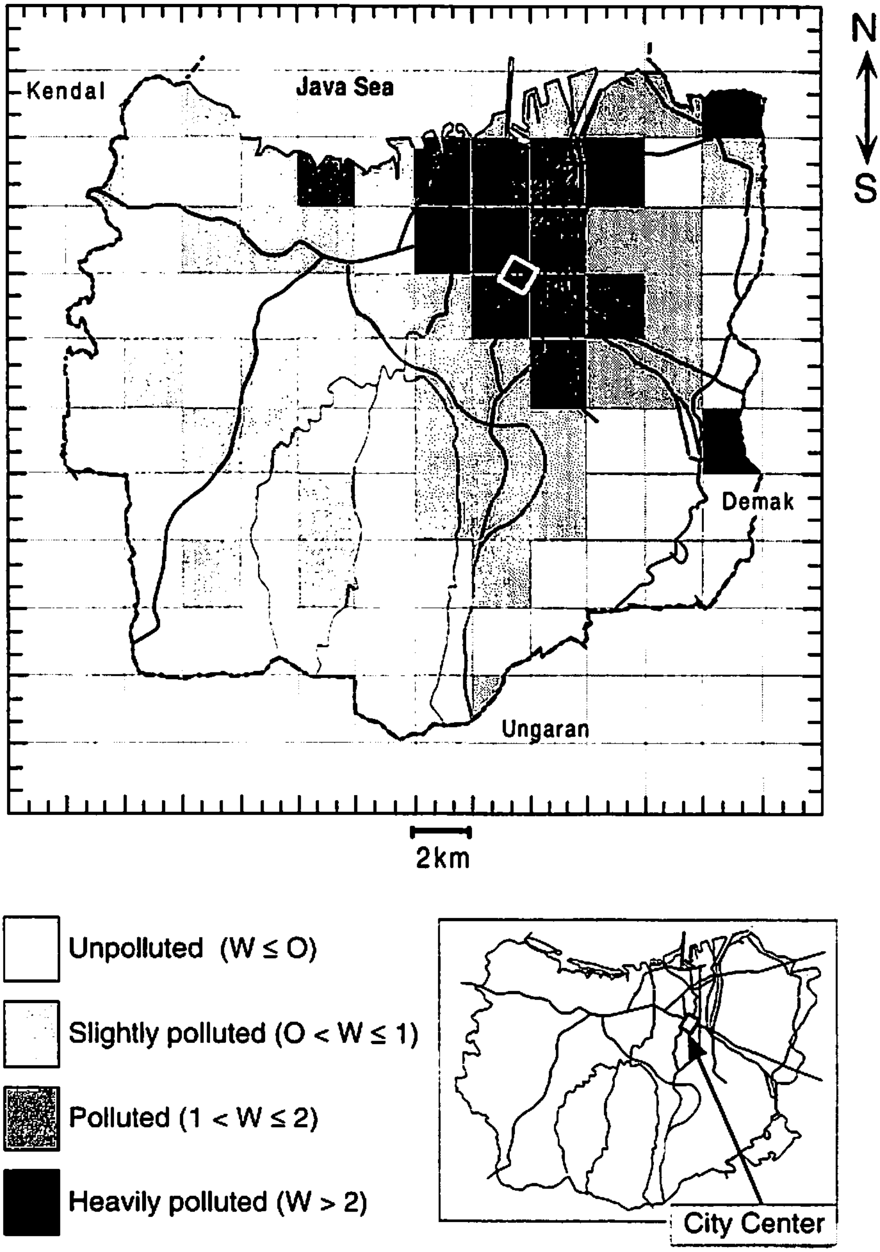


Table 3. Summary of metal concentrations in sediments in (in µg/g), when the data for polluted sites were omitted (using the 95th percentile as a cut-off level).

Metal	Number of sites	Mean	SD
Zn	96	132.2	138
Pb	96	25.6	24.2
Cu	96	40.7	1.8

SD = standard of deviation

Figure 4. Spatial Distribution of Combined Metals Contamination in Sediments from Urban Streams of Semarang



4. Discussion

The skewness of metals distributions, especially Pb and Zn, found in this study is not uncommon for spatial distribution of metals. A similar finding was reported by Von Steiger *et al.* (1996) based on a study on the distribution of heavy metals (Pb, Cd, Cu and Zn) in soil of Weinfelden in north-east Switzerland.

Concentrations of metals in sediment found in the present study are not extreme compared to metal concentrations in sediment of other parts of South East Asia. Prudente *et al.*, (1994) reported respective ranges of metal concentrations in surface sediments from three inflowing rivers of Manila Bay, i.e. Marikina River, Pasig River and Rivers in Bulacan of 18-31, 66-137 and 36-198 μg/g dry wt for Pb; 74-169, 236-1560 and 95-313 μg/g dry wt for Zn; 28-79, 110-189 and 36-98 μg/g dry wt for Cu. A study on total leachable trace metal concentrations in surface sediments of Kuala Juru and Kuala Muda rivers in the western coast of Peninsular Malaysia, by Mat & Maah (1994), revealed concentration with standard deviations of 4.5 ± 0.5 μg/g and 4.2 ± 0.8 μg/g for Cd; 36.8 ± 12.1 μg/g and 10.8 ± 3.3 μg/g for Cu; 64.2 ± 11.5 μg/g and 46.7 ± 2.8 μg/g for Pb; 233 ± 63 μg/g and 39.4 ± 2.2 μg/g for Zn, respectively.

In this study, an empirical approach was used for deriving reference values for the three metals. Except for Pb, these values are similar to the target values defined in the Environmental Quality Objectives in the Netherlands (Anonymous, 1994$_b$). Sediments which meet these quality objectives can generally be considered to be multifunctional and can be spread on land as dredging sludge without restrictions.

The present approach, can be extended if one would wish to distinguish the anthropogenic contribution of metals as separated from their natural occurrences. Other approaches which can be considered in conjunction to this present study include the determination of the anthropogenic input of the metals, either using a separation of the mobile fraction of metals (e.g. Fernandes *et al.*, 1994) or a normalization procedure (Din, 1992 & 1995). The former approach is based on the leaching of the mobile fraction of metals with strong acid (0.5 M HCl) during 16 h continuous shaking. The latter applied a normalization procedure for heavy metal data, using aluminium as the reference metal. Regression analysis was used to relate the concentration of each metal to the concentration of aluminium. This procedure seems to be promising for separating the natural and anthropogenic input of these metals in a particular area.

The approach developed in the present study is prospective in the light of the need to define sediment quality standards for the region. It will be useful for the future monitoring of urban metal pollution in Semarang as well as other cities in South East Asia, where anthropogenic influences as well as continuous discharges of municipal and industrial effluents into urban streams are expected to increase.

5. Acknowledgments

Funding for this research was provided by the Universitas Katolik Soegijapranata and Department of Ecology and Ecotoxicology, Vrije Universiteit, Amsterdam. The authors would like to thank Mr. Felix Sholeh Kuntoro and Mr. Novi Susetyo Adi for the assistance in the collection and preparation of the samples, Mr. Luigi Sanna for the drawings, and Ir. D. Bambang Sudarsono, MS. for providing maps of Semarang.

6. References

Adams, W.J.; R.A. Kimerle & J.W. Barnett Jr. (1992). Sediment quality and aquatic life assessment. Environ. Sci. Technol. 26: 1865-1875.

Anonymous (1993a). Measuring sediment toxins in Surabaya. Eco-Sounder. 9: 14-16.

Anonymous (1993b). Kotamadya Daerah Tingkat II Semarang Dalam Angka. Kantor Statistik, Semarang.

Anonymous (1994a). Timah hitam mencemari sayuran di Jakarta. KOMPAS, November 1, p. 3.

Anonymous (1994b). Environmental Quality Objectives in the Netherlands. Risk Assessment and Environmental Quality Division, Directorate for Chemicals, External Safety and Radiation Protection, Ministry of Housing, Spatial Planning and the Environment. The Hague.

Astuti; Y.; N.H. Suprapti & M. Hadi (1991). Keanekaragaman benthos sebagai bioindikator pencemaran logam Pb, Hg dan Cd di pantai utara Jawa Tengah. In R.B. Darmojo; A.G. Soemantri; R. Miranda & J. Herminanto (eds.): Bunga Rampai PIP - UNDIP II. Lembaga Penelitian Universitas Diponegoro, Semarang, pp. 5-13.

Berger, B. & R. Dallinger (1993). Terrestrial snails as quantitative indicators of environmental metal pollution. Environ. Monit. Assess. 25: 65-84.

Cotrufo, M.F.; A.V. De Santo; A. Alfani; G. Bartoli & A. De Cristofaro (1995). Effects of urban heavy metal pollution on organic matter decomposition in *Quercus ilex* L. Woods. Environ. Pollut. 89: 81-87.

Dallinger, R.; B. Berger & S. Birkel (1992). Terrestrial isopods: useful biological indicators of urban metal pollution. Oecologia 89: 32-41.

Darmojo, R.B.; A.G. Soemantri; H.R. Sunoko & R. Miranda (1985). Monitoring logam berat di perairan pantai Semarang. In R.B. Darmojo; R. Hartono; A.G. Soemantri & R. Miranda (eds): Bunga Rampai PIP - UNDIP I. Lembaga Penelitian Universitas Diponegoro, Semarang, pp. 17-22.

Depledge, M.H.; T.L. Forbes & V.E. Forbes (1993). Evaluation of cadmium, copper, zinc, and iron concentrations and tissue distribution in the benthic crab, *Dorippe granulata* (De Haan, 1841) from Tolo Harbour, Hongkong. Environ. Pollut. 81: 15-19.

Din, Z.B. (1992). Use of aluminium to normalize heavy-metal data from estuarine and coastal sediments of the Straits of Malacca. Mar. Poll. Bull. 24: 484-491.

Din, Z.B. (1995). Natural and anthropogenic trace-metal input into the coastal and estuarine sediments of the straits of Malacca. Bull. Environ. Contam. Toxicol. 55: 666-673.

Fernandes, H.M.; E.D. Bidone; L.H.S. Veiga & S.R. Patchineelam (1994). Heavy-metal pollution assessment in the coastal lagoons of Jacarepagua, Rio de Janeiro, Brazil. Environ. Pollut. 85: 259-264.

Kratz, W. (1996). Ecotoxicological bioindication: PAH, PCB and heavy metals studied in the natural resource monitoring programme of Berlin

(Germany). In N.M. van Straalen & D.A. Krivolutsky: Bioindicator Systems for Soil Pollution. NATO ASI Series. Kluwer Acad. Publ. Dordrecht. pp. 223-232.
Low, K.S. & G. Balamurugan (1991). Urbanization and urban water problems in South East Asia: A case of unsustainable development. J. Environ. Man. 32: 195-209.
Mackey, A.P. & M.C. Hodgkinson (1995). Concentrations and spatial distribution of trace metals in mangrove sediments from the Brisbane River, Australia. Environ. Pollut. 90: 181-186.
Mat, I. & M.J. Maah (1994). Sediment trace metal concentrations from the mudflats of Kuala Juru and Kuala Muda of Malaysia. Bull. Environ. Contam. Toxicol. 53: 740-746.
Pizl, V. & G. Josens (1995). Earthworm communities along a gradient of urbanization. Environ. Pollut. 90: 7-14.
Prudente, M.S.; H. Ichishashi & R. Tatsukawa (1994). Heavy metal concentration in sediments from Manila Bay, Philippines and inflowing rivers. Environ. Pollut. 86: 83-88.
Sokal, R.R. & F.J. Rohlf (1995). Biometry, The Principles and Practice of Statistics in Biological Research. 3rd ed. W.H. Freeman and Co. New York.
Supriharyono, H.R. Sunoko & B.T. Basuki (1989). Monitoring logam berat di perairan pantai Semarang. Lembaga Penelitian Universitas Diponegoro, Semarang. (unpublished)
Von Steiger, B,; R. Webster, R. Schulin & R. Lehmann (1996). Mapping heavy metals in polluted soil by disjunctive kriging. Environ. Pollut. 94: 205-215.
Zar, J.H. (1984). Biostatistical Analysis. Prentice-Hall Inc., Englewood-Cliffs.

CHAPTER 4

Associations Between Trace Metals in Sediment, Water and Guppy, *Poecilia reticulata* (Peters) from Urban Streams of Semarang, Indonesia

With Cornelis A.M. Van Gestel, Rudo A. Verweij and Nico M. Van Straalen

ABSTRACT

An answer was sought to the question whether the amount of metal in aquatic biota reflects the concentrations in the sediment and water, and whether the physico-chemical properties of the water and sediment have any influence on the suspected relationship. A study was made of 101 small streams in the city of Semarang, Central Java, Indonesia. Data on fish occurrence in 63 streams of the greater Semarang indicated that the guppies did not avoid the highly polluted sites. No significant difference in body weight between sites was found. Significant differences were found in metal body concentrations (Pb and Zn) between fish collected from sites with different degrees of pollution. A significant declining trend of Pb concentrations with increasing organism size was observed, whereas for two other metals, Zn and Cu, the concentrations did not depend on the body weight. Apparently, body concentrations of these two metals are regulated and maintained at a certain concentration. As to the relationships between metal concentrations in water, sediment and fish, water and sediment parameters, and fish dry weight, the presence of significant multiple correlations and bivariate correlation (in 17 out of 91 pairs of variables) indicated that, in general, abiotic parameters and body size had no influence on the metal flux from sediment to water, and into fish. Results of partial correlation analyses further suggested that metal concentrations in the sediments were the most important factor governing the metal body concentrations of fish. The present study indicates that the guppy *P. reticulata* from urban streams is a potential bioindicator for urban metal pollution, especially with respect to their (1) spatial distribution over sites of all pollution regimes, and (2) variation in metal accumulation levels reflecting the degree of pollution.

1. Introduction

Residues of contaminants in biota are often used to assess environmental quality. Measurements in biota may supplement measurements in the physical environment. Several animal species have been used for studies on metal residues, e.g. terrestrial isopods, *Porcellio scaber* (Hopkin *et al.*, 1989, Dallinger *et al.*, 1992, Hopkin, 1993), terrestrial snails, *Arianta arbustorum* (Berger & Dallinger, 1993), earthworm species (Weigman, 1991, Pizl & Josens, 1995, Kratz, 1996), Arachnida and Formicidae (Rabitsch, $1995_{a,b}$).

To provide a more comprehensive insight into the extent of metal contamination, it will be of substantial use to connect information on the spatial distribution of metals in the physical environment (i.e. sediment and water) with information from a biological matrix (i.e. fish) in a defined spatial scale. The research presented here seeks to answer the question whether the amount of metal in the biota reflects the concentrations in the sediment and water, and to find out whether the physico-chemical properties of water and sediment have any influence on the suspected relationship.

Previous studies on three metals, i.e. lead (Pb), copper (Cu) and zinc (Zn), found that the spatial distributions of these metals in sediments of the urban area of Semarang are as follows : (i) the highest Zn concentrations are in the coastal grids; (ii) the highest Pb concentrations are clustered in the centre of the city; (iii) there are two hot spots, i.e. one site in the north-east with extremely high Cu (but normal Zn and Pb), and one site in the south-east with high Zn and Pb (but normal Cu). Seventy five percent of the sites have low metal concentrations, i.e. Pb < 30.2 µg/g, Zn < 174 µg/g and Cu < 49.0 µg/g (Widianarko *et al.*, subm., Chapter 3).

An evaluation based on the calculated value of the combined contamination index, W, was proposed (details can be found in Widianarko *et al.*, subm., Chapter 3) to classify the degree of metal contamination of each individual site in a single index. A site is classified as unpolluted if W is less than or equal to zero ($W \leq 0$); a site is classified as slightly polluted if W is larger than zero and less than or equal to one ($0 < W \leq 1$); a site is classified as polluted if W is larger than one and less than or equal to two ($1 < W \leq 2$); a site is classified as heavily polluted if W is larger than two ($W > 2$). According to this classification, among the 101 sites in the greater Semarang area, 51, 36, 9 and 5 sites were categorized as unpolluted, slightly polluted, polluted and heavily polluted, respectively (Widianarko *et al.*, subm., Chapter 3).

The research presented here is aimed at contributing to data on trace metals in a tropical urban ecosystem, which up to now are still under represented in global terms. There is a wide gap to be filled, specifically if one considers the abundance of data from the industrialized world compared to the limited data from tropical regions (Davies, 1992). Furthermore, by incorporating data from the whole area of the city, both "interesting" areas (polluted areas) and unpolluted areas, where usually the data are scarce, are taken into account.

2. Materials and Methods

2.1. Sampling Sites

Sediment, water and fish samples were collected from 101 grids (2 km x 2 km) laid out over the whole area of Semarang, the fifth largest city of Indonesia. In each grid five sediment, three water and five fish (guppy, *Poecilia reticulata*) samples were collected systematically from the largest stream provided the guppies were present.

2.2. Handling and Measurement of the Samples

2.2.1. Sediment

The field collection of sediment samples and its subsequent laboratory preparations and measurements on pH, and dry matter content of these samples are described in Widianarko *et al.* (subm., Chapter 3). Prior to further analyses the sediment powder was kept in a plastic (PE) bag and stored at room temperature. A part of this powder was used for gravimetric determination of organic matter content using a muffle oven with a successive increase of temperature (1 hour : 200 °C, 1 hour : 400 °C and 6 hours: 500 °C), and another part was used for metal analyses.

2.2.2. Water

Three water samples of approximately 1 L each were collected from each site and kept in a plastic container and transported to the laboratory. The pH measurements of these samples were done using a digital pH meter (Hanna Instruments, HI 8418) which was routinely calibrated using buffer solutions of pH 4 and 7.

Total Suspended Solids (TSS) content of the water was measured by three successive filtrations of 250 mL water samples through plain filter paper of known dry weight under vacuum (Allen, 1988). After filtration, the residue was oven dried to constant weight (105 °C, 30 min) and put in the desiccator for 15 min. and then weighed using an electronic microbalance (Ohaus AP 250 D).

To prepare water samples for metal analyses, 100 mL of water was filtered using a millipore vacuum filter successively through plain and 0.45 µm filter papers. Ten mL of the filtrate was collected and acidified to pH = 2 using concentrated HNO_3 (65%) and stored in the refrigerator (4 °C).

2.2.3. Fish

From each site five individuals of male guppy (approximately of uniform size) were caught and transported to the laboratory in plastic (polyethylene) bags with original water. Upon arrival in the laboratory, two body size parameters, body length and body weight (fresh and dry) were measured.

For the metal analyses, fish were killed in ether and wrapped individually in aluminium foil. Dry weight determination was done by putting fresh fish samples in an oven (60 °C, 18 h). Prior to a metal analyses these oven-dried samples were stored in a desiccator.

2.3. Metal Analyses

Metal analyses were carried out at the laboratory of the Department of Ecology and Ecotoxicology, Vrije Universiteit Amsterdam. Analyses of metal concentrations in sediments are described in Widianarko *et al.* (subm., Chapter 3).

Determination of metal concentrations in the water and in fish were done using a graphite furnace Atomic Absorption Spectrophotometer Perkin Elmer 1100 B. No prior treatment was needed for the determination of metals in the acidified water samples. Oven-dried fish samples were digested in a mixture of HNO_3 and $HClO_4$ (Ultrex grade, 7:1). The digestion was done according the method of Van Straalen & Van Wensem (1986). Prior to the measurement, the pellet remaining after digestion was dissolved in 1 mL of 0.1 M HNO_3 (Ultrex grade).

2.4. Data Analysis

Several statistical techniques were applied in the data analysis, including a G-test, analysis of variance (ANOVA) followed by appropriate post-hoc comparison of means, linear regression, multiple linear correlation, bivariate correlation, and partial correlation. All computations were undertaken using statistical software for PC under Windows environment, i.e. Statistica version 4.5 (1993) and SPSS version 6.1 (1994).

A G-test of independence (Sokal & Rohlf, 1995) was used to establish whether the presence of the guppy, *Poecilia reticulata* (Peters) was dependent on the degree of pollution of the streams.

A one-way ANOVA with unbalanced data (see Zar, 1984) was used to test the differences in body weight, metal concentration and metal body burden of the guppy, *P. reticulata* , between urban streams with different degrees of metal contamination (expressed in W values). To fulfil the normality assumption a logarithmic transformation was applied to the data prior to the ANOVA computation (Steel & Torrie, 1981).

A linear regression technique (Sokal & Rohlf, 1995) was used to assess the allometric relationship between metal body concentration and dry weight expressed as equation (1):

$$C = a\,X^{b} \qquad \text{or} \qquad \log C = \log a + b \log X \tag{1}$$

where X is dry weight (mg), C is metal body concentration (µg/g), a and b are constants (regression parameters).

Three different correlation techniques, i.e. bivariate correlation, multiple correlation and partial correlation, were used to evaluate the relationships present among 14 variables in this study. These variables include metal concentrations in sediment, water and fish, pH and total suspended solids content of the water, pH and organic matter content of the sediment, and fish dry weight.

Partial correlation was used to overcome the inability of bivariate correlation to take into account the interactions of any of the other variables on the two variables in question. Partial correlation solves this problem because it considers the correlation between each pair of variables while holding the value of one of the other variables constant (Sokal & Rohlf, 1995; Zar, 1984; Nurosis, 1994).

Decisions on the degree of significant correlations among all the variables in this study were done following the procedure in Chatfield & Collins (1980), which is based on the magnitude of the correlation coefficient. Large correlations are those whose absolute value exceeds 0.70 and small correlations are those whose absolute value is less than 0.25.

3. Results

Out of 101 sampling sites the fish, *Poecilia reticulata*, was only present at 63 sites. According to the site classification based on the value of the combined contamination index, W, these sites fell into four categories, i.e. 30 unpolluted, 20 slightly polluted, 8 polluted and 5 heavily polluted sites. Results of the G-test revealed that the presence of fish was not dependent on the degree of metal pollution of the sites (Table 1). All heavily polluted sites and most of the polluted ones had fish; slightly polluted and non-polluted sites had fish in around 50% of the cases.

Table 1. Distribution of fish (*Poecilia reticulata*) occurrence over sites with different degrees of metal contamination

Fish Present*	Degree of metal contamination				TOTAL
	Non - Polluted	Slightly Polluted	Polluted	Heavily Polluted	
No	21	16	1	0	38
Yes	30	20	8	5	63
TOTAL	51	36	9	5	101
% Presence	58.8	55.6	88.9	100	

* G-value = 8.92 ($p<0.05$)

Table 2. Body weight, metal concentration and metal body burden of *Poecilia reticulata* from streams with different degrees of metal contamination (all values are mean ± standard deviation)

Parameter	Degree of metal contamination*)			
	Non - Polluted	Slightly Polluted	Polluted	Heavily Polluted
No. of samples	150	100	40	25
Body weight**) (mg)	20.1 ± 4.7	18.3 ± 5.3	16.9 ± 3.9	19.0 ± 4.8
Concentration (µg/g)				
Lead	0.8^{a} ± 0.7	1.2^{b} ± 0.6	3.2^{c} ± 2.8	6.9^{c} ± 7.7
Copper	3.4 ± 1.1	2.6 ± 1.0	4.6 ± 5.8	6.4 ± 9.3
Zinc	207^{a} ± 39.2	223^{b} ± 43.6	343^{c} ± 238	271^{bc} ± 74.4
Body burden (ng/fish)				
Lead	15.4^{a} ± 13.7	21.3^{b} ± 12.1	47.8^{c} ± 34.1	135^{c} ± 177
Copper	69.5^{a} ± 33.5	49.1^{b} ± 25.0	91.9^{ab} ± 150	87.4^{ab} ± 94.5
Zinc	4111 ± 1055	4052 ± 1342	6308 ± 6548	5274 ± 2410

Different superscripts show significant differences ($p<0.01$), based on the LSD test following one-way ANOVA of log-transformed data.

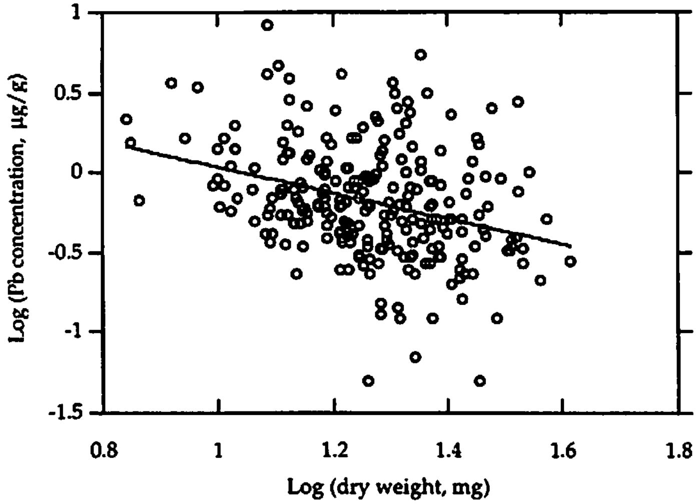
*) The degree of metal contamination of each individual site was classified according to the calculated value of combined contamination index, W, based on metal concentration in the sediment.

**) measured as dry weight

Results of ANOVA showed that there was no significant difference in body weight between sites, but there were significant differences in metal body concentration and body burden between fish collected from sites with different degrees of pollution (Table 2). With regard to metal concentrations, significant differences were found for Pb and Zn. For the body burdens, only Pb showed a significant difference. No significant difference was found for Cu presented either as concentration or as body burden.

Only fish from non-polluted and slightly polluted streams were used for evaluation of the allometric relationship between metal body concentration and body weight. Only Pb concentrations showed a significant association with the

Figure 1.a. Regression plot of internal Pb concentrations and body weight of *Poecilia reticulata*



body weight (Figure 1.a). Pb concentrations in fish tended to decrease with the increase of body weight. Concentrations of two other metals, Zn and Cu, appeared to be independent of body weight. The linear regression method did not provide an acceptable fit for the relationship between the concentration of these metals (Zn and Cu) and body weight of fish (see Figure 1.b and 1.c, the insignificant fitted lines were plotted as dashed lines). A highly significant (p <

Figure 1.b. Regression plot of internal Zn concentrations and body weight of *Poecilia reticula*

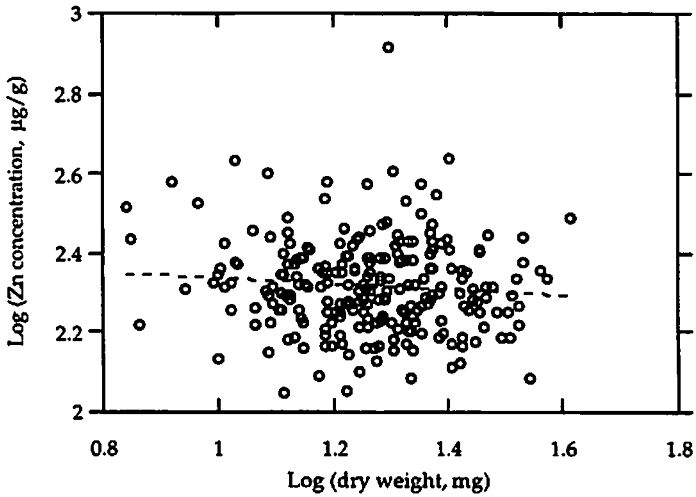
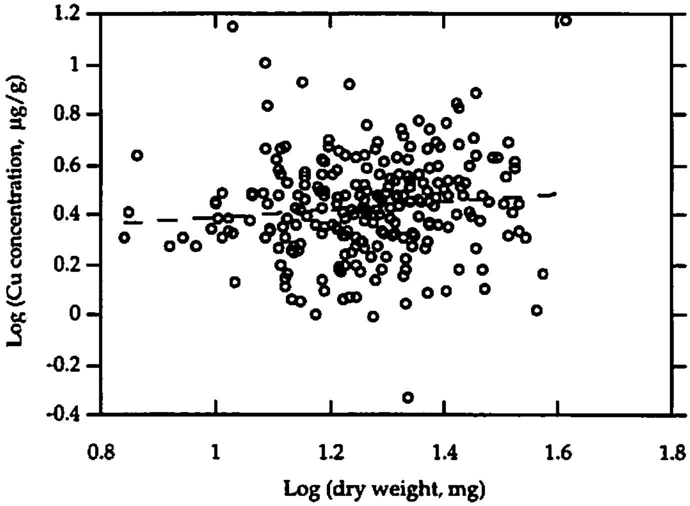


Figure 1.c. Regression plot of internal Cu concentrations and body weight of *Poecilia reticulata*



0.01) regression coefficient was found for Pb. For Zn and Cu the estimated values of the regression coefficients were not significantly different from zero. Parameter estimates of the linear regressions and their standard errors are listed in Table 3.

A significant coefficient of multiple correlation ($p < 0.05$, $R = 0.61$) was found for the multiple association among 14 variables, i.e. Pb concentrations in sediment, water and fish; Cu concentrations in sediment, water and fish; Zn concentrations in sediment, water, fish; pH and total suspended solids content

Table 3. Parameter estimates for linear regression between metal body concentration and body weight of fish (*Poecilia reticulata*) from non-polluted and slightly polluted streams

Metal	Parameter (± Standard Error)	
	intercept	slope coefficient
Lead	0.84 ± 0.19	-0.80 ± 0.15*
Zinc	2.41 ± 0.07	-0.07 ± 0.05
Copper	0.02 ± 0.12	0.16 ± 0.09

* = regression coefficient significantly different from zero (p< 0.01)

Table 4. Correlation between metal concentrations in guppies and concentrations in and properties of corresponding water and sediment

Variable	PbFish	PbSed	PbWat	CuFish	CuSed	CuWat	ZnFish	ZnSed	ZnWat	DWFish	OMSed	pHSed	pHWat	TSS
PbFish	1													
PbSed	0.758*	1												
PbWat	-0.209	-0.053	1											
CuFish	0.177	0.014	-0.076	1										
CuSed	0.348*	0.081	-0.163	0.724*	1									
CuWat	-0.039	-0.033	0.414*	0.266*	0.232	1								
ZnFish	0.271*	0.343*	0.057	0.504*	0.066	0.035	1							
ZnSed	0.696*	0.753*	-0.165	0.019	0.282*	-0.068	0.243*	1						
ZnWat	-0.017	-0.021	0.393*	0.049	0.031	0.327*	-0.060	-0.030	1					
DWFish	-0.125	0.107	0.224	0.011	-0.208	0.163	0.009	-0.040	-0.030	1				
OMSed	-0.053	-0.064	-0.118	-0.238	0.042	-0.294*	-0.156	0.144	-0.220	-0.160	1			
pHSed	-0.021	0.031	-0.150	-0.094	-0.116	-0.154	0.083	0.040	-0.100	0.035	0.032	1		
pHWat	0.018	0.114	-0.265*	-0.196	-0.141	-0.145	-0.059	0.043	-0.140	-0.090	0.046	0.413*	1	
TSS	-0.111	0.078	0.085	0.019	-0.115	-0.024	-0.113	-0.180	0.182	0.056	-0.030	0.006	0.147	1

Note : **PbFish, CuFish** and **ZnFish** are lead, copper and zinc concentrations in the guppy, respectively. **PbSed, CuSed** and **ZnSed** are lead, copper and zinc concentrations in the sediment, respectively. **PbWat, CuWat** and **ZnWat** are lead, copper and zinc concentrations in the water, respectively. **OMSed** and **pHSed** are organic matter content and pH of the sediment, respectively. **pHWat** is pH of the water, and **TSS** is total suspended solids.

of the water, pH and organic matter content of the sediment, and fish dry weight. The application of the simple bivariate correlation on these variables resulted in 91 bivariate correlation coefficients (Table 4). Only 17 out of these 91 coefficients were significant.

From Table 4 it can be seen that concentrations of all metals (Pb, Cu and Zn) in the body of *P. reticulata* were significantly correlated with the concentrations of the respective metals in the sediment. Large correlation coefficients (> 0.7) were shown by Pb and Cu, whereas Zn showed a small correlation coefficient (< 0.25). A significant correlation between metal concentration in the water and in the fish was only found for Cu.

It is interesting to note that concentrations of Zn in fish were correlated with Pb and Cu in fish, and with Pb in sediment. Zn concentrations in sediment were correlated with concentrations of the two other metals, Pb and Cu, in the same medium. Pb in water was negatively correlated with pH of the water. Significant correlations were also present between Zn in water and the corresponding concentrations of Pb and Cu. Concentrations of Cu in water, however, were correlated with Pb in water and organic matter content of the sediment. Finally, pH of the sediment was significantly correlated with pH of the water.

To identify any hidden interactions that cannot be taken into account by the simple bivariate correlation technique, a partial correlation analysis was conducted. By controlling sediment pH, organic matter conternt of the sediment, water pH and total suspended solids content of the water, it was found that only three significant correlations remained, namely between concentrations of the metals in fish (Pb, Cu and Zn) and their corresponding concentrations in sediment (see Table 5). In all cases, the values of partial correlation coefficients were slightly larger than the corresponding values for bivariate correlation. When the inclusion of controlled variables was done step-wise the same results were revealed.

4. Discussion

The data on fish occurrence in 63 streams of the greater Semarang indicated that guppies (*Poecilia reticulata*) did not avoid the highly polluted sites. Apparently, fish are attracted by the organic waste at these sites and can survive the metal pollution. This fish species has been known to be well established in polluted water with a high amount of organic waste in South East Asia (Chou & Lam, 1989). This implies that guppy fish is a promising bioindicator species. The presence of guppies in polluted sites will allow the determination of changes in various ecotoxicological parameters induced by the metal pollution.

No simple interpretation can be made on significant differences in metal concentrations (Pb and Zn) and metal burdens (Pb) between fish from sites with different contamination levels. One possible explanation might be that fish from polluted and highly polluted streams have developed a physiological

Table 5. Partial correlation between metal concentrations in *Poecilia reticulata*, in sediment, and in water (controlling for pH and organic matter content of sediment; pH and TSS of water)

Metal	Variable	Sediment	Water	Fish
Lead	Sediment	1		
	Water	-0.03	1	
	Fish	0.77**	-0.21	1
Copper	Sediment	1		
	Water	0.23	1	
	Fish	0.76**	0.19	1
Zinc	Sediment	1		
	Water	0.08	1	
	Fish	0.26*	-0.11	1

* = significant ($p < 0.05$); ** = highly significant ($p < 0.01$)

adaptation by accumulating more metals when facing excessive metal concentrations in their environment. Studies on metal accumulation in animals from sites with different distances from an emission source showed such a tendency (see e.g. Hopkin *et al.*, 1989; Dallinger *et al.*, 1992; Berger & Dallinger, 1993; Pizl & Josens, 1995).

A significant declining trend of Pb concentrations with increasing organism size was observed. This accumulation phenomenon is one of the three types of metal concentration-size relationships which include increases, decreases and no change in concentration with increased body weight as demonstrated by several studies on aquatic invertebrates (Smock, 1983). Ray *et al.* (1980) reported that small individuals of the polychaete *Nereis virens* accumulated larger amounts of Cd per unit of body weight than larger individuals, in association with a larger uptake rate of Cd in small individuals. The decreasing metal concentration with body size might indicate that surface adsorption is an important mode of accumulation (Smock, 1983).

Clearly, for the two essential metals, Zn and Cu, the concentrations were not dependent on the body weight. Apparently, body concentrations of these two metals are regulated and maintained at a certain concentration. The existing theory on the influence of body size on the accumulation of trace metals in animals as exemplified by several bioaccumulation studies on different aquatic and terrestrial species (e.g. Moriarty *et al.*, 1984; Janssen & Bedaux, 1989; Krantzberg, 1989; Van Hattum *et al.*, 1991; Mersch *et al.*, 1996) does not appear to hold for essential metals.

As to the relationships between metal concentrations in water, sediment and fish, water and sediment parameters, and fish dry weight, the presence of significant multiple correlations and bivariate correlations (in 17 out of 91 pairs of variables) indicates that, in general, abiotic parameters and body size have no influence on the metal flux from sediment to water, and into fish. It can be inferred that the significant multiple associations among 14 variables in the present study were mostly dominated by the association between metal concentrations in sediments and those in the fish. This is in contrast with other bioaccumulation studies which have clearly shown the role of abiotic parameters in the uptake of toxicants, including metals, by animals (see e.g. Schrap & Opperhuizen, 1989; Van Hattum *et al.*, 1991; Landrum & Faust, 1991; Van Hattum *et al.*, 1993; Ankley *et al.*, 1994, Bervoets *et al.* 1996)

Based on the results of simultaneous bivariate correlation analysis, this study demonstrated some significant relationships which are relatively self-evident, such as relationships between concentrations of all metals (Pb, Cu and Zn) in sediment and the corresponding metal concentrations in fish, and between Cu concentrations in the water and Cu concentrations in the fish, or between pH of the sediment and pH of the water. Some of the other significant relationships are not so trivial, e.g. relationships between Zn concentrations in all compartments, i.e. sediment, water and fish, and concentrations of the two other metals (Pb and Cu) in the corresponding compartments, or between concentrations of Cu and Pb in water. It seems that, although the spatial distribution is specific for each metal (cf. Widianarko *et al.*, subm., Chapter 3), on the average the metals occur in association.

Partial correlation analysis was applied, basically, to identify and to isolate the most important variables which possibly have some influence on the flux of metals between the different compartments in the ecosystem studied. Results of partial correlation analyses suggest that metal concentrations in the sediments are the most important factor governing the metal body concentration of fish, since none of the abiotic parameters of the sediment and water were significant covariates.

The results of the present study indicate that the guppy *P. reticulata* from urban streams is a potential bioindicator for urban metal pollution, especially with respect to its (1) spatial distribution over sites of all pollution regimes, and (2) variation in metal accumulation related to the degree of pollution. Accordingly this fish species can be considered a suitable candidate for biomonitoring programmes.

5. Acknowledgement

Funding for this research was provided jointly by the Universitas Katolik Soegijapranata and Department of Ecology and Ecotoxicology, Vrije Universiteit, Amsterdam. The authors would like to thank Mr. Felix Sholeh Kuntoro and Mr. Novi Susetyo Adi for the assistance in the collection and preparation of the samples.

6. References

Allen, S.E. (1988). Chemical Analysis of Ecological Materials. Blackwell Scientific Publ. Oxford.

Bervoets, L.; R. Blust & R. Verheyen (1996). Uptake of zinc by the midge larvae *Chironomus riparius* at different salinities: Role of speciation, acclimation, and calcium. Environ. Toxicol. Chem. 15: 1423-1427.

Berger, B. & R. Dallinger (1993). Terrestrial snails as quantitative indicators of environmental metal pollution. Environ. Monit. Assess. 25: 65-84.

Chatfield, C. & A.J. Collins (1980). Introduction to Multivariate Analysis. Chapman and Hall. London.

Chou, L.M. & T.J. Lam (1989). Introduction of exotic aquatic species in Singapore. In S.S. Silva (ed.): Proc. of a Workshop on Introduction of Exotic Aquatic Organisms in Asia. Spec. Publ. Asian Fish Soc. 3: 91-97.

Dallinger, R.; B. Berger & S. Birkel (1992). Terrestrial isopods: useful biological indicators of urban metal pollution. Oecologia 89: 32-41.

Davies, B.E. (1992). Trace metals in the environment, retrospect and prospect. In D.C. Adriano (ed.): Biogeography of Trace Metals in Invertebrates. Lewis Publishers. Boca Raton. pp. 383-399.

Hopkin, S.P. (1989). Ecophysiology of Metals in Terrestrial Invertebrates. Elsevier Applied Science, London.

Hopkin, S.P.; C.A.C. Hames & S. Bragg (1989). Terrestrial isopods as biological indicators of zinc pollution in the Reading area, South England. Monitore zool. ital. (N.S.) Monogr. 4: 477-488.

Janssen, M.P.M. & J.J.M. Bedaux (1989). Importance of body-size for cadmium accumulation by forest litter arthropods. Neth. J. Zool. 39: 194-207.

Krantzberg, G. (1989). Metal accumulation by chironomid larvae: the effects of age and body weight on metal body burdens. Hydrobiologia 188/189: 497-506.

Kratz, W. (1996). Ecotoxicological bioindication: PAH, PCB and heavy metals studied in the natural resource monitoring programme of Berlin (Germany). In N.M. van Straalen & D.A. Krivolutsky (eds.): Bioindicator Systems for Soil Pollution. NATO ASI Series. Kluwer Acad. Publ. Dordrecht. pp. 223-232.

Landrum, P.F. & W.R. Faust (1991). Effect of variation in sediment composition on the up take rate coefficient for selected PCB and PAH congeners by the amphipod, *Diporeia sp.* In M.A. Mayes & M.G. Barron (eds.): Aquatic Toxicology and Risk Assessment. Fourteenth Volume, ASTM STP 1124, Philadelphia. pp. 263-279.

Mersch, J.; P. Wagner & J-C. Pihan (1996). Copper in indigenous and transplanted zebra mussels in relation to changing water concentrations and body weight. Environ. Toxicol. Chem. 15: 886-893.

Moriarty, F.; H.M. Hanson & P. Freestone (1984). Limitations of body burden as an index of environmental contamination: Heavy metals in fish *Cottus gobio* L. from the River Ecclesbourne, Derbyshire. Environ. Pollut. (Series A) 34: 297-320.

Norusis, M.J. (1994). SPSS Advanced Statistics 6.1. SPSS Inc. Chicago.

Pizl, V. & G. Josens (1995). Earthworm communities along a gradient of urbanization. Environ. Pollut. 90: 7-14.

Rabitsch, W.B. (1995a). Metal accumulation in arthropods near a lead/zinc smelter in Arnoldstein, Austria. II. Formicidae. Environ. Pollut. 90: 239-247.

Rabitsch, W.B. (1995b). Metal accumulation in arthropods near a lead/zinc smelter in Arnoldstein, Austria. III. Arachnida. Environ. Pollut. 90: 249-257.

Ray, S., D. McLeese & D. Pezzack (1980). Accumulation of cadmium by *Nereis virens*. Arch. Environ. Contam. Toxicol. 9: 1-8.

Schrap, S.M. & A. Opperhuizen (1989). Preliminary investigations on the influence of suspended sediments on the bioaccumulation of two chlorobenzenes by the guppy (*Poecilia reticulata*). Hydrobiologia 188/189: 573-576.

Smock, L.A. (1983). Relationships between metal concentrations and organism size in aquatic insects. Freshwater Biol. 13: 313-321.

Sokal, R.R. & F.J. Rohlf (1995). Biometry, The Principles and Practice of Statistics in Biological Research. 3rd ed. W.H. Freeman and Co. New York.

Van Hattum, B.; K. Timmermans & H.A.J. Govers (1991). Abiotic and biotic factors influencing in situ trace metal levels in macroinvertebrates in freshwater ecosystems. Environ. Toxicol. Chem. 10: 275-292.

Van Hattum, B.; G. Korthals; N.M. Van Straalen; H.A.J. Govers & EN.G. Joosse (1993). Accumulation patterns of trace metals in freshwater isopods in sediment bioassays - influence of substrate characteristics, temperature and pH. Wat. Res. 27: 669-684.

Van Straalen, N.M. & J. Van Wensem (1986). Heavy metal content of forest litter arthropods as related to body-size and trophic level. Environ. Pollut. Ser. A. 42: 209-221.

Weigman, G. (1991). Heavy metal levels in earthworms of a forest ecosystem influenced by traffic and air pollution. Wat. Air & Soil Pollut. 57-58: 655-663.

Widianarko, B.; R.A. Verweij; C.A.M. Van Gestel & N.M. Van Straalen. Spatial distribution of trace metals in sediments from urban streams of Semarang, Central Java, Indonesia. [Submitted, Chapter 3]

Zar, J.H. (1984). Biostatistical Analysis. Prentice-Hall Inc., Englewood-Cliffs.

CHAPTER 5

Heavy Metals in Urban Streams of Semarang: Bioindication Potential of Body Size and Reproductive Parameters of the Guppy *Poecilia reticulata* (Peters)

ABSTRACT

The potential use of the guppy, *Poecilia reticulata* (Peters), as a bioindicator is assessed based on a comparison of body size and reproductive parameters between populations from unpolluted and metal polluted streams in Semarang, Indonesia. Among body size parameters, body length and body weight showed significant differences between polluted and unpolluted sites. The same did not hold for the size structure. Smaller body lengths were observed for male guppies from the polluted stream, but this was not the case for the females. In terms of total energy content of male fish, there was a significant difference between sites. This is in contrast with the indication shown by body size measurements. Among reproductive parameters observed in this study, it was shown that sex ratio and the ratio of pregnant to total females are promising parameters for bioindication. Combining these two ratios, it can be inferred that guppy populations in polluted ecosystems tend to have fewer females, which have a higher reproductive activity than those in unpolluted ecosystems. Slight and insignificant differences were observed for the number of reproductive units (juveniles + eggs) and reproductive allocation of individual pregnant females from different sites.

1. Introduction

Urban areas are the places where most of the anthropogenic pollution is being produced. Urban pollution is of high concern because the human population is intensely exposed. To deal with this problem properly, regulatory authorities need to know the extent and degree of pollution. Complementary to the routinely applied chemical measurements, biomonitoring may be another instrument that can be used to assess environmental quality. The basic principle of biomonitoring is the identification of environmental quality through its ecological receptors, i.e. organisms living in the ecosystems (Spellerberg, 1991; Van Straalen, 1993).

Several animal and plant species have been proposed for biomonitoring programmes of various environmental pollutants. For metal pollution in soils the terrestrial isopod *Porcellio scaber* (Hopkin *et al.*, 1989, Dallinger *et al.*, 1992), the terrestrial snail *Arianta arbustorum* (Berger & Dallinger, 1993), and earthworm species (Weigman, 1991, Pizl & Josens, 1995, Kratz, 1996) have been used. Plant species used for the same purpose include a.o. Scots pine, *Pinus sylvestris* L. (Kratz, 1996), vegetables: cabbage, carrot, lettuce, leek and endive (Sauve, 1996; Voutsa *et al.*, 1996), and ryegrass (Sauve, 1996).

Although concentrations of toxic substances in biota give evidence of bioavailability, simply measuring residues in biota is insufficient. There is a need to couple residue levels with effects of chemicals on the organisms. Therefore, ecological studies are needed to indicate what residue levels are of concern by assessing the biological impairment of bioindicator organisms exposed to the chemicals. Using ecological studies it is possible to demonstrate the actual damage resulting from a pollution event.

A study on the spatial distribution of trace metals in Semarang conducted previously revealed that three metals: lead (Pb), copper (Cu) and zinc (Zn) were present in measurable concentrations in sediments of urban streams (Widianarko *et al.*, subm.$_a$, Chapter 3). A further study (Widianarko, subm., Chapter 4) also showed that concentrations of all metals (Pb, Cu and Zn) in the body of *Poecilia reticulata*, a predominant fish species in the urban streams, are significantly correlated with the concentrations of the respective metals in the sediment.

To what extent the presence of these metals has an impact on organisms living in the water body is unknown. Freshwater sediments play an important role in the chemical exchange processes in the aquatic environment, namely between particulate, dissolved and biological phases (Reynoldson & Day, 1993). Many contaminants, which are relatively insoluble in water, are known to be adsorbed by suspended particulate organic matter which eventually settles to the bottom sediment.

The study of Widianarko *et al.* (subm.$_a$, Chapter 3) revealed that of 101 sample sites in the greater Semarang area, 51 sites are unpolluted, 36 sites are slightly polluted, 9 sites are polluted and 5 sites are heavily polluted. Furthermore, having a majority of sites unpolluted, offered the possibility to derive background concentrations of the metals which is urgently needed to provide a sound basis for environmental management (Davies, 1992). In this case, Semarang can probably be used as a model for other medium-sized cities in Indonesia, since no such a study has been done elsewhere in Indonesia.

There may be many other species in the aquatic environment which are equally or even more sensitive to changes in water quality, but fish, by their size and by the attention paid to them, are excellent indicator organisms. Freshwater fish have often been adopted as the "sentinel" organisms for the health of the freshwater environment, because they are capable of inhabiting all zones of the aquatic habitat where the turbulence and flow rate of the water, its oxygen content and other constituents of water quality permit (Solbe, 1993). As a logical consequence for the next few decades the majority of studies on the ecology of freshwater fish will be dealing with various impacts of human activity on the freshwater environment (Larkin, 1992).

The "wild" guppy, *Poecilia reticulata* (Peters), which is common in urban streams of Semarang, was used in this study. This fish species is a prospective candidate bioindicator, and has already been used for laboratory toxicity tests. *P. reticulata* is an exotic species that has adapted very well to the South East Asian urban streams, after its introduction in the 1930's for mosquito control (Chou & Lam, 1989). In Singapore, *P. reticulata* is one of the most common fishes dominating the drains, canals, reservoirs and most open-water bodies

(Ng *et al.*, 1993). It is reported that this species has survived in polluted waters with free ammonia concentrations of a few hundred mg/L (Chou & Lam, 1989). Although this fish has shown its persistence in streams contaminated with a wide range of urban wastes, so far almost no ecotoxicological field studies have been made using this species.

In this study the potential use of the guppy, *P. reticulata* , as a bioindicator of urban metal pollution was assessed through a comparison between guppy populations from non-polluted and metal-polluted streams. This comparison was made for several parameters related to body size and reproduction. An attempt is made to evaluate the effects of elevated levels of metals in urban streams and to identify possible thresholds for ecological effects that might be used as benchmarks in biomonitoring programmes.

2. Materials and Methods

2.1 Study Area

Based on a previous study (Widianarko *et al.*, subm.a, Chapter 3) two streams in the South-Western part of Semarang, i.e. Purwosari (C_1) and Kreo (C_2), were selected to represent unpolluted streams. The polluted site was represented by streams from an industrial area, LIK-Bugangan Baru (D_1) in East-Semarang, and from a business district, Jalan Jendral Sudirman(D_2) in West-Semarang. Selected water quality parameters of these streams are presented in Table 1.

From each stream, 101 to 104 individual fish were collected randomly on a single sampling occasion in August 1995 and transported alive to the laboratory for further analysis.

Table 1. Water quality parameters of non-polluted (C_1, C_2) and polluted (D_1,D_2) streams in Semarang, Indonesia.

Location	pH	Dissolved oxygen (mg/L)	(%)	Total Suspended solids ± SD (mg/L)	Salinity (‰)
C_1	6.43 - 6.57	5.4	70	13.5 ± 1.2	0
C_2	7.79 - 8.13	6.6	86	48.3 ± 41.4	0
D1	7.41 - 7.43	1.9	26	46.4 ± 18.9	2
D2	7.61 - 7.94	6.2	77	5.4 ± 7.1	1

* Dissolved oxygen was measured in the afternoon at water temperature of 27° to 30° C.

2.2. Metal Analyses

Metal analyses were carried out at the laboratory of the Department of Ecology and Ecotoxicology, Vrije Universiteit Amsterdam. Analyses of metal concentrations in sediments, water and fish has been described in Widianarko *et al.* (subm.$_a$, Chapter 3 and Chapter 4).

2.3. Measurement of Body Size and Reproductive Parameters

Upon arrival in the laboratory, sex ratio, number of pregnant females, fresh weight and body length were determined from the samples of each location (C_1, C_2, D_1 and D_2). For pregnant females, fresh weight of reproductive tissues (eggs and juveniles) was determined apart from the fresh weight of somatic tissues. After weighing, the numbers of eggs and juveniles in each individual pregnant female were also recorded. After the completion of the above measurements, all fish samples were placed in an oven at 60 ^oC, 18 h, and the dry weights were determined.

The energy content of the fish was calculated based on the concentrations of protein and fat which were determined gravimetrically according to procedures outlined by Reznick (1983). Only male fishes were used in this measurement. Oven-dried fish tissue was extracted individually in a Soxhlett extraction apparatus using anhydrous ether. The extraction lasted for 6 hours until a constant weight was reached. After extraction, the sample was ashed in a muffle furnace at 550 ^oC for 6 hours. The difference between dry weight and weight after extraction is taken to be the fat content (ether extract) in the tissue, which was then multiplied by a factor of 39.75 to arrive at the energy content in kJ. The difference between the weights after extraction and after ashing is the protein content of the tissue, which multiplied by 23.85 gives the energy content in kJ.

2.4. Data Analysis

Due to its comparative nature, one-way ANOVA followed by a post-hoc test was the main statistical method employed in this study (Zar, 1984). The exceptions were the data which are more suitable to descriptive analysis, i.e. size structure, sex ratio, and pregnant/total female ratio. The size structure of the fish populations was derived from a frequency distribution of body lengths.

Following one-way ANOVA, the Least Significant Difference, LSD (Zar, 1984) or its modification, i.e. the Bonferroni test (Norusis, 1994), was used as the post-hoc test. To fulfil the normality and homogeneity of variance assumptions, if necessary, data transformations were done according to principles outlined by Steel & Torrie (1981). All computations were undertaken using SPSS for Windows version 6.1.

3. Results

Concentrations of Pb, Cu and Zn of the four streams selected for comparative study of the guppy population are depicted in Table 2. According to Widianarko *et al.* (subm.$_a$, Chapter 3) the degree of metal contamination in the Semarang areas can be classified into four categories, i.e. unpolluted, slightly polluted, polluted and heavily polluted. The selected sites represented unpolluted and heavily polluted sites.

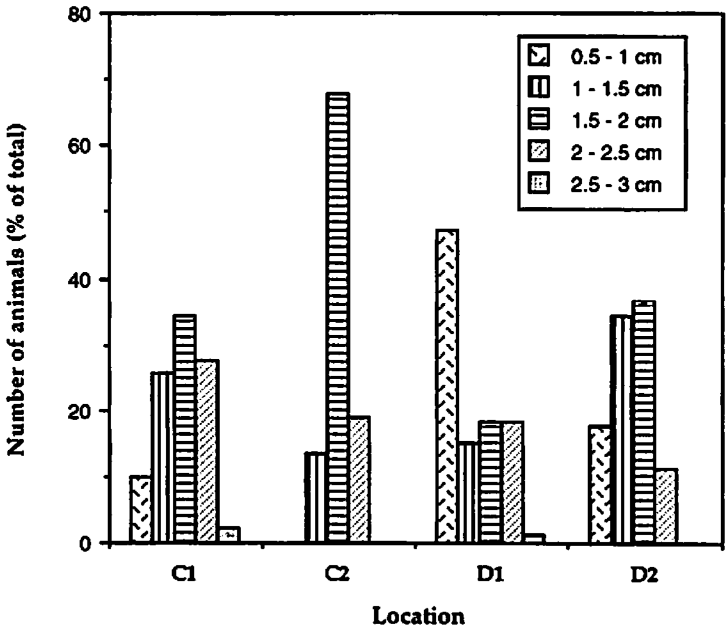
Table 2 shows that Pb and Zn concentrations in sediments from unpolluted (C_1 and C_2) and heavily polluted sites (D_1 and D_2) are significantly different. This does not hold for Cu, because there was no significant difference between C_2 and D_1 and D_2. A similar pattern was demonstrated by the concentrations of Pb and Zn in fish. No significant difference in concentrations of Cu in fish was detected between C_1 and D_1, D_2, as well as between C_2 and D_1 and D_2. With regard to the presence of metal in water, concentration of Pb from C_1 was much higher than expected. This concentration was significantly different from those of other sites.

Table 2. Metal concentrations in the sediment, water and *Poecilia reticulata* from non-polluted (C_1, C_2) and polluted streams (D_1,D_2) in Semarang, Indonesia.

Substrate		Metal		
		Lead	Copper	Zinc
Sediment (µg/g)	C_1	5.8 ± 6.2^a	19.7 ± 2.6^a	58.7 ± 8.2^a
	C_2	13.3 ± 3.9^b	53.6 ± 1.2^b	77.3 ± 5.6^a
	D_1	2666 ± 1941^c	65.4 ± 45.4^b	1257 ± 703^b
	D_2	344 ± 51.8^d	57.2 ± 19.6^b	585 ± 88.7^c
Water (µg/L)	C_1	37.0 ± 4.8^a	9.5 ± 7.1	39.7 ± 3.3
	C_2	8.5 ± 4.2^b	5.2 ± 0.4	43.5 ± 12.1
	D_1	3.0 ± 0.6^b	3.8 ± 0.6	61.2 ± 4.2
	D_2	6.2 ± 2.8^b	4.5 ± 1.5	53.8 ± 15.9
Fish (µg/g)	C_1	0.45 ± 0.19^a	2.34 ± 1.18^a	167 ± 21.0^a
	C_2	0.33 ± 0.11^a	3.98 ± 1.11^b	189 ± 38.5^a
	D_1	19.8 ± 15.4^b	2.23 ± 0.77^{ab}	404 ± 72.8^b
	D_2	4.35 ± 0.38^c	2.48 ± 0.67^{ab}	232 ± 16.5^c

- All values are means + standard deviation. Sample size = 5 (sediment & fish) & 3 (water).
- Different superscripts show significant differences between locations ($p < 0.05$), based on the LSD test following the one-way ANOVA of log-transformed data.

Figure 1. Size structure (body length) of guppy populations sampled in non-polluted (C1, C2) and polluted (D1, D2) streams in Semarang, Indonesia



Size structures of the fish populations at each sampling location were developed based on body-length data using five intervals, i.e. 0.5 - 1 cm; 1 - 1.5 cm; 1.5 - 2 cm; 2 - 2.5 cm and 2.5 - 3 cm. Figure 1 shows that the differences in size structure between unpolluted and polluted sites are inconclusive. If data from non-polluted and polluted streams were pooled, respectively, a significant difference was revealed.

There was no trend showing an association between metal pollution and the distribution of fish body lengths; However, in terms of the averages of body length and dry weight of overall fishes (male + female + unidentified), significant differences ($p<0.05$) between populations from unpolluted and heavily polluted sites were detected (see Table 3). When a separated analysis was done for each sex, only male fish showed significant differences in body length. When data for C_1 and C_2; D_1 and D_2 were pooled to respectively represent non-polluted and polluted streams, it can be shown that male fish from polluted streams had a significantly smaller body length (Table 3)

Only negligible differences were observed for other body size parameters, i.e. coefficient of weight and length relationship. Results of the measurements on the energy content of male guppies were inconclusive (Table 3). Significant differences occurred between C_1 and C_2, D_1, but there was no difference between C_1 and D_2, D_1 and D_2 (Table 3).

In unpolluted streams the ratio of female/male individuals were 2.5:1 and 3.3:1, whereas in polluted streams this ratio reduces to 1.4:1 and 0.9:1 (Table 4,

Table 3. Body size parameters of guppy populations sampled in non-polluted (C_1, C_2) and Polluted (D_1, D_2) streams in Semarang, Indonesia.

Parameter	Non-polluted		Polluted	
	C1	C2	D1	D2
<u>Overall</u>				
Body length (cm)	1.7 ± 0.5^a	1.8 ± 0.3^a	1.5 ± 0.4^b	1.3 ± 0.6^b
Body weight (mg, dry weight)	12.0 ± 9.3^a	12.1 ± 5.6^a	7.3 ± 5.7^b	7.4 ± 8.7^b
Body size coefficient (a^1)	2.04 ± 0.08	2.00 ± 0.10	1.99 ± 0.07	1.99 ± 0.08
<u>Males</u>				
Body length (cm)	2.16 ± 0.05^a	2.05 ± 0.06^a	1.77 ± 0.05^b	2.10 ± 0.05^{ab}
Body length^2 (cm)	2.11 ± 0.22^a		1.93 ± 0.29^b	
Energy content (kJ/g)	28.8 ± 1.14^a	24.0 ± 1.50^b	30.6 ± 1.27^c	29.1 ± 1.68^{ac}
Energy content (kJ/g)	26.39 ± 2.77^a	30.33 ± 3.23^b		
<u>Females</u>				
Body length (cm)	2.10 ± 0.08	2.00 ± 0.08	1.97 ± 0.10	2.36 ± 0.13
Body length^2 (cm)	2.05 ± 0.63		2.11 ± 0.53	

- C_1, C_2 and D_1, D_2 are respective representatives of clean and polluted streams
- Overall = male + female + unidentified
- All values are means $\pm$ standard deviation, except for male and female body length, and estimates of body size coefficient, a, followed by the standard errors.
- Sample size for overall body size comparison = 90 (C_1, C_2 & D_1) and 87 (D_2). Sample size for body length comparison of male fish = 22 (C_1), 18 (C_2), 27 (D_1) and 24 (D_2). Sample size for body length comparison of female fish = 57 (C_1), 61 (C_2), 37 (D_1) and 21 (D_2). Sample size for energy content (male only) =10.
- Different superscripts show significant differences ($p < 0.05$), based on the modified LSD (Bonferroni) test following the one-way anova, except for pooled comparisons (non-polluted *versus* polluted streams) based on t-tests.

1 coefficient of the length-weight relationship, Weight = a • $(\text{Length})^3$

2 data of body length are pooled (C_1 & C_2; C_1 & D_2) for a comparison of between non-polluted and polluted streams

Figure 2). The value of the pregnant to total female ratio (Figure 3) indicates the proportion of pregnant individuals among the females is about 1:5 in unpolluted streams, whereas in polluted streams it is higher.

Other reproductive success parameters observed in this study were fecundity and reproductive allocation or reproductive allotment. Fecundity can be measured as the number of eggs and juveniles per individual pregnant

Table 4. Reproductive parameters of guppy populations sampled in non-polluted (C_1, C_2) and polluted (D_1, D_2) streams in Semarang, Indonesia.

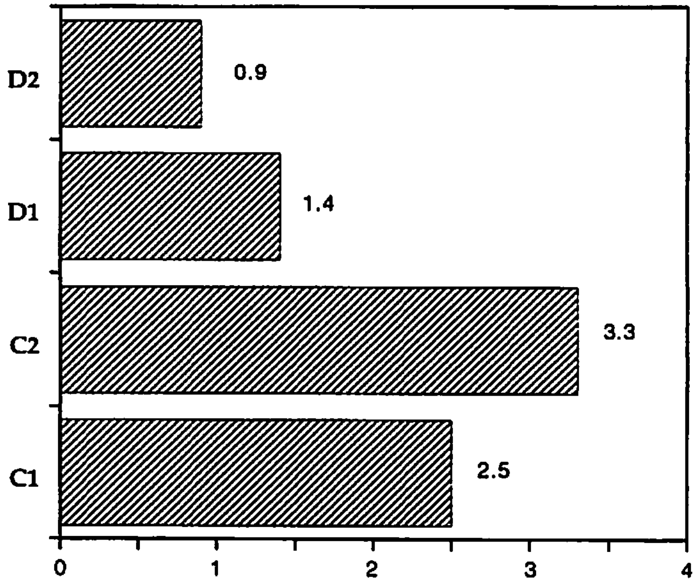
Parameter	Location			
	C_1	C_2	D_1	D_2
Sex ratio (f/m)	2.5	3.3	1.4	0.9
Percentages pregnancies of total females	19.6	20.0	32.2	58.8
Fecundity				
Juveniles/female	30.3 ± 21.9^{a}	7.5 ± 4.0^{b}	12.4 ± 5.3^{ab}	4.8 ± 3.3^{b}
Eggs/female	13.0 ± 7.0	12.5 ± 4.8	10.8 ± 7.9	9.8 ± 9.1
(Juveniles + eggs)/ female	24.8 ± 17.3^{a}	14.2 ± 7.3^{ab}	11.8 ± 6.2^{b}	8.9 ± 7.4^{b}
Reproductive allocation	19.6 ± 5.9	21.9 ± 9.2	20.9 ± 16.6	19.8 ± 11.1

- Fecundity = number of juvenile and/or egg beared by individual pregnant female,
- Reproductive allocation = percentage of reproductive tissues (eggs and or juveniles) of total body weight of individual pregnant female on a dry matter basis.
- All values are means + standard deviation. Sample size = 10 (C_1), 11 (C_2) & 12 (D_1 & D_2).
- Different superscripts show significant differences ($p < 0.05$), based on the LSD test following one-way ANOVA of square root-transformed data.

female. As shown in Table 4, no clear indication was available on the difference in fecundity, either expressed in number of juveniles or number of eggs. In terms of the total reproductive tissue, i.e. juveniles + eggs of individual pregnant females, a slightly more indicative result was obtained. The result of the statistical analysis on this parameter, however, was inconclusive. This may be related to the reasonably large variation in the data.

Reproductive allocation, expressed as the percentage weight of reproductive tissues to the dry body weight of individual pregnant females, did not show any significant difference between populations (Table 4). Large data variation occurred in measurements at all sites.

Figure 2. Sex ratio of guppy populations sampled in non-polluted (C1, C2) and polluted (D1, D2) streams in Semarang, Indonesia



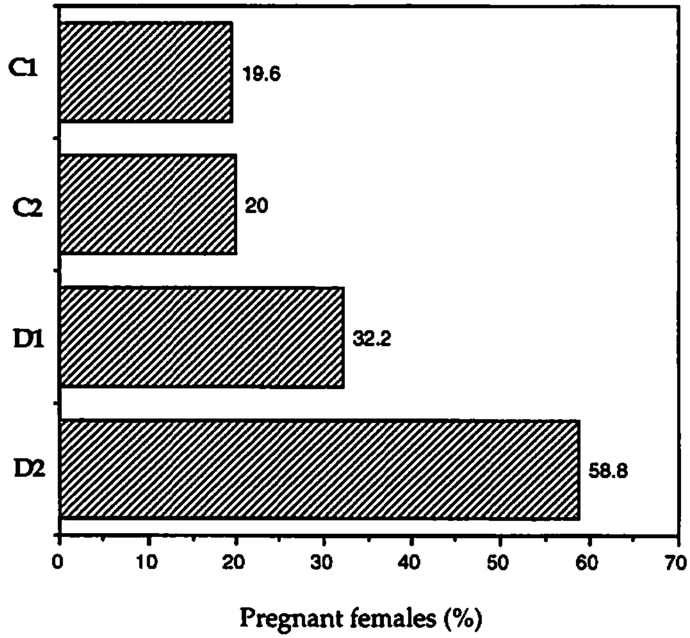
4. Discussion

The concentrations of metals in fish were well correlated with the concentration in sediment, but not with the concentration in water. This observation is in accord with the result of a correlation study conducted previously (Widianarko *et al.*, subm.b, Chapter 4). An extremely high Pb concentration in the water was measured in one of the unpolluted sites (Table 2). It seems that measurements in the water phase of the streams should be conducted repeatedly, to obtain a good impression of the degree of contamination. Significant differences in Pb and Zn concentrations in sediments as well as in fish from unpolluted and polluted sites might imply that the future monitoring in urban streams of Semarang can be better focused on these two metals.

Suppression of growth, as reflected by the smaller body size of the male guppies in the present study, is similar to those demonstrated for guppy (*P. reticulata*) populations under predation threat (Frazer & Gilliam, 1992); but, interestingly, in the present study female fish did not demonstrate this reduction of growth. Although it is not statistically significant, body lengths of female fish from polluted streams were slightly larger than those from non-polluted streams.

A significant difference in total energy content of male fish from non-polluted and polluted sites, which is in contrast with the corresponding results

Figure 3. Percentage of pregnancies of total female guppies sampled in non-polluted (C1, C2) and polluted (D1, D2) streams in Semarang, Indonesia



of body size measurements, might indicate a difference in tissue composition between fish from different sites. A further study is needed to clarify this phenomenon.

Among reproductive parameters observed in this study, it was shown that sex ratio and the ratio of pregnant to total females are promising parameters for bioindication. The latter is one of the simplest measures of reproductive success. Combining these two ratios, it can be inferred that guppy populations in polluted ecosystems tend to have fewer females, which show higher reproductive activity than those in unpolluted ecosystems.

For fecundity, a slight indication of a lower number of juveniles + eggs of individual pregnant females from polluted streams might, again, support the analogy between the presence of pollutants and predators as described above. In a long-term study on a population of Trinidian guppies in natural streams, the absence of predators resulted in bigger, longer living guppies with fewer and bigger offspring (Reznick *et al.*, 1996).

Insignificant differences in the reproductive allocation of pregnant female fish from different sites did not conform with the life history theory, which predicts that disturbances in a habitat will result, in increased reproductive efforts (Reznick *et al.*, 1990; Donker *et al*, 1993a). In a study on metal-adapted populations of the soil isopod *Porcellio scaber*, Donker *et al.* (1993b) concluded that the isopods at metal-contaminated sites were selected for early reproduction and increased reproductive allocation. The same evidence was demonstrated in a study on the guppy *P. reticulata* under predation pressure of the cichlid *Crenicichla alta* which preys predominantly on large, sexually mature size classes of guppies (Reznick *et al.*, 1990).

Based on the above findings, it can be seen that body size and reproductive parameters used in this study provide different levels of responses towards metal pollution. Among body size parameters, body length and body weight have shown a significant difference. The same does not hold for the size structure, although studies on other species suggested that this parameter is sensitive to metal pollution (see e.g. Van Capelleveen, 1987; Hopkin, 1989). The use of body size parameters for bioindication of chronic pollution, such as urban metal pollution, is promising, especially with regard to the nature of fish growth. Body size in fish is very distinctive, because it typically continues throughout life (McDowall, 1994). Furthermore, it has been established that many evolutionary strategies relate to body size (McDowall, 1994, Reznick, 1996). So, it can be expected that body size will be one of the most affected parameters during the long-term chronic exposure to metals.

Suitable candidates for evaluation of metal pollution among reproductive parameters, include sex ratio and the total-to-pregnant female ratio. These parameters are simple to measure, and have a direct implication for the reproduction capacity of a population. The use of reproductive parameters is common in ecotoxicology. These parameters are suitable for the evaluation of chronic pollution using fish as biomonitor organisms (Barnthouse *et al.*, 1987). Ecologically, the role of reproductive parameters is crucial, since reproductive success is a key to the existence of the population (Donaldson, 1990).

In this type of comparative field study, it is difficult to prove that there is a cause-effect relationship (see e.g. Donker *et al.*, 1993b), since various factors other than metal concentrations in the sediment, such as pH, nutrition, and the presence of organic contaminants, can play an additional role. Body size, for example, as the outcome of growth is highly flexible and subject to both genetic and environmental factors, such as water temperatures and food availability (McDowall, 1994). To establish cause-effect relationships, the above promising results should be confirmed by laboratory experiments.

5. Acknowledgements

Funding for this research was provided by the Universitas Katolik Soegijapranata and Department of Ecology and Ecotoxicology, Vrije Universiteit, Amsterdam. I am grateful to Prof. Dr. N.M. Van Straalen and Dr. Ir. C.A.M. Van Gestel for stimulating comments and suggestions, and to Mr. R.A. Verweij for assistance in the metal analysis. Thanks also goes to Mr. Felix Sholeh Kuntoro and Mr. Novi Susetyo Adi for the assistance in the collection and preparation of the samples.

6. References

Barnthouse, L.W., G.W. Suter, A.E. Rosen & J. Beauchamp (1987). Estimating responses of fish populations to toxic contaminants. Environ. Toxicol. Chem. 6: 811-824.

Berger, B. & R. Dallinger (1993). Terrestrial snails as quantitative indicators of environmental metal pollution. Environ. Monit. Assess. 25: 65-84.

Chou, L.M. & T.J. Lam (1989). Introduction of exotic aquatic species in Singapore. In S.S. Silva (ed.): Proc. of a Workshop on Introduction of Exotic Aquatic Organisms in Asia. Spec. Publ. Asian Fish Soc. 3: 91-97.

Dallinger, R.; B. Berger & S. Birkel (1992). Terrestrial isopods: useful biological indicators of urban metal pollution. Oecologia 89: 32-41.

Davies, B.E. (1992). Trace metals in the environment: retrospect and prospect. In D.C. Adriano (ed.): Biogeochemistry of Trace Metals. Lewis Publishers. Boca Raton. pp. 1-17.

Donaldson, E.M. (1990). Reproductive indices as measures of the effects of environmental stressors in fish. In S.M. Adams (ed.): Biological indicators of stress in fish. Am. Fish. Symp. 8: 109-122.

Donker, M.H.; H.E. Van Capelleveen & N.M. Van Straalen (1993a). Metal contamination affects size-structure and life-history dynamics in isopod field populations. In R. Dallinger & P.S. Rainbow (eds.): Ecotoxicology of Metals in Invertebrates. Lewis Publ. Boca Raton. pp. 383-399.

Donker, M.H.; C. Zonneveld & N.M. Van Straalen (1993b). Early reproduction and increased reproductive allocation in metal-adapted populations of the terrestrial isopod *Porcellio scaber*. Oecologia 96: 316-323.

Frazer, D.F. & J.F. Gilliam (1992). Nonlethal impacts of predator invasion: facultative suppression on growth and reproduction. Ecology 73: 959-970.

Hopkin, S.P. (1989). Ecophysiology of Metals in Terrestrial Invertebrates. Elsevier Applied Sciences, London.

Hopkin, S.P.; C.A.C. Hames & S. Bragg (1989). Terrestrial isopods as biological indicators of zinc pollution in the Reading area, South England. Monitore zool. ital. (N.S.) Monogr. 4: 477-488.

Kratz, W. (1996). Ecotoxicological bioindication: PAH, PCB and heavy metals studied in the natural resource monitoring programme of Berlin (Germany). In N.M. Van Straalen & D.A. Krivolutsky: Bioindicator Systems for Soil Pollution. NATO ASI Series. Kluwer Acad. Publ. Dordrecht. pp. 223-232.

Larkin, P.A. (1992). Future prospects and their implications for research on the ecology of freshwater fish. Ecology of Freshwater Fish 1: 1-4.

McDowall, R.M. (1994). On size and growth in freshwater fish. Ecology of Freshwater Fish 3: 67-79.

Ng, P.K.L.; L.M. Chou & T.J. Lam (1993). The status and impact of introduced freshwater animals in Singapore. Biol. Conservation 64: 19-24.

Norusis, M.J. (1994). SPSS Advanced Statistics 6.1. SPSS Inc. Chicago.

Pizl, V. & G. Josens (1995). Earthworm communities along a gradient of urbanization. Environ. Pollut. 90: 7-14.

Reznick, D.A. (1983). The structure of guppy life histories: the tradeoff between growth and reproduction. Ecology 64: 862-873.

Reznick, D.A.; H. Bryga & J.A. Endler (1990). Experimentally induced life-history evolution in a natural population. Nature 346: 357-359.

Reznick, D.A.; F.H. Shaw; F.H. Rodd & R.G. Shaw (1996). Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science 275: 1934-1937.

Sauve, S.; N. Cook; W.H. Hendershot & M.B. McBride (1996). Linking plant tissue concentrations and soil copper pools in urban contaminated soils. Environ. Pollut. 94: 153-157.

Solbe, J.F.D.L.G. (1993). Freshwater fish. In P. Calow (ed.): Handbook of Ecotoxicology. Vol. I. Blackwell Sci. Publ. Oxford-London. pp. 66-82.

Spellerberg, I.F. (1991). Monitoring Ecological Change. Cambridge University Press. Cambridge.

Steel, R.G.D. & J.H. Torrie (1981). Principles and Procedures of Statistics, A Biometrical Approach. Internat. Ed. Mc Graw-Hill Book Co. Singapore.

Van Capelleveen, H.E. (1987). Ecotoxicity of heavy metals for terrestrial isopods. PhD Thesis, Vrije Universiteit, Amsterdam.

Van Straalen, N.M. (1993). Open problems in the derivation of soil quality criteria from ecotoxicity experiments. In F.G.J. Ahrendt, R. Annokkee, R. Bosman & W.J. Van den Brink (eds): Contaminated Soil '93. Kluwer Acad. Publ., Dordrecht, pp. 315-326.

Van Straalen, N.M. & J. Van Wensem (1986). Heavy metal content of forest litter arthropods as related to body-size and trophic level. Environ. Pollut. Ser. A. 42: 209-221.

Voutsa, D.; A. Grimanis & C. Samara (1996). Trace elements in vegetables grown in industrial area in relation to soil and air particulate matter. Environ. Pollut. 94: 325-335.

Weigman, G. (1991). Heavy metal levels in earthworms of a forest ecosystem influenced by traffic and air pollution. Wat. Air & Soil Pollut. 57-58: 655-663.

Widianarko, B.; R.A. Verweij; C.A.M. Van Gestel & N.M. Van Straalen. Spatial distribution of trace metals in sediments from urban streams of Semarang, Central Java, Indonesia. [submitted$_a$]

Widianarko, B.; C.A.M. Van Gestel; R.A. Verweij & N.M. Van Straalen. Associations between trace metals in sediment, water and guppy, *Poecilia reticulata* (Peters) from urban streams of Semarang, Indonesia. [submitted$_b$]

Zar, J.H. (1984). Biostatistical Analysis. Prentice-Hall Inc., Englewood-Cliffs.

CHAPTER 6

Toxicokinetics-Based Survival Analysis In Bioassays Using Non-Persistent Chemicals

With Nico M. Van Straalen

ABSTRACT

Present statistical analysis of survivorship data obtained from bioassays using non-persistent toxicants does not explicitly take into account that exposure decreases with time due to degradation of the toxicant. Such a situation typically occurs in static aquatic toxicity tests and in soil tests. We develop a model for the analysis of survival data obtained in such experiments and show how estimates for the initial LC_{50}, the degradation rate constant, and the elimination rate constant may be obtained from observations on time-dependent survival rates. The model assumes linear one-compartment toxicokinetics with exponentially decreasing input. The hazard rate, i.e. the instantaneous probability rate of dying, is assumed to be directly proportional to the internal concentration. An explicit expression for time and concentration dependent survival is obtained. The model predicts that, with increasing exposure time, survival will approach a non-zero baseline value for certain initial concentrations. Application of the model is illustrated by curve fitting to experimental observations, made over 6 weeks, for the effect of diazinon on the terrestrial isopod *Porcellio scaber*. The fit to data is fairly good, but the parameter estimates tend to have a high coefficient of variation. A considerable increase in precision may be obtained if the degradation rate constant is given a fixed value, for example, following from chemical residue analysis of the medium. The approach is applicable to all situations where, due to loss of the toxicant during the test, mortality shows no further increase after a certain exposure period.

1. Introduction

The environmental hazards of potentially toxic chemicals are usually assessed on the basis of toxicity experiments in which selected organisms are exposed to a range of dose levels, and exposure response relationships are estimated. The results of these experiments are often used to derive maximum acceptable concentrations of chemicals in the environment.

The design of toxicity experiments, the interpretation of the results obtained from them, and the consequent derivation of environmental standards usually start from the premise that the exposure level is constant. This premise, however, is relevant only in the case of persistent chemicals. For non-persistent chemicals, constant exposure will occur only when there is constant infusion into the exposure environment.

The exposure concentration in actual toxicity experiments is often not constant. This may be due to various factors, such as degradation, sorption, volatilization, and uptake by organism. Usually this is considered to be a nuisance and extensive measures are taken in the laboratory to make exposure as constant as possible. In aquatic toxicity tests, this may be achieved by flow-through systems (Mount & Brungs, 1967). When concentration changes are moderate, a time-weighted average of the actual concentration is usually taken as a substitute for constant exposure (*e.g.* Kraak *et al.*, 1994). Soil tests, in contrast, are essentially static and a constant exposure level cannot be achieved if the chemical is not persistent (Van Straalen & Van Gestel, 1993). Often, effects are attributed to the nominal initial concentration, without taking the concentration decrease in the course of the experiment into account when analysing the data.

In the field, constant exposure will be realized only in the case of persistent toxicants in a well buffered environment, *e.g.* heavy metals in soil. In almost all other cases, exposure is not constant. This may vary from erratic fluctuations, *e.g.* chemicals in river water with variable flow and discharges, to peaks followed by a gradual decrease, *e.g.* pesticides applied to an agricultural field.

For non-persistent chemicals, such as pesticides, the half-life, or degradation time, is a very important variable determining ecological effects. Exposure concentrations in toxicity tests are characterized by an initial peak at time zero, followed by a gradual decrease. There is no theoretical framework for dealing with these non-constant exposures in the standard statistical analysis of concentration response experiments (Forbes, 1993). Strictly speaking, the concept of LC_{50} loses its meaning because it is not clear to what external concentration the effects should be attributed.

Some authors have proposed the use of a parametric approach in the analysis of toxicity data, based on the one compartment kinetics model (see, *e.g.* Kooijman, 1981; Landrum *et al.*, 1991 and McCarty *et al.*, 1992). In these types of models the LC_{50} decreases with exposure time due to the gradual accumulation of toxicants in the organism. This approach is based on the concept of the critical body residue (McCarty & Mackay, 1993). In the present contribution, we adopt a toxicokinetics based parametric survival analysis, like the authors cited above, but we present a new model for LC_{50}-time curves incorporating degradation kinetics.

The proposed model was developed following an approach outlined by Bedaux & Kooijman (1994), in which they relate survivorship to toxicokinetics by assuming that the hazard rate, the instantaneous probability rate of dying, is related to the concentration of the toxicant in the organism. Application of the model is illustrated by curve fitting using observations on the toxicity of the organophosphorus insecticide diazinon to the terrestrial isopod *Porcellio scaber*.

2. Derivation of The Model

In line with the nature of non-persistent chemicals, the first assumption of the model is that the concentration of the chemical in the environment decreases with time. We assume first order kinetics and write the external concentration, C(t), as:

$$C(t) = C_0 e^{-k_0 t} \tag{1}$$

where:

C_0 = initial external concentration (*e.g.* in µg/g),

k_0 = rate constant for degradation of the chemical in the medium (*e.g.*, in day^{-1}).

The change of exposure with time is illustrated in Fig. 1a.

The second assumption is that the kinetics of the concentration in the body follow a one-compartment model. This can be written as:

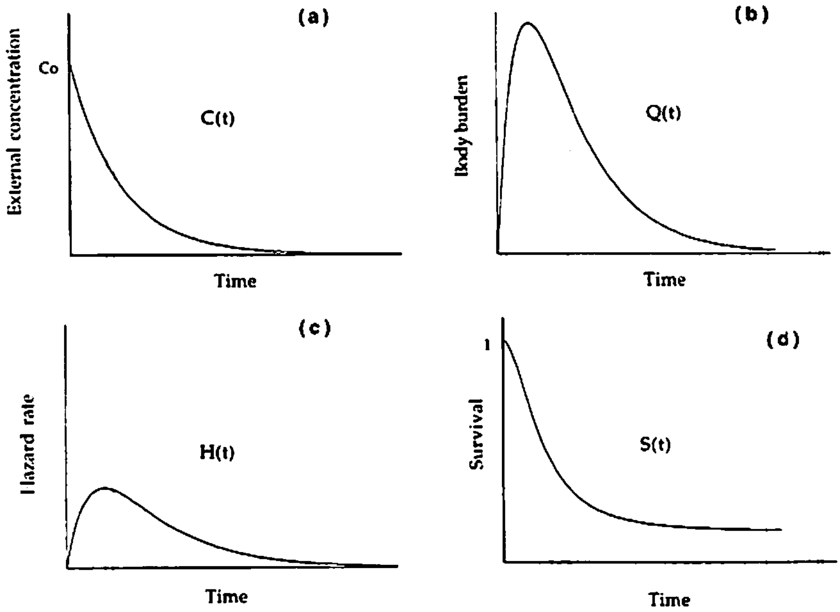


Figure 1. Curves showing exposure concentration (a), internal body concentration (b), hazard rate (c) and survival probability (d), as predicted by the toxicokinetics based survival model proposed in this paper.

$$\frac{dQ}{dt} = k_1\, C(t) - k_2\, Q(t) \tag{2}$$

where:

$Q(t)$ = internal concentration at time t (μg/g),

k_1 = rate constant for uptake (day^{-1}),

k_2 = rate constant for elimination, and other loss processes from the body, such as metabolism (day^{-1}).

Most toxicity experiments start with animals transferred from a clean environment, so equation (2) can be integrated with the initial condition, $Q(0) = 0$. Application of standard techniques (*e.g.* Laplace transforms, see Jacques, 1972), yields:

$$Q(t) = \frac{k_1 C_0}{k_2 - k_0}\left(e^{-k_0 t} - e^{-k_2 t}\right) \tag{3}$$

The curve for the internal body concentration, Q(t), is different from the one for the constant exposure single compartment model. The curve has a "hump", it starts with an increase directly after the beginning of the exposure period (Fig 1b), but this is followed by a decrease thereafter. The decrease is characterized by two exponentials, one due metabolism and elimination, and one due to degradation in the environment. Equation (3) has the constant exposure model as a special case, when k_0 is given the value zero.

To proceed from toxicokinetics to mortality, a further assumption is made, namely the hazard rate = instantaneous probability rate of dying is directly proportional to the internal concentration. We therefore introduce a proportionality constant q, and write the hazard rate, H(t), as:

$$H(t) = \theta\, Q(t) = \theta \left\{\frac{k_1 C_0}{k_2 - k_0}\left(e^{-k_0 t} - e^{-k_2 t}\right)\right\} \tag{4}$$

The curve for the hazard rate as a function of time (eq. 4) has the same shape as the internal concentration (see Fig. 1b and 1c). The proportionality constant, θ, is a measure for the toxicity of the chemical. Bedaux & Kooijman (1994) introduced a similar constant, called "killing rate", k_+, in our notation: $k_+ = \theta\, k_1/k_2$.

Using equation (4) it is now possible to construct survival as a function of time. By definition, survival probability, S(t) can be derived from the hazard rate, H(t) as:

$$S(t) = \exp\left[-\int_0^t H(s)\, ds\right] \tag{5}$$

where s is a (dummy) integration variable.

Under the initial condition $S(0) = 1$, and assuming no sources of mortality other than the toxicant, the right-hand side of eq. (5) can be solved analytically, allowing $S(t)$ to be written as:

$$S(t) = \exp\left[\theta \frac{k_1 C_0}{k_2 - k_0}\left\{\frac{1}{k_2}\left(1 - e^{-k_2 t}\right) - \frac{1}{k_0}\left(1 - e^{-k_0 t}\right)\right\}\right] \tag{6}$$

The survival curve (eq. 6) takes the form of an S-shaped decrease, followed by a baseline survival, a "floor" (Fig. 1d). A floor in the survival curve appears because after some time the toxicant has disappeared from the environment and all animals still surviving will then suffer no further mortality. Residual survival can take values between one and zero, depending on the parameters. It can be shown, using eq. (6), that the natural logarithm of the "floor", $\ln S(\infty)$, is directly proportional to C_0, k_1 and q, and inversely proportional to k_2 and k_0.

In the context of the present model, the classical concept of the LC_{50}, as the median lethal concentration in the environment that causes 50% mortality after a certain exposure time, has lost its meaning, because the external concentration is not constant. Instead, we may define a new toxicity parameter, the *Initial Median Lethal Concentration* (ILC_{50}). This can be defined as *the external initial concentration that will cause 50% mortality after a defined exposure time.*

Obviously, ILC_{50} will depend on exposure time, and this dependence is contained in eq. (6). When seen as a function of both time, t, and initial concentration, C_0, equation (6) defines a curved surface in a three dimensional space, with axes t, C_0 and S. On this surface we can find all points for which $S(t, C_0) = {}^1/_2$. The corresponding values for C_0 define ILC_{50} as a function of time. Rearranging equation (6) the following equation is obtained:

$$ILC_{50} = \left(\frac{\ln 2\, k_2 k_0}{\theta\, k_1}\right)\left(\frac{k_0 - k_2}{k_0(1 - e^{-k_2 t}) - k_2(1 - e^{-k_0 t})}\right) \tag{7}$$

The first factor of equation (7) has a special interpretation, namely the value of ILC_{50} when t approaches infinity. So, we may call this the ultimate ILC_{50}, denoted by μ. This analysis allows us to reparameterize equation (6), replacing the parameters θ and k_1 (which cannot be estimated separately because they occur as a product) by the more meaningful parameter μ. Rearranging equation (6) gives:

$$S(t) = \exp\left[\frac{C_0 \ln 2}{\mu(k_2 - k_0)}\left\{k_0(1 - e^{-k_2 t}) - k_2(1 - e^{-k_0 t})\right\}\right] \tag{8}$$

This expression may be used for the analysis of experimental data; using observations on survival as a function of time, t, and initial concentration, C_0, estimates may be obtained for the three parameters, k_0, k_2, and μ.

3. Application Of The Model

The model described above was applied to mortality observations in toxicity tests using the terrestrial isopod *Porcellio scaber*. Experiments were performed using F_1 generation animals collected from the Dieng plateau (Central Java, Indonesia). The animals were cultured in a glass terrarium filled with a layer of moistened sand covered with a layer of leaf litter (*Polyalthia longifolia*). The sweet potato, *Ipomoea batatas*, was used as a food supplement.

An alluvial soil collected from a place with no known history of pesticide use (Chinese cemetery area at Ambarawa) was used in the experiments. This type of soil is one of the major soil types of Central Java, distributed over approximately 20% of the total area, and has a composition of 75% clay, 13% silt and 11% sand, with an organic matter content (determined using dichromate titration) of 2.5% (w/w dry weight). The pH of the soil ranged from 5.4 to 5.5. Prior to use, the soil was sieved (1.5 mm) and dried for 24 h at 105 ^oC.

During the experiments isopods (10 - 20 mg) were kept in PVC pots (diameter = 5 cm, height = 5 cm), filled with 30 g dry weight soil, to which distilled water was added to obtain a moisture level of 40% (v/w). The lid and bottom of these pots were made of nylon gauze. Each pot contained five isopods. In each pot two slices of fresh sweet potato (± 20 x 2 x 5 mm) were offered twice a week. The pots were placed on a plastic tray containing a 1 cm thick layer of sand. To maintain the moisture level of the soil, distilled water was supplied to the trays. The experiment was carried out at temperatures of 18.0 to 26.5 ^oC and a relative air humidity varying from 46 to 70%.

A commercial formulation of diazinon (Diazinon 60 EC, PT. Petrokimia kayaku) was used. The diazinon was diluted with acetone (99.5% purity) and distilled water was then added for further dilution. The solutions were added to the soil and mixed thoroughly. Control soils were treated with acetone only. All soils were left for 24 h, to facilitate evaporation of the acetone, before the isopods were placed in each pot.

The experiment lasted for six weeks, using seven concentration levels of diazinon, 0 (acetone control), 2.00, 2.83, 4.00, 5.66, 8.00, and 11.31 μg active ingredient per g dry weight soil. The concentrations were based on the results obtained from a range finding experiment (not reported). Ten pots (50 individuals) were used for each concentration. Mortality inspection was done on a daily basis. Results of this experiment are presented as weekly survival rate data (Table 1, Fig. 2).

As shown in Fig. 2, the data conformed qualitatively to the predictions of the model, that is, a residual survival ("floor") appeared, due to some animals surviving after the degradation of the insecticide. This phenomenon was especially apparent at the highest initial concentrations, 8.00 and 11.31 μg/g.

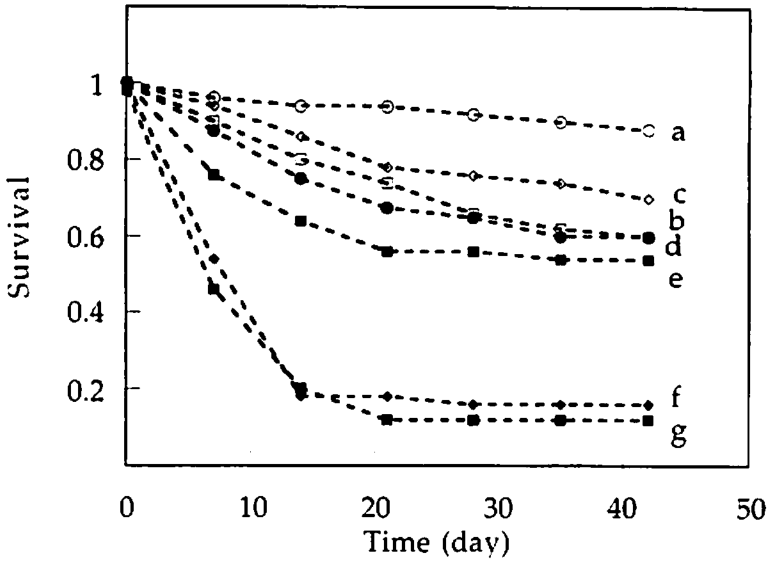


Figure 2. **Survival of the terrestrial isopod *Porcellio scaber* for various initial concentrations of diazinon in soil. Survival is given as a fraction of the initial number of animals in each treatment (50). Nominal initial concentrations, based on the active ingredient per mass of dry soil, are as follows: a = solvent control, b = 2.00 µg/g, c = 2.83 µg/g, d = 4.00 µg/g, e = 5.66 µg/g, f = 8.00 µg/g, and g = 11.31 µg/g.**

Table 1. **Survival rate of the terrestrial isopod *Porcellio scaber* under various exposure levels of the organophosphorous insecticide diazinon, mixed into a soil substrate. Each entry gives the total number of animals surviving out of 50 at the start of the experiment.**

Time (days)	Nominal initial concentration in soil (µg/g)					
	2.00	2.83	4.00	5.66	8.00	11.31
0	50	50	50	50	50	49
7	45	47	43	38	27	23
14	40	43	38	32	9	10
21	37	39	34	28	9	6
28	33	38	33	28	8	6
35	31	37	30	27	8	6
42	30	35	30	27	8	6

We used the survival data from Table 1 to estimate the parameters k_0, k_2 and μ in equation (8), divided by the initial number of animals in each treatment (50). The curve fitting was done using the "Nonlin" module in the SYSTAT software package, run on a Macintosh microcomputer. The iteration procedure converged to the same final estimates from various start values. Parameter estimates are given in Table 2, and the expected survival based on these estimates is reproduced in Table 3; because the model predicts 100% survival for all $C_0 = 0$ and all $t = 0$, the first row of the data table does not contribute to the parameter estimation and was disregarded when estimating variances for the estimates.

Table 2. Parameter estimates for the toxicokinetics based survival model applied to data in Table 1

Parameter	Unit	Estimate	SD	CV(%)
k_0	day^{-1}	0. 116	0.104	90
k_2	day^{-1}	0.363	0.564	155
μ	µg/g	4.409	1.562	13

Note: k_0 = degradation rate constant, k_2 = elimination rate constant, μ = initial concentration causing an ultimate mortality of 50%, SD = standard deviation of the estimate, CV = coefficient of variation.

Table 3. Expected survival data obtained after fitting equation (8) to the data in Table 1, using parameter estimates from Table 2.

Time (days)	Nominal initial concentration in soil (µg/g)					
	2.00	2.83	4.00	5.66	8.00	11.31
7	44.3	42.2	39.3	35.5	30.9	25.3
14	40.0	36.4	31.9	26.5	20.4	14.1
21	38.0	33.9	28.9	23.0	16.7	10.6
28	37.2	32.9	27.6	21.6	15.3	9.4
35	36.8	32.4	27.1	21.0	14.7	8.8
42	36.6	32.2	26.9	20.7	14.4	8.6

Comparison of Tables 1 and 3 shows that the fit of the model is fairly good (corrected $R^2 = 0.845$), but the residuals are not distributed homogeneously over the data matrix. The model underestimates survival at the intermediate initial concentrations and overestimates survival at the highest and the lowest concentrations. This may indicate concentration dependent effects that are not accomodated in the model, *e.g.* uptake being not proportional to C_0 or the actual C_0 being not proportional to the nominal C_0.

The parameter estimates have a high coefficient of variation (CV), and this is especially true for k_0 and k_2 (Table 2). The high CVs of the estimates can be reduced significantly if there is an independent estimate for the degradation rate constant (k_0). This may be the case if the concentration of the chemical is monitored in the course of the experiment using chemical residue analysis of the medium. Table 4 shows that if k_0 is given a fixed value, the estimates for k_2 and μ increase significantly in precision, although the estimated means do not differ much from the three parameter estimation exercise (see Table 2).

4. Discussion

An interesting feature of the derivation is that it ultimately leads to a relatively simple mathematical expression (eq. 8), which provides a possibility to estimate, from survival data, the degradation rate of a chemical (k_0), the elimination rate (k_2) and the ultimate ILC_{50} (μ); the latter was defined as the initial concentration in the medium that is just high enough ultimately to kill 50% of the animals before it disappears due to degradation in the medium.

As the model contains only three parameters, the assumptions leading to its erection are necessarily a simplified representation of reality. One point in particular is the assumption that the toxicokinetics are invariant across doses and with time. This assumption will not hold if rate constants change

Table 4. Parameter estimates for the time dependent ILC_{50} model when the degradation rate constant (k_0) is given a fixed value of 0.116 day^{-1}

Parameter	Unit	Estimate	SD	CV(%)
k_2	day^{-1}	0.361	0.169	47
μ	µg/g	4.410	0.313	7

Note: k_2 = elimination rate constant, μ = initial concentration causing an ultimate mortality of 50%, SD = standard deviation of the estimate, CV = coefficient of variation.

with exposure, *e.g.* due to behavioral changes during accumulation, affecting uptake, or induced metabolism, affecting depuration. The data elaborated in this paper indicated that concentration dependent uptake might well be a significant factor contributing to differences between observations and model expectations (Table 3).

Another possible point for extension concerns the way in which the hazard rate is assumed to depend on the body burden of the toxicant. Instead of our proposal, assuming direct proportionality, it could be useful to assume a threshold concentration for the body burden, below which the hazard rate is zero (Bedaux & Kooijman, 1994). This would, however, lead to a fourth parameter to be estimated from the data. It is our experience that bioassay data are rarely precise enough to allow the estimation of more than three parameters. The assumption of no threshold for the hazard rate may be reasonable for nerve toxins such as diazinon, however, it will not hold for toxicants whose effects are preceded by a gradual accumulation process in some storage compartment, such as heavy metals.

The estimate obtained for the degradation rate of diazinon (k_0 = 0.116 day^{-1}) is in reasonable agreement with the literature. Using residue data obtained in the field (United Kingdom), reported by Edwards (1976), the degradation rate of diazinon may be estimated as 0.053 day^{-1}. In a soil microcosm study, conducted at 29°C, Vink (1995) estimated a value of 0.0776 day^{-1} from chemical analysis of diazinon in tropical litter, with an initial exposure concentration of 1.86 mg/g. It should be pointed that our estimates for the degradation rate constant are derived from survival data, so they possibly include processes other than degradation that lead to a decrease in the bioavailability of the toxicant, such as volatilization and sorption. It is obvious from the present analysis (Tables 2 and 4) that estimates for toxicity parameters can be greatly improved if survival data are supplemented with actual measurements of the chemical's concentration changes.

Ecotoxicity studies taking non-constant exposure conditions into account are limited. Several authors have addressed the question of how to predict mortality under variable conditions, when the exposure regime is given as input and the LC_{50} is known from constant exposure conditions. There are few studies, however, that have addressed the inverse problem, that is how to estimate toxicological criteria from mortality observations with non-constant exposure (see Meyer *et al.*, 1995; Van Hattum, 1995).

Southworth *et al.* (1978) indicate a decreasing aqueous concentration during short-term bioaccumulation in *Daphnia pulex* for seven polycyclic aromatic hydrocarbons (PAH). In sediment bioassays, a decline in bioavailability of sediment associated contaminants, with increased contact time, has also been observed (see *e.g.* Varanasi *et al.*, 1985 and Landrum, 1989). Landrum *et al.* (1992) suggested that the effect of aging, *i.e.* increased contact time, is an important factor, in addition to organic matter concentration and composition, which should be considered when developing standard protocols for laboratory sediment bioassays.

Our model could be extended by modifying the input function, C(t), with respect to the various possible forms of chemodynamics in the environment, ranging from simple exponential decrease to erratic fluctuations, however, a more sophisticated input function will demand more mathematical elaboration and considerably more data.

5. Acknowledgements

The authors are grateful to Drs. Axel Groenveld and Drs. Maaike van Rijn (supported by CDCS-VU) for performing the ecotoxicity experiments at the Faculty of Biology, Universitas Kristen Satya Wacana, Salatiga, Indonesia, to Dr. Paul G. Doucet for mathematical discussions, to Dr. C.A.M. van Gestel and Dr. J.J.M. Bedaux for comments on the manuscript, to Miranda Aldham Breary M.Sc. for correcting the English style, to the Universitas Katolik Soegijapranata and the Department of Ecology and Ecotoxicology, Vrije Universiteit which provided a fellowship and hospitality to the first author (BW). Comments from two anynomous reviewers on an earlier version of this paper were very helpful.

6. References

Bedaux, J.J.M. & S.A.L.M. Kooijman (1994). Statistical analysis of bioassays, based on hazard modeling. Environmental and Ecological Statistics 1: 303-314.

Edwards, C.A. (1976). The uptake of two organophosphorus insecticides by slugs. Bull. Environ. Contam. Toxicol. 16: 406-410.

Forbes, T.L. (1993). The design and analysis of concentration-response experiments. In P. Calow (ed.): Handbook of Ecotoxicology, Blackwell, Oxford, UK, pp. 438-459.

Jacques, J.A. (1972). Compartmental Analysis in Biology and Medicine. Elsevier Publ. Co., Amsterdam.

Kooijman, S.A.L.M. (1981). Parametric analysis of mortality rates in bioassays. Water Res. 15: 107-119.

Kraak, M.H.S.; D. Lavy; H. Schoon; M. Toussaint; W.H.M. Peeters & N.M. Van Straalen. (1994). Ecotoxicity of mixtures of metals to the zebra mussel *Dreissena polymorpha*. Environ. Toxicol. Chem. 13: 109-114.

Landrum, P.F. (1989). Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod, *Pontoporeia hoyi*. Environ. Sci. Technol. 23: 588-595.

Landrum, P.F.; B.J. Eadie & W.R. Faust (1991). Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphiphod *Diporeia sp*. Environ. Toxicol. Chem. 10: 35-46.

Landrum, P.F.; B.J. Eadie & W.R. Faust (1992). Variation in the bioavailability of polycyclic aromatic hydrocarbons to the amphipod *Diporeia* (spp.) with sediment aging. Environ. Toxicol. Chem. 11: 1197-1208.

McCarty, L.S. & D. Mackay (1993). Enhancing ecotoxicological modeling and assessment. Environ. Sci. Technol. 27: 1719-1727.

McCarty, L.S.; D. Mackay; A.D. Smith; G.W. Ozburn & D.G. Dixon. (1992). Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: neutral narcotic organics. Environ. Toxicol. Chem. 11: 917-930.

Meyer, J.S.; D.D. Gulley; M.S. Goodrich; D.C. Szmania & A.S. Brooks. (1995). Modeling toxicity due to intermittent exposure of rainbow trout and common shiners to monochloramine. Environ. Toxicol. Chem. 14: 165-175.

Mount, D.I. & W.A. Brungs. (1967). A simplified dosing apparatus for fish toxicological studies. Water Res. 1: 21-29.

Southworth, G.R.; J.J. Beauchamp & P.K. Schmieder (1978). Bioaccumulation potential of polycyclic hydrocarbons in *Daphnia pulex*. Water Res. 12: 973-977.

Van Hattum, A.G.M. (1995). Bioaccumulation of sediment-bound contaminants by the freshwater isopod *Asellus aquaticus* (L). Ph.D. Thesis. Vrije Universiteit, Amsterdam.

Van Straalen, N.M. & C.A.M. Van Gestel. (1993). Soil invertebrates and micro-organisms. In P. Calow (ed.): Handbook of Ecotoxicology, Blackwell, Oxford, UK, pp. 251-277.

Varanasi, U.; W.L. Reichert; J.E. Stein; D.W. Brown & H.R. Sanborn (1985). Bioavailability and biotransformation of aromatic hydrocarbons in benthic organisms exposed to sediment from urban estuary. Environ. Sci. Technol. 19: 836-841.

Vink, K. (1995). Tropical isopods: ecological aspects and ecotoxicological application. Ph.D. Thesis. Vrije Universiteit, Amsterdam.

CHAPTER 7

Toxicokinetics and Toxicity of Zinc under Time-Varying Exposure in the Guppy, *Poecilia reticulata* (Peters)

with F.X.S. Kuntoro, C.A.M. Van Gestel and N.M. Van Straalen

ABSTRACT

In real world situations, constant exposure to toxicants is a very special case. Mostly, levels of toxic substances released into environment are highly variable and fluctuate in time. The present report deals with one specific type of time/varying exposure, a "diluted pulse". The objectives of the present studies were: (1) to determine the toxicokinetics of Zn in *Poecilia reticulata*, (2) to analyse the effect of time-varying Zn exposure concentrations on the survival of the guppies, and (3) to evaluate the applicability of a toxicokinetics-based survival model (Widianarko & Van Straalen, 1996) to survival data obtained. A toxicokinetics experiment using a constant Zn concentration was done in addition to two independent toxicity experiments with time decreasing exposure concentrations. In terms of toxicokinetics and mortality patterns, the field guppy responded to Zn exposure in a predictable manner, so it was feasible to use it for further ecotoxicological analysis and interpretations. In accordance with the model, baseline survival was found in both toxicity experiments. In general, values of parameter estimates of toxicokinetics and dynamics of Zn concentrations were close to estimated values from direct parameter estimation. The model fitted reasonably well for the lower initial concentration levels and tended to overestimate survival rates at higher concentrations. During the course of the two experiments, Zn concentrations in fish seemed to be independent of the initial exposure concentrations, and so were lethal body concentrations. In conclusion the present study suggests: (1) the use of conventional toxicity experiments, when coping with urban-industrial discharges of toxicants assuming a constant exposure concentration, might be misleading, and (2) a slightly more complicated toxicity experiment, allowing for time varying exposure, with an endpoint as simple as mortality, can provide a useful insight into the toxicokinetics and toxicodynamics of toxicants.

1. Introduction

In the real world, constant exposure to toxicants is a very special case. Mostly, levels of toxic substances released into the environment are highly variable. Constant exposure will be realised only in the case of persistent toxicants in a well buffered environment, *e.g.* heavy metals in soil. In almost all other cases, exposure is not constant. This may vary from erratic fluctuations to peaks followed by a gradual decrease. Concentrations of environmental pollutants can be variable due to varying rates of input and dilution, changes in chemical form and solubility, and degradation. Variable toxicant exposures can be found in a wide range of environmental settings, including agricultural, urban and industrial environments.

Various terms have been used to describe the patterns of time variable exposures, including pulse, plug, spike, episodic, fluctuating and intermittent exposures. In general these patterns can be simplified into two types of variable exposure: (1) pulse exposure which involves one or more isolated and brief exposure periods, and (2) fluctuating exposure which can be defined as a continuous exposure to varying toxicant concentrations (Hickie *et al.*, 1995).

Clearly, conventional toxicological approaches and concepts based on the premise of constant exposure to chemicals may no longer be adequate to deal with time variable exposure. Therefore, variable exposure has become an attractive field of study, both in experimental (see e.g. Hodson *et al.*, 1983; Holdway *et al.*, 1994; Barry *et al.*, 1995a & 1995b) and modelling studies (Mancini, 1983; Landrum *et al.*, 1991; McCarty *et al.*, 1992; Bedaux & Kooijman, 1994; Hickie *et al.*, 1995; Meyer *et al.*, 1995; Widianarko & Van Straalen, 1996). Most of the proposed models to deal with time variable exposure are based on the concept of critical body residues, which integrates toxicokinetics and the effect of exposure time on toxicity (McCarty & Mackay, 1993; Hickie *et al.*, 1995). This approach is promising since some studies showed that toxicity resulting from pulse exposures is largely controlled by the accumulation and elimination rates of toxicants in the exposed organisms (Hickie *et al.*, 1995).

The present study deals with a pulse exposure followed by exponential decay ("diluted pulse"). This type of exposure is not an uncommon phenomenon, and can be found both in terrestrial and aquatic environments. In the case of metal contamination in aquatic environments, such as urban streams, a diluted pulse type of exposure can occur when chemical discharges are released intermittently during production processes, so there will be dilution driven by the flow and volume of water in the streams.

Zinc is used in a variety of industrial processes. It enters aquatic environments as a result of mining and industrial and domestic effluent. Zinc is one of the "grey list" metals (Taylor *et al.*, 1985) which is, in high concentrations, known to be toxic to aquatic plants (see e.g. Huebert & Shay, 1992) and animals (see e.g. Giesy *et al.*, 1980; Timmermans *et al.*, 1992). In fish, the major toxic effects of elevated concentrations of waterborne Zn are disturbances of acid base and ionic regulation, e.g. impairment of branchial uptake of Ca^{2+}, disruption of gill tissues and hypoxia (Köck & Bucher, 1997). Besides its potential toxicity, Zn is an essential element which can be regulated by fish over a wide range of body concentrations (Ahsanullah & Williams, 1991; Köck & Bucher, 1997). Deficiency of Zn may lead to failure in reproduction, ranging from reduced fecundity to total inhibition of reproduction (see Caffrey & Keating, 1997). Furthermore, Zn has been shown to be a key structural component of more than 300 enzymes. In most of these enzymes, Zn is involved directly in catalysis, interacting with the substrate molecules undergoing transformation (Berg & Shi, 1996).

The "wild" field guppy, *Poecilia reticulata* (Peters), which is easily found in urban streams of Semarang, was used in this study. This fish is a suitable test species for toxicity experiments. *P. reticulata* is an exotic species which has adapted very well to the South East Asian urban streams, after its introduction in the 1930's for mosquito control (Chou & Lam, 1989). In Singapore, *P.*

reticulata is one of the most common fish dominating the drains, canals, reservoirs and most open water bodies (Ng *et al.*, 1993). It is reported that this species has survived in polluted waters with free ammonia concentrations of a few hundred mg/L (Chou & Lam, 1989). Previous field studies indicate that guppy from urban streams are a potential bioindicator for urban metal pollution, especially with respect to their (1) spatial distribution over sites of all pollution regimes, and (2) variation in metal accumulation levels reflecting the degree of pollution (Widianarko *et al.*, subm.$_a$, Chapter 4). Another field study by Widianarko (subm., Chapter 5) proposes that the potential use of *P. reticulata* as a bioindicator can be related to body size parameters (body length and body weight) and reproductive parameters (sex ratio and the ratio of pregnant to total females).

The objectives of the present studies were: (1) to determine the toxicokinetics of Zn in *P. reticulata,* (2) to analyse the effect of time decreasing Zn exposure concentrations on the survival of guppies, and (3) to evaluate the applicability of our toxicokinetics based survival model (Widianarko & Van Straalen, 1996) to the present survival data.

2. Materials and Methods

2.1. Fish and water

Poecilia reticulata were collected at the River Kreo, one of the non-polluted streams in Semarang, Central Java, Indonesia (Widianarko *et al.*, subm.$_b$, Chapter 3). The use of a metal wire framed plastic net was sufficient to catch the guppies. Caught fish were transported to the laboratory and kept in a 20 L plastic container. The average body weight of guppies was 88.4 mg with a standard error (SE) of 6.2 mg.

The fish were kept in a 120 L glass aquarium for two weeks to acclimatise them to the laboratory conditions. Drinking water supplied by the municipality water company of Semarang was used during acclimatisation and the actual experiment. The physico-chemical properties of the water used in the experiments (average of 10 measurements) were: temperature 25-27°C, pH 6.9-7.4, dissolved oxygen (DO) 6.4-7.1 mg/L and hardness (according to APHA, 1975) 168-178 mg $CaCO_3$/L.

2.2. Experimental Set Up

All experiments were done at the Biology Laboratory, Faculty of Agricultural Technology, Universitas Katolik Soegijapranata, Semarang. Three independent experiments, i.e. one toxicokinetics experiment using a constant Zn concentration and two short term semi-static toxicity experiments with decreasing concentrations of Zn, were conducted. Zinc chloride, $ZnCl_2$, with a purity of 98% (E-Merck, Germany) was used for all treatments. No food was offered to the fish during the experiments.

2.2.1. Toxicokinetics Experiment

Eighty guppies were sub-divided over 4 replicates, each consisting of twenty individuals kept in 2 L polyethylene containers filled with one litre of drinking water. During the first 5 days, fish were exposed to a nominal concentration of 10 mg Zn/L, from which they were moved to containers with clean water and kept in to the 10th day. Starting from day 0, one live guppy was taken from each replicate container every 24 hours. Water samples for Zn analysis were collected at day 0 and day 5 of the accumulation period, and during the depuration period: on the clean water replacement day (day 5) and at the end of experiment (day 10).

2.2.2. Toxicity Experiment I

Five nominal Zn concentrations, 10, 14.14, 20, 28.28 and 40 mg/L, with four replicates each, were applied in this experiment. In total 200 *P. reticulata* individuals were used, allowing random assignments of ten guppies to each replicate. Two litre polyethylene containers filled with one litre of water containing Zn were used for each replicate. Every 8 hours, 10 percent of the Zn contaminated water was replaced by clean water. Accordingly, mortality of the guppies was also monitored every 8 hours. At each observation time, all dead fish were collected for measurements of the lethal body concentration. Four water samples were taken from all replicates of all treatment levels every 24 hours. Two live guppies, i.e. from replicate 1 and 4 of each treatment, were also sampled for metal analysis, at the same time interval. Consequently, enumeration of fish survival was recorded only for replicates 2 and 3 of each treatment level. This experiment lasted for 72 hours.

2.2.3. Toxicity Experiment II

Basically this experiment was similar to the previous one. It differed only in the level and number of concentrations used and the time intervals of water replacement. Four nominal Zn concentrations, 5.6, 10, 18 and 32 mg/L, were used in this experiment. Replacement of 10 percent of the Zn contaminated water in each replicate of all treatment levels with clean water took place every 12 hours. Accordingly mortality observations and collection of dead fishes were done every 12 h. Collection of water and live fish samples, and enumeration of fish survival were done similarly to experiment I. This experiment lasted for 144 h.

2.3. Metal Analysis

Preparation of water and fish samples prior the Zn analysis was described in Widianarko *et al.* (submitted; Chapter 4). Metal analyses were carried out at the laboratory of the Department of Ecology and Ecotoxicology, Vrije Universiteit Amsterdam.

Determination of metal concentrations in the water and in fish were done using a flame Atomic Absorption Spectrophotometer Perkin Elmer 1100 B. No prior treatment was needed for the determination of metals in the acidified water samples. Oven dried fish samples were digested in a mixture of HNO_3 and $HClO_4$ (Ultrex grade, 7:1). The digestion was done according to the method of Van Straalen & Van Wensem (1986). Prior to the measurement, the pellet remaining after digestion was dissolved in 1.5 mL of 0.1 M HNO_3 (Ultrex grade). Certified reference material (bovine liver, BCR Reference Material no. 185, EC-Community Bureau of Reference) was routinely digested and analysed to maintain quality control. The result of this analysis, i.e. Zn concentration of 129.6 ± 5.0 µg/g, was in agreement with the reference value (123 µg/g).

2.4. Data Analysis

2.4.1. Toxicokinetics

A single compartment model was used to describe the toxicokinetics of Zn in the guppy. To deal with the fact that Zn is a regulated metal, we introduced an additional parameter, A, to represent the basal physiological Zn concentration, assuming that this Zn concentration is maintained at a constant level. The model assumes that an organism is exposed to a constant concentration C, followed by transfer to a concentration of zero from $t=t_c$ onwards. The toxicokinetics are then described by the following equation:

$$Q(t) = A + \frac{k_1 C}{k_2} \left[\{1 - e^{-k_2 t}\} - H(t_c)\{1 - e^{-k_2 (t-t_c)}\}\right] \qquad (1)$$

where

$Q(t)$	=	Zn concentration in fish at time t (µg/g)
A	=	basal physiological Zn concentration in fish (µg/g)
C	=	Zn concentration in water (mg/L)
k_1	=	rate constant for uptake (µg day^{-1})
k_2	=	rate constant for elimination (day^{-1})
t	=	time (days)
t_c	=	time at the start of the depuration period (day)
$H(t_c)$	=	a step function: $H = 0$ for $0 \leq t \leq t_c$, and $H = 1$ for $t > t_c$

2.4.2. Survival analysis

The decreasing water concentrations of Zn with time in the toxicity experiments I and II can be described according to the first order kinetics as:

$$C(t) = C_0 \, e^{-k_0 t} \tag{2}$$

where:
C_0 = initial Zn concentration in water (mg/L)
k_0 = rate for the decrease of Zn concentration (day^{-1})

A simultaneous parameter estimation was performed for each experiment to obtain a series of initial concentrations (C_0), assuming a common value for the decrease rate (k_0) for all initial concentrations. These estimations were done using the "Nonlin" module in SYSTAT software package, run on a Macintosh microcomputer.

Results of both toxicity experiments I and II were analysed using a toxicokinetics based survival model proposed previously by Widianarko & Van Straalen (1996). This model allows the estimation of toxicokinetic parameters and the dynamics of a toxicant, i.e. decreasing concentration, directly from the corresponding survival data. The model can be written as:

$$S(t) = \exp\left(\frac{C_0 \ln 2}{\mu(k_2 - k_0)}\left\{ k_0(1-e^{-k_2 t}) - k_2(1-e^{-k_0 t})\right\}\right) \tag{3}$$

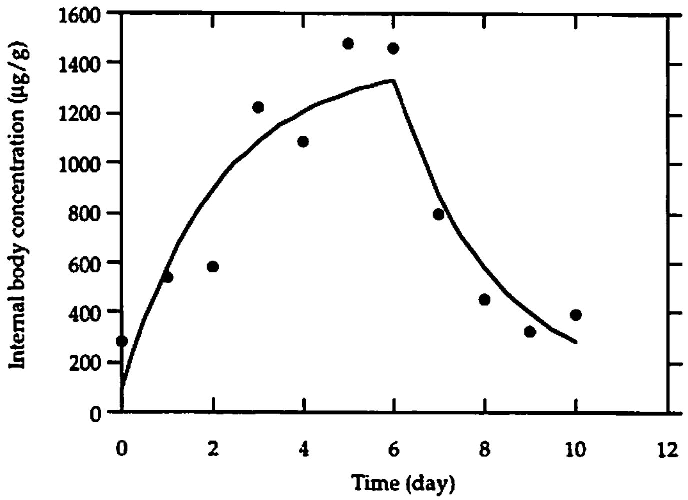
Where
$S(t)$ = survival rate at time t
C_0 = initial external concentration (mg/L)
k_0 = decrease rate of the external concentration (day^{-1})
k_2 = elimination rate (day^{-1})
μ = the ultimate initial LC_{50} (mg/L)
t = time (days)

Parameter estimation for the survival analysis was done using an APL function "RGNOR" based on the Newton-Raphson algorithm, developed by Prof. Dr. S.A.L.M. Kooijman, Department of Theoretical Biology, Vrije Universiteit Amterdam. Eight parameters for the first experiment (C_{01}, C_{02}, C_{03}, C_{04}, C_{05}, k_0, μ and k_2) and seven parameters for the second experiment (C_{01}, C_{02}, C_{03}, C_{04}, k_0, μ and k_2) were estimated simultaneously from the survival data.

3. Results

During the toxicokinetics experiment, the actual concentration of Zn in the water was lower than the nominal one. During the accumulation period, the actual concentration decreased from 7.34 mg/L (day 0) to 2.48 mg/L (day 5). During the depuration period, Zn concentration in the water slightly increased

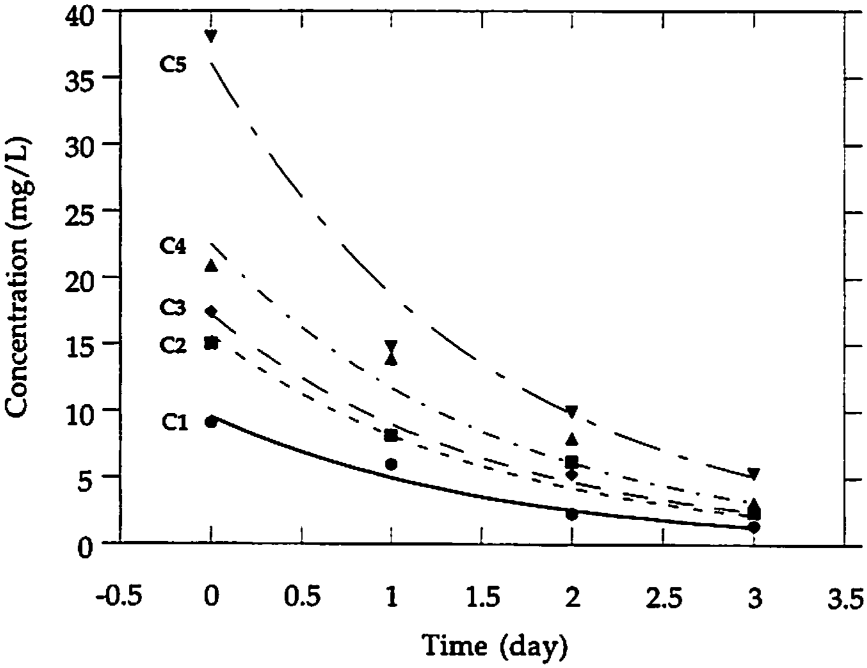
Figure 1. Uptake and elimination of Zn in guppy exposed to a constant Zn concentration in water from time 0 to 6 and to clean water from time 6 onwards. The data points are averages over four measurements. The continuous line is a curve fitted to the data according to equation (1) in the text.



from 0.20 mg/L (day 5) to 0.43 mg/L at the end of experiment (day 10). Combining the Zn concentrations over time in water and in fish (Figure 1), and the mass balance calculation of Zn in the test system, it can be inferred that these changes of Zn concentrations in the water can only be partly attributed to the accumulation and elimination of Zn by the guppies. Curve fitting of the accumulation and elimination kinetics, based on the Zn concentrations in the fish (Figure 1), resulted in estimated values (± SD) of the initial Zn concentration in fish, A, the accumulation rate, k_1 and the elimination rate, k_2 of 91.0 ± 122 µg/g, 83.5 ± 14.2 day^{-1} and 0.463 ± 0.096 day^{-1}, respectively. Based on these values, it can be calculated that the Biological Concentration Factor (BCF) and $t_{0.5}$, biological half-life, of Zn in guppies were 180 and 1.5 day, respectively.

The dynamics of the Zn concentrations in water of the experiments with decreasing exposure concentration are depicted in Figures 2 and 3. Simultaneous parameter estimations of the change of Zn concentration in water with time resulted in different decay rates, i.e. 0.652 day^{-1} in experiment I and

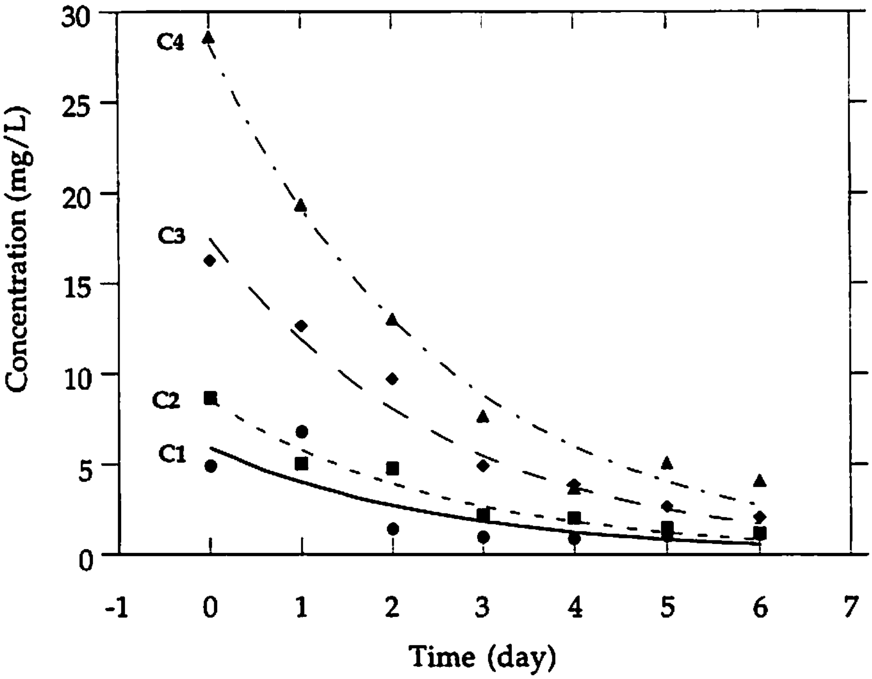
Figure 2. Decreasing concentrations of Zn in the water in the toxicity experiment I. The data points are averages over four measurements. The continuous line is a curve fitted to the data according to equation (2) in the text.



0.375 day^{-1} in experiment II. Measured and estimated initial concentrations (C_0s) and estimated decrease rate (k_0) of Zn in experiments I and II are listed in Table 1.

As expected from the model, baseline survival was found in both toxicity experiments I and II. Fish mortality was found under all Zn treatment levels (Table 2 and 3). Clean water replacement every 8 hours in experiment I halted the fish mortality after 32 hours, except for the lowest initial concentration (9.09 mg/L). This lowest treatment unexpectedly also induced a higher mortality rate compared to higher Zn treatments (i.e. 15.01 and 17.42 mg/L). A very steep mortality rate was demonstrated by fish under the highest treatment level (38.08 mg/L), leading to only 10 % survival after 32 hours until the end of the experiment (72 hours).

Figure 3. Decreasing concentrations of Zn in the water in the toxicity experiment II. The data points are averages over four measurements. The continuous line is a curve fitted to the data according to equation (2) in the text.



In toxicity experiment II, fish mortality under all treatment levels was according to expectation. Lower Zn treatment levels resulted in lower mortality rates compared to those at higher treatment levels. Clean water replacement every 12 hours did not seem to halt fish mortality. Only in the lowest Zn treatment level (4.94 mg/L) fish mortality levelled off at 60 hours, whereas in the higher Zn concentrations fish mortality continued until 120 hours (28.6 mg/L), 132 hours (8.67 mg/L) and the end of experiment, 144 hours (16.25 mg/L).

Curve fitting of the survival data of both toxicity experiments I and II, using the toxicokinetics-based model (Eq. 3), resulted in the parameter estimates listed in Table 1. It can be seen that in general, parameters for toxicokinetics and dynamics of Zn concentrations estimated from the survival data were close to estimated values from the toxicokinetics experiment (Table 1). High standard errors were found for the estimates of the ultimate initial LC_{50} (μ) and the elimination rate constant (k_2). The value of μ was somewhat lower for experiment I than for experiment II.

Expected survival can be calculated based on estimated values of parameters of the toxicokinetics based survival model (Eq. 3). The expected

Table 1. Measured initial Zn concentrations (from four replicates) and parameter estimates of dynamics of Zn concentrations in water and toxicokinetic parameters in toxicity experiments with *Poecilia reticulata* exposed to a diluted Zn pulse in water. All values are ± SE.

Parameter		Actual	Parameters estimated from measured Zn in water	Parameters estimated from observed fish survival
Experiment I				
Initial concentrations				
(mg/L)	C_{01}	9.09 ± 0.59	9.54 ± 0.86	9.86 ±0.67
	C_{02}	15.01 ± 3.59	15.58 ± 0.87	15.93 ± 0.69
	C_{03}	17.42 ± 0..65	17.20 ± 0.88	16.77 ± 0.69
	C_{04}	20.91 ± 5.95	22.51 ± 0.88	23.82 ± 0.73
	C_{05}	38.08 ± 1.55	36.07 ± 0.94	33.70 ± 0.78
Decrease rate				
(day^{-1})	k_0		0.652 ± 0.044	0.634 ± 0.018
Ultimate initial				
LC_{50} (mg/L)	μ			6.40 ± 3.59
Elimination rate				
(day^{-1})	k_2			0.434 ± 0.338
Experiment II				
Initial concentrations				
(mg/L)	C_{01}	4.94 ± 0.04	5.87 ± 0.66	6.23 ± 0.47
	C_{02}	8.67 ± 0.31	8.45 ± 0.67	8.41 ± 0.48
	C_{03}	16.25 ± 0.44	17.30 ± 0.71	17.80 ± 0.54
	C_{04}	28.60 ± 0.87	27.07 ± 1.40	28.49 ± 0.54
Decrease rate				
(day^{-1})	k_0		0.375 ± 0.020	0.383 ± 0.013
Ultimate initial				
LC_{50} (mg/L)	μ			9.10 ± 4.13
Elimination rate				
(day^{-1})	k_2			0.488 + 0.444

Figure 4. Concentration of Zn in guppies under different initial concentrations in water (Experiment I)

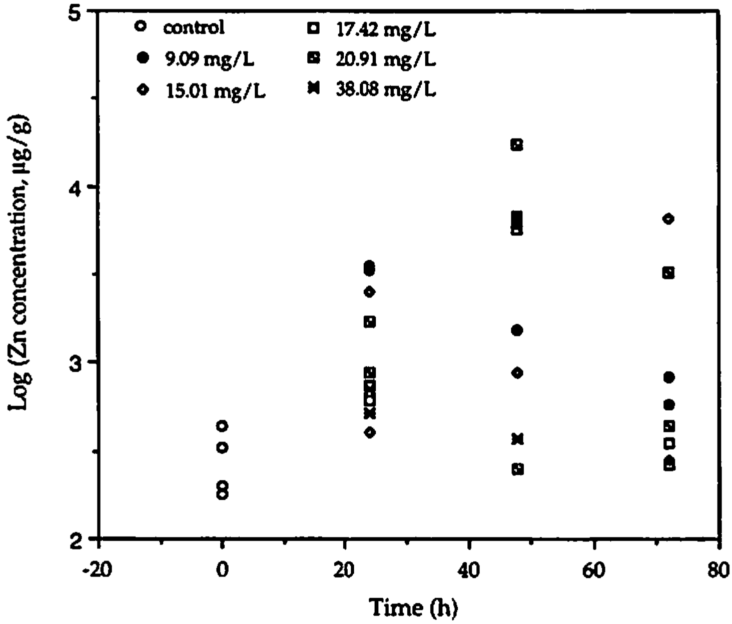


Table 2. Number of surviving *Poecilia reticulata* exposed to a diluted pulse, with different Zn concentrations in water (Experiment I). Observed values are followed by expected values between brackets.

Time (h)	Initial Zn Concentration Level*				
	C_{01}	C_{02}	C_{03}	C_{04}	C_{05}
0	20 (20)	20 (20)	20 (20)	20 (20)	20 (20)
8	20 (19.7)	19 (19.5)	18 (19.5)	18 (19.4)	15 (19.0)
16	19 (19.0)	19 (18.4)	16 (18.3)	14 (17.6)	5 (16.8)
24	19 (18.0)	19 (16.9)	16 (16.8)	11 (15.6)	3 (14.0)
32	17 (17.0)	18 (15.3)	15 (15.1)	11 (13.4)	2 (11.4)
40	16 (15.9)	17 (13.8)	15 (13.5)	11 (11.4)	2 (9.1)
48	13 (14.8)	17 (12.3)	15 (12.0)	11 (9.7)	2 (7.2)
56	13 (13.9)	17 (11.1)	15 (10.7)	11 (8.2)	2 (5.7)
64	13 (13.0)	17 (10.0)	15 (9.6)	11 (7.0)	2 (4.6)
72	12 (12.2)	17 (9.0)	15 (8.6)	11 (6.1)	2 (3.7)

* see Table 1 for corresponding concentration

number of surviving guppies according to the model are included in Tables 2 and 3, respectively for experiments I and II. These tables show that the model fitted reasonably well for the lower initial concentration levels and tended to overestimate the survival rates at higher concentrations. Generally, a better fit between data and model was found for experiment I than for experiment II.

It can be seen from Figures 4 and 5 that during the course of experiments I and II Zn concentrations in surviving fish seemed to be independent of the initial exposure concentrations. Compared to the Zn level in control fish, clearly elevated Zn concentrations in guppies were found both for experiments I and II (Figure 4 and 5).

In this study lethal body concentration is defined as the concentration of Zn measured in the fish when it was found dead. The scatter plots of lethal body concentrations from experiments I and II (Figure 6 and 7), however, suggest that there is no difference between initial exposure concentrations. Lethal body concentrations seemed to be more or less constant with exposure time. Exceptionally, a lower range of lethal body concentrations was found in the period from 60 to 84 hours in experiment II.

Table 3. Number of surviving *Poecilia reticulata* exposed to a diluted pulse, with different Zn concentrations in water (Experiment II). Observed values are followed by expected values between brackets.

Time (h)	Initial Zn Concentration Level*			
	C_{01}	C_{02}	C_{03}	C_{04}
0	20 (20)	20 (20)	20 (20)	20 (20)
12	19 (19.8)	20 (19.7)	19 (19.5)	7 (19.4)
24	19 (19.3)	19 (19.1)	19 (18.2)	5 (18.1)
36	19 (18.7)	19 (18.3)	17 (16.6)	5 (16.5)
48	19 (18.1)	19 (17.4)	16 (15.0)	5 (14.8)
60	18 (17.4)	18 (16.6)	16 (13.5)	5 (13.2)
72	18 (16.8)	18 (15.8)	16 (12.1)	4 (11.9)
84	18 (16.2)	18 (15.0)	16 (11.0)	3 (10.7)
96	18 (15.7)	17 (14.4)	16 (10.0)	3 (9.7)
108	18 (15.2)	17 (13.8)	16 (9.2)	2 (8.9)
120	18 (14.8)	16 (13.3)	15 (8.5)	1 (8.2)
132	18 (14.5)	15 (12.9)	15 (7.9)	1 (7.6)
144	18 (14.2)	15 (12.5)	14 (7.5)	1 (7.2)

* see Table 1 for corresponding concentration

Figure 5. Concentration of Zn in guppies under different initial concentrations in water (Experiment II)

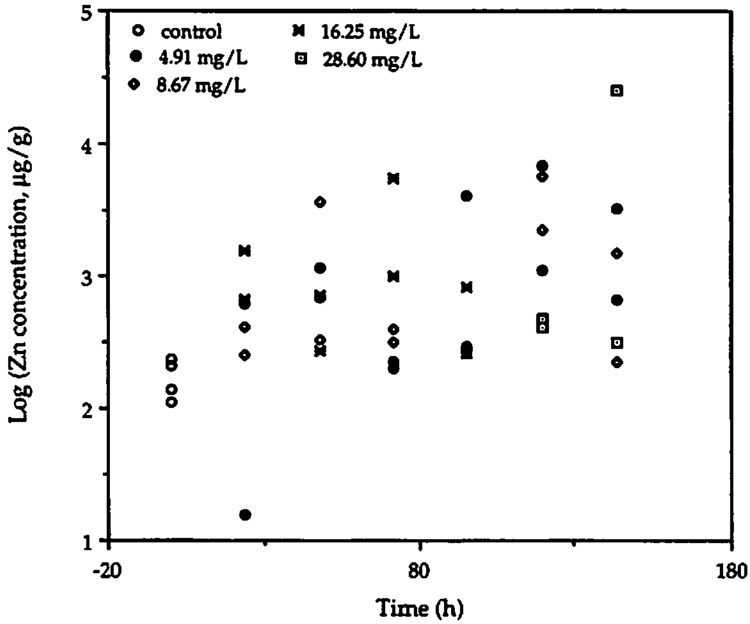


Figure 6. Lethal body burden of Zn in guppies under different initial concentrations in water (Experiment I)

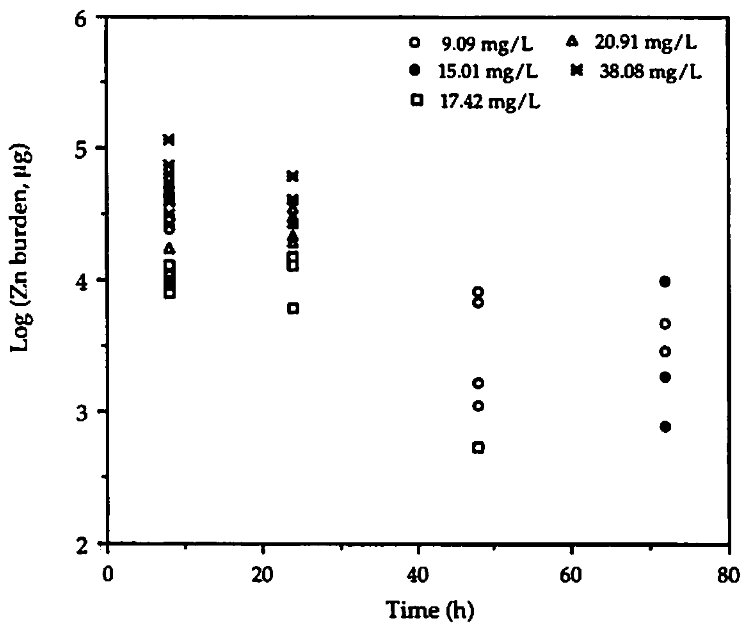
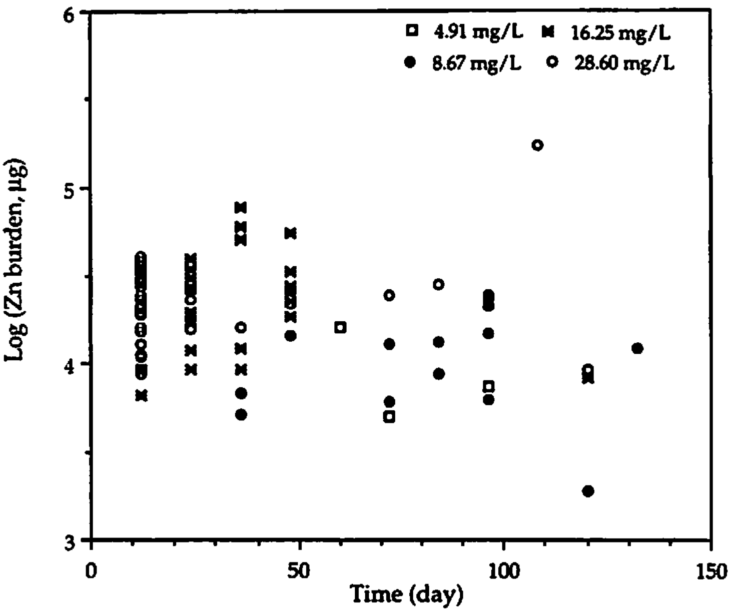


Figure 7. Lethal body burden of Zn in guppies under different initial concentrations in water (Experiment II)



4. Discussion

In addition to the bioindication potentials of *Poecilia reticulata* as demonstrated in previous field studies (Widianarko *et al.*, Widianarko, subm., Chapter 4; Widianarko, subm, Chapter 5), the present study clearly showed the usefulness of this fish species as a test animal in aquatic toxicity experiments with metals. In terms of toxicokinetics and mortality patterns, the field guppy from urban streams of Semarang responded to Zn exposure in a predictable manner, so it is feasible to use it for further ecotoxicological analysis and interpretations.

The basal physiological Zn concentration in fish (A) derived from the toxicokinetics curve fitting was lower than the actual measurement on control fish, but it showed very large variation. Three independent measurements (each with four replicates) showed that internal concentrations (± SD) of the fish used in the control (day 0) of the toxicokinetics experiment, and the toxicity experiments I and II were 282.4 ± 70.1 µg/g, 287.5 ± 121.0 µg/g and 174.8 ± 58.4 µg/g, respectively. This may be due to the inadequacy of the constancy assumption of basal physiological Zn concentration in the toxicokinetics model applied. According to Köck and Bucher (1997), the physiological needs of Zn as an essential element, can be satisfied over a wide range of internal body concentrations.

The high accumulation rate (k_1) found in this study implies that guppies can rapidly take Zn from water, since no dietary Zn was offered during the experiment. It indicates that Zn uptake from water by guppies is an important pathway. No consensus exists in the literature on the relative importance of Zn uptake in fish from dietary or aqueous sources (see e.g. Giesy *et al.*, 1980; Dallinger *et al.*, 1987; Timmermans *et al.*, 1992; Köck & Bucher, 1997).

Due to the fact that the elimination rate (k_2) was also high, and accordingly the biological half-life (t_{05}) was short, the Biological Concentration Factor (BCF) of Zn in guppies was relatively low. BCFs found in this study, however, are much higher compared to results of a study on other fish species. Giesy *et al.* (1980) reported that accumulation and elimination rates (k_1 and k_2) for Zn in freshwater crayfish, *Procambarus acutus acutus* were only 0.83 - 0.84 and 0.029 - 0.080 day^{-1}, respectively under Zn exposure levels of 50 and 100 µg/L. In addition to species differences, the discrepancy in the values of the kinetics parameters may be due to the difference in Zn exposure concentrations. Giesy *et al.* (1980) showed that uptake of Zn tended to be proportional to the water concentration. Another possible explanation for this discrepancy may be body size. Newman & Mitz (1988), in a study of mosquito fish, *Gambusia affinis*, found that the elimination rate constant (k_2) of Zn decreased with increasing fish size.

Elimination rates found in the present study are also high compared to those reported for freshwater isopods (*Asellus aquaticus*). Van Hattum *et al.* (1993), in a mesocosm study using a contaminated sediment (Zn = 319 µg/g), found that elimination rates of Zn were 0.019 to 0.026 day^{-1}. Values of toxicokinetics parameters from the present study, except for elimination rate, were, however, not extremely high. In a toxicokinetics study using 1.0 mg/L Zn on caddisfly larvae, Timmermans *et al.* (1992) reported values of k_1, k_2, BCF and t_{05} of 121 day^{-1}, 0.131 day^{-1}, 924 and 5 days, respectively. It seems that under acute exposure consentrations (10 mg/L), guppies tend to increase their elimination rates (k_2), so the biological half-life of Zn is shortened. A half life of 1.5 day is indeed very short for an essential element; but it may be that excretion is the main mechanism to avoid or to cope with increasing aquatic concentration of Zn, as demonstrated by a study on the decapod *Palaemon elegans* (Rainbow & White, 1989).

Parameter estimates for the dynamics of Zn concentration, initial concentrations (C_0s) and decrease rate (k_0), consistently reflected the different rates of water replacement between experiments I and II. The simulated decreasing concentrations seemed to agree with the expectation and resulted in baseline survival of guppies, which was similar to the response of the terrestrial isopod *Porcellio scaber* in a previous study using the non-persistent pesticide diazinon (Widianarko & Van Straalen, 1996; Chapter 6). High mortality in the initial period of the toxicity experiments, especially in experiment I where mortality halted at 32 h, is a common phenomenon in pulsed exposure toxicity tests in which test animals are exposed only shortly to a high concentration of a toxicant followed by a decrease of exposure concentrations or absence of the toxicant. Holdway *et al.* (1994) demonstrated that a one hour pulse exposure to the pesticides fenvalerate and esfenvalerate resulted in the most mortality of larval Australian crimson spotted rainbow fish (*Melanotaenia fluviatilis*) within the first 24 hours.

Estimated values of initial exposure concentrations (C_0s), decreasing rates of exposure concentrations (k_0) and elimination rates (k_2) of Zn found from the curve fitting of survival data of experiments I and II using the toxicokinetics-based model (Eq. 3) were convincingly close to the corresponding values from the independent toxicodynamics and toxicokinetics curve fittings. This shows that the model is capable of explaining the variation of survival data in terms of toxicokinetic and toxicodynamic parameters of Zn in *P. reticulata.* High standard errors for the estimates of the ultimate initial LC_{50} (μ) and elimination rate (k_2) may be related to the nature of the data. In fact, these two were the most problematic parameters to estimate during the iteration processes, in which start values needed to be close to the final values to make the iteration converge. These high standard errors may be responsible for the poor fit of the model (Eq. 3) on the survival data from the higher exposure concentrations. More chronic survival data may be needed to further validate the model (Eq. 3) for high exposure concentrations. The agreement of estimated values for elimination rates (k_2) from the toxicokinetics experiment (0.463 day^{-1}) and those from the diluted pulse experiments (0.434 day^{-1} and 0.488 day^{-1}) are of particular interest. This constancy may be attributed to the range of Zn concentrations used in the two toxicity experiments which were close to the Zn level applied in the toxicokinetics experiment.

As soon as we deal with time varying exposure concentrations of toxicants, such as decreasing exposure, the classical concept of LC_{50} does not hold. In this case, the parameter μ, i.e. initial concentration causing 50% of mortality at infinite time, can be regarded as a new measure characterizing the concentration mortality relationship. Basically μ is a chronic toxicity parameter, but interestingly it can be estimated from changes in mortality including the acute phase. In the light of the range of available LC_{50} values for Zn in marine and freshwater animals, the values of μ in the present study, i.e. 6.4 and 9.1 mg/L for ± 10% decreasing exposure concentrations every 8 h and 12 h, respectively, are not extraordinary. According to Taylor *et al.* (1985) the range of 96 h LC_{50}s for Zn in marine fish is 4.0 - 100 mg/L. For freshwater fish, Köck & Bucher (1997) provided LC_{50} values of 1.9 - 2.3 mg Zn/L for rainbow trout and brown trout. Ahsanullah (1976) reported 96h LC_{50} values for seven marine invertebrate species in the range of 0.58 - 13.1 mg Zn/L. For larvae of the crab (*Paragrapsus quadridentatus*) the corresponding value was 1.23 mg Zn/L (Ahsanullah & Arnott, 1978). In a chronic experiment using the shrimp *Callianasa australiensis*, Ahsanullah *et al.* (1981) found Zn LC_{50} values of 10.2 and 1.15 mg/L for 4 and 14 days, respectively.

Body concentrations of Zn in surviving guppies and the corresponding lethal body concentrations seemed to be independent of the initial exposure concentrations. No particular explanation, other than individual variation, can be assigned to the relatively low values for lethal body concentrations during the period of 60 to 84 hours in experiment II. The fact that concentrations in surviving fish are not very different from those in fish found dead indicates that some fishes can survive while their body contains Zn levels comparable to the lethal body concentration. Besides the resistance variation between individuals, this result further suggests that Zn in the body of fish is not regulated within a narrow range, but may vary between relatively wide limits (Ahsanullah & Williams, 1991; Köck & Bucher, 1997). It also suggests that perhaps only a small part of the internal Zn concentration is relevant for toxicity (e.g. Zn in gills), explaining why mortality is better related to the external concentration of Zn than to the total body concentration. A similar mechanism was proposed for the effect of metals on filtration rate of freshwater mussels (Kraak *et al.*, 1994).

Finally, the present study suggests: (1) When coping with urban industrial discharges of toxicants the use of conventional toxicity experiments, assuming a constant exposure concentration, may be misleading, because the toxicity values derived thereafter will overestimate the real toxicity when the exposure concentrations varied, and (2) a slightly more complicated toxicity experiment allowing for a time varying exposure, with an endpoint as simple as mortality, can provide useful insight into the toxicokinetics and toxicodynamics of toxicants.

5. Acknowledgements

Funding for this research was provided by the Universitas Katolik Soegijapranata, Semarang, Indonesia and Department of Ecology and Ecotoxicology, Vrije Universiteit, Amsterdam. The authors are grateful to Mr. R.A. Verweij for assistance in the metal analysis.

6. References

Ahsanullah, M. (1976). Acute toxicity of cadmium and zinc to seven invertebrate species from Western Port, Victoria. Aust. J. Mar. Freshwater Res. 27: 187-196.

Ahsanullah, M. & G.H. Arnott (1978). Acute toxicity of copper, cadmium and zinc to larvae of the crab *Paragrapsus quadridentatus* (H. Milne Edwards), and implications for water quality criteria. Aust. J. Mar. Freshwater Res. 29: 1-8.

Ahsanullah, M.; D.S. Negilski & M.C. Mobley (1981). Toxicity of zinc, cadmium and copper to the shrimp *Callianassa australiensis*. I. Effects of individual metals. Mar. Biol. 64: 299-304.

Ahsanullah, M. & A.R. Williams (1991). Sublethal effects and bioaccumulation of cadmium, chromium, copper and zinc in the marine amphipod *Allorchestes compressa*. Marine Biol. 108: 59-65.

APHA (1975). Standard methods for examination of water and wastewater. 14th ed. American Public Health Association, American Water Works Association and Water Pollution Control Federation, Washington, D.C.

Barry, M.J.; D.C. Logan; J.T. Ahokas & D.A. Holdway (1995a). Effects of esfenvalerate pulse-exposure on the survival and growth of larval Australian crimson-spotted rainbow fish *(Melanotaenia fluviatilis)*. Environ. Toxicol. Wat. Qual. 10: 267-274.

Barry, M.J.; D.C. Logan, R.A. Van Dam; J.T. Ahokas & D.A. Holdway (1995b).

Effects of age and weight-specific respiration rate on toxicity of esfenvalerate pulse-exposure to the Australian crimson-spotted rainbow fish (*Melanotaenia fluviatilis*). Aquat. Toxicol. 32: 115-126.
Bedaux, J.J.M. & S.A.L.M. Kooijman (1994). Statistical analysis of bioassay based on hazard modelling. Environ. Ecol. Statistics 1: 303-314.
Berg, J.M. & Y.G. Shi (1996). The galvanization of biology: a growing appreciation for the roles of zinc. Science 271: 1081-1085.
Caffrey, P.B. & K.I. Keating (1997). Results of zinc deprivation in daphnid culture. Environ. Toxicol. Chem. 16: 572-575.
Chou, L.M. & T.J. Lam (1989). Introduction of exotic aquatic species in Singapore. In S.S. Silva (ed.): Proc. of a Workshop on Introduction of Exotic Aquatic Organisms in Asia. Spec. Publ. Asian Fish Soc. 3: 91-97.
Dallinger, R.; F. Prosi; H. Segner & H. Back (1987). Contaminated food uptake of heavy metals by fish: a review and a proposal for further research. Oecologia 73: 91-98.
Giesy, J.P.; J.W. Bowling & Henry J. Kania (1980). Cadmium and zinc accumulation and elimination by freshwater crayfish. Arch. Environ. Contam. Toxicol. 9: 683-697.
Hickie, B.E.; L.S. McCarty & D.G. Dixon (1995). A residue-based toxicokinetic models for pulse-exposure toxicity in aquatic systems. Environ. Toxicol. Chem. 14: 2187-2197.
Hodson, P.V.; B.R. Blunt; U. Borgmann; C.K. Minns & S. McGraw (1983). Effect of fluctuating lead exposures on lead accumulation by rainbow trout *Salmo gairdneri*. Environ. Toxicol. Chem. 2: 225-238.
Holdway, D.A.; M.J. Barry; D.C. Logan; D. Robertson; V. Young & J.T. Ahokas (1994). Toxicity of pulse-exposed fenvalerate and esfenvalerate to larval Australian crimson-spotted fish (*Melanotaenia fluviatilis*). Aquat. Toxicol. 28: 169-187.
Huebert, D.B. & J.M. Shay (1992). Zinc toxicity and its interaction with cadmium in the submerged aquatic macrophyte *Lemna trixulca* L. Environ. Toxicol. Chem. 11: 715-720.
Köck, G. & F. Bucher (1997). Accumulation of zinc in rainbow trout (*Oncorhynchus mykiss*) after waterborne and dietary exposure. Bull. Environ. Contam. Toxicol. 58: 305-310.
Kraak, M.H.S.; M. Toussaint; D. Lavy & C. Davids (1994). Short-term effects of metals on the filtration rate of the zebra mussel *Dreissena polymorpha*. Environ. Pollut. 84: 139-143.
Landrum, P.F., B.J. Eadie & W.R. Faust (1991). Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphiphod *Diporeia sp*. Environ. Toxicol. Chem. 10: 35-46.
Mancini, J.L. (1983). A method for calculating the effects on aquatic organisms, of time varying concentrations. Water Res. 17: 1335-1362.
McCarty, L.S., D. Mackay, A.D. Smith, G.W. Ozburn & D.G. Dixon (1992). Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: neutral narcotic organics. Environ. Toxicol. Chem. 11: 917-930.
McCarty, L.S. & D. Mackay (1993). Enhancing ecotoxicological modelling and assessment. Environ. Sci. Technol. 27: 1719-1727.
Meyer, J.S., D.D. Gulley, M.S. Goodrich, D.C. Szmania & A.S. Brooks (1995). Modeling toxicity due to intermittent exposure of rainbow trout and common shiners to monochloramine. Environ. Toxicol. Chem. 14: 165-175.
Newman, M.C. & S.V. Mitz (1988). Size dependence of zinc elimination and uptake from water by mosquito fish (Baird and Girard). Aquat. Toxicol. 12: 17-32.
Ng, P.K.L.; L.M. Chou & T.J. Lam (1993). The status and impact of introduced freshwater animals in Singapore. Biological Conservation 64: 19-24.
Rainbow, P.S. & S.L. White (1989). Comparative strategies of heavy metal accumulation by crustaceans: zinc, copper and cadmium in a decapod, an

amphipod and a barnacle. Hydrobiologia 174: 245-262.

Taylor, D.; B.G. Maddock & G. Mance (1985). The acute toxicity of nine "grey list" metals (arsenic, boron, chromium, copper, lead, nickel, tin, vanadium and zinc) to two marine fish species: Dab (*Limanda limanda*) and grey mullet (*Chelon labrosus*). Aquat. Toxicol. 7: 135-144.

Timmermans, K.R.; E. Spijkerman; M. Tonkes & H. Govers (1992). Cadmium and zinc uptake by two species of aquatic invertebrate predators from dietary and aqueous sources. Can. J. Fish. Aquat. Sci. 49: 655-662.

Van Hattum, B.; G. Korthals; N.M. Van Straalen; H.A.J. Govers & EN.G. Joosse (1993). Accumulation patterns of trace metals in freshwater isopods in sediment bioassays - influence of substrate characteristics, temperature and pH. Water Res. 27: 669-684.

Van Straalen, N.M. & J. Van Wensem (1986). Heavy metal content of forest litter arthropods as related to body-size and trophic level. Environ. Pollut. Ser. A. 42: 209-221.

Widianarko, B. & N.M. Van Straalen (1996). Toxicokinetics-based survival analysis in bioassays using nonpersistent chemicals. Environ. Toxicol. Chem. 15: 402-406.

Widianarko, B. Heavy metals in urban streams of Semarang: bioindication potential of body size and reproductive parameters of the guppy (*Poecilia reticulata*). [Submitted]

Widianarko, B.; C.A.M. Van Gestel; R.A. Verweij & N.M. Van Straalen. Associations between trace metals in sediment, water and guppy, *Poecilia reticulata* (Peters) from urban streams of Semarang, Indonesia. [submitted$_a$]

Widianarko, B.; R.A. Verweij; C.A.M. Van Gestel & N.M. Van Straalen. Spatial distribution of trace metals in sediments from urban streams of Semarang, Central Java, Indonesia. [submitted$_b$]

CHAPTER 8

Discussion and Summary

The potential threats of urban pollution, which can be expected to continue to increase, are certainly a challenging area of scientific research. By the year 2000, in South Asia alone, at least 15 cities will have over one million inhabitants, and three megacities each consisting of over 10 million inhabitants (Low & Balamurugan, 1991). Consequently, a high degree of environmental stress may be expected in the vicinity of these cities.

Since ecotoxicology is the study of toxic effects of chemical and physical agents on living organisms, especially on populations and communities within defined ecosystems, it is imperative for this scientific discipline to play an important role in urban environmental management. Unfortunately only a few ecotoxicological studies, especially in South East Asia (SEA), have been directed to urban pollution problems (Chapter 2).

A survey of three years (1993-1995) data showed that SEA scientists have addressed a wide variety of areas with a heavy reliance on well established methods, i.e. toxicity studies (single species) and spatial distribution of toxicants. Heavy metals are the most studied (41.7 %) environmental contaminants followed by non-pesticide organic pollutants (22.2%), pesticides (19.4%) and atmospheric gases (16.7%). Among the type of ecosystems studied, the urban-industrial ecosystem was listed as the least studied ecosystem (Chapter 2).

The future of urban ecotoxicology studies in South East Asia, as in other parts of the world, holds great promises. Ecotoxicology (or environmental toxicology) receiving increasing attention and shows significant growth in South East Asia (see Widianarko *et al.*, 1994). Much more work has to be done in urban ecotoxicology, both in theoretical fields and in environmental management practices. Any forms of support to facilitate this important field of research should be raised easily, since urban problems are related directly to the existence of the majority of human being. Furthermore, South East Asian cities accurately represent fast-growing urban areas, so they will be a suitable model for other parts of the world. This is, thus, a chance for ecotoxicological research in SEA to pursue in the above direction.

The research presented in this thesis has demonstrated that with its special characteristics, urban pollution cannot be studied using conventional ecotoxicological approaches and methods. The traditional assessment of ecotoxicological impacts is often based on a presumption of the 'balance of nature'. On the contrary, urban pollution is typified by three features : (1) the spatial distribution of pollutants in the urban ecosystem tends to be patchy or aggregated, (2) in most of the areas the exposure of pollutants occurs at relatively low doses over extended periods, and (3) in some areas high peaks of pollutants occurs during short periods. Consequently, urban ecotoxicology has to take into account the spatial and temporal heterogeneity of the toxicant. This will lead to a development of new field of landscape ecotoxicology (Cairns & Niederlehner, 1996).

Spatial Distribution of Metals

Elevated concentrations of heavy metals, such as lead (Pb), cadmium (Cd), copper (Cu) and zinc (Zn), are usually associated with the impact of urbanization. Therefore, it is common to use concentrations of these metals as indicators of urbanization. Among the different urban pollutants, toxic metals, such as lead, cadmium, zinc and copper, play an important role. The study on the spatial distribution of metals in the urban area of Semarang presented in this research provided a full picture of the extent of the city's metals contamination. This information also covered the status of trace metal pollution in the sediment of coastal areas which is of considerable importance for the public health when seafood from this area is caught for consumption (Mat & Maah, 1994; Mat *et al.*, 1994).

The skewed spatial distributions of metals in Semarang found in this study for Pb and Zn (Chapter 3) are not uncommon (see e.g. Von Steiger *et al.*, 1996). Excepts for some sites with extremely high metal concentrations, i.e. Zn up to 1257 μg/g, Pb up to 2666 μg/g and Cu up to 448 μg/g, the major proportion of the sites had a low metal concentration. Concentrations of metals in sediment found in the present study are not extreme compared to metal concentrations in sediment of other parts of South East Asia (see e.g. Mat & Maah, 1994; Prudente *et al.*, 1994). Seventy five percent of the sites have low metal concentrations, i.e. Pb < 30.0 μg/g, Zn < 172 μg/g and Cu < 49.3 μg/g. Due to the above facts, it seems justified to derive the average background concentrations of Cu, Zn and Pb. The proposed reference values are 25.6 μg/g, 132 μg/g and 40.7 μg/g, respectively for Pb, Zn and Cu.

These values are similar to the ones presently considered as "background concentrations" in Dutch environmental policy (Anonymous, 1994), however the "background" concentration for Pb seems to be higher (Table 1). Derivation of these values is certainly empirical. In the light of the absence of sediment quality standards, these reference values can be used to derive a combined metal contamination index, W. Based on W values, four categories, i.e. unpolluted, slightly polluted, polluted and heavily polluted, are proposed. The present approach is prospective in the light of the need of defining sediment quality standards for the region. It will be useful for the future monitoring of urban metal pollution in Semarang, as well as, other cities in South East Asia, where anthropogenic influences, as well as, continuous discharges of municipal and industrial effluents into urban streams are expected to increase.

Table 1. Proposed reference values for metals in sediments, derived from analyses in the Semarang area, Indonesia. The reference values are compared to background concentrations adopted in the evaluation of sediment pollution in the Netherlands (Anonymous, 1994)

Metal	Reference value Semarang (μg/g)	Background concentration the Netherlands (μg/g)
Cu	41	36
Pb	26	85
Zn	132	140

Association of Metals in Sediment, Water and Fish

The data on fish occurrence in 63 streams of the greater Semarang indicated that guppies (*Poecilia reticulata*) did not avoid the highly polluted sites (Chapter 4). This implies that the guppy fish is a promising bioindicator species. The presence of guppies at polluted sites will allow the determination of changes in various ecotoxicological parameters induced by metal pollution.

No simple interpretation can be made on significant differences in metal concentrations (Pb and Zn) and metal burdens (Pb) between fish from sites with different contamination levels. One possible explanation might be that fish from polluted and highly polluted streams have developed a physiological adaptation by accumulating more metals when facing excessive metal concentrations in their environment. Studies on metal accumulation in animals from sites at different distances from an emission source showed such a tendency (see e.g. Hopkin *et al.*, 1989; Dallinger *et al.*, 1992; Berger & Dallinger, 1993; Pizl & Josens, 1995).

A significant declining trend of Pb concentrations with increasing organism size was observed (Chapter 4). This accumulation phenomenon is one of the three types of metal concentration size relationships which include increases, decreases and no change in concentration with increased body weight as demonstrated by several studies on aquatic invertebrates (Smock, 1983). Clearly, for the two essential metals, Zn and Cu, the concentrations were not dependent on the body weight. Apparently, body concentrations of these two metals are regulated and maintained at a certain concentration.

As to the relationships between metal concentrations in water, sediment and fish, water and sediment parameters, and fish dry weight, the presence of significant multiple correlations and bivariate correlations (in 17 out of 91 pairs of variables) indicates that, in general, abiotic parameters and body size have no influence on the metal flux from sediment to water and into fish. Significant multiple associations among 14 variables in the present study were mostly dominated by the association between metal concentrations in sediments and those in the fish (Chapter 4). This is in contrast to other bioaccumulation studies which have clearly shown the role of abiotic parameters in the uptake of toxicants, including metals, by animals (see e.g. Van Hattum *et al.*, 1991). Results of partial correlation analyses suggest that metal concentrations in the sediments are the most important factor governing the metal body concentration of fish, since none of the abiotic parameters of the sediment and water were found to be significant covariates.

Results of the present study indicate that the guppy *P. reticulata* from urban streams is a potential bioindicator for urban metal pollution, especially with respect to its (1) spatial distribution over sites of all pollution regimes, and (2) variation in metal accumulation related to the degree of pollution.

Bioindication Potential of *Poecilia reticulata*

Suppression of growth, as reflected by the smaller body size of the male guppies in the present study (Chapter 5), is similar to those demonstrated for guppy (*P. reticulata*) populations under predation threat (Frazer & Gilliam, 1992); but, interestingly, in the present study female fish did not demonstrate this reduction of growth. Although it is not statistically significant, body lengths of female fish from polluted streams were slightly larger than those from non-polluted streams.

A significant difference in the total energetic content of male fish from non-polluted and polluted sites, which is in contrast to the corresponding results of body size measurements, might indicate a difference in tissue composition between fish from different sites. A further study is needed to clarify this phenomenon.

Among reproductive parameters observed in this study, it was shown that sex ratio and the ratio of pregnant to total females are promising parameters for bioindication. The latter is one of the simplest measures of reproductive success. Combining these two ratios, it can be inferred that guppy populations in polluted ecosystems tend to have fewer females, which have a higher reproductive activity than those in unpolluted ecosystems. For fecundity, a slight indication of a lower number of juveniles + eggs of individual pregnant females from polluted streams might, again, support the analogy between the presence of pollutants and predators as described above. In a long term study on a population of Trinidian guppies in natural streams, the absence of predators resulted in bigger, longer living guppies with fewer and bigger offspring (Reznick *et al.*, 1996).

Based on the above findings, it can be seen that body size and reproductive parameters used in this study provide different levels of responses towards metal pollution. Among body size parameters, body length and body weight show a significant difference. The same does not hold for the size structure, although studies on other species suggested that this parameter is sensitive to metal pollution (see e.g. Van Capelleveen, 1987; Hopkin, 1989).

The use of body size parameters for bioindication of chronic pollution, such as urban metal pollution, is promising, especially with regard to the nature of fish growth. Body size in fish is very distinctive, because it typically continues throughout life (McDowall, 1994). Furthermore, it has been established that many evolutionary strategies relate to body size (McDowall, 1994, Reznick *et al.*, 1996). So, it can be expected that body size will be one of the most affected parameters during the long-term chronic exposure experiments to metals.

Sex ratio and total to pregnant ♀ ratio are suitable candidates parameters for evaluation of metal pollution. These parameters are simple to measure, and have a direct implication for the reproduction capacity of a population.

In this type of comparative field study, it is difficult to prove that there is a cause effect relationship (see e.g. Donker *et al.*, 1993), since various other factors than metal concentrations in the sediment, such as pH, nutrition and the presence of organic contaminants, can play an additional role. The above promising results should be confirmed by laboratory experiments to establish cause effect relationships.

Model for Diluted Pulse Exposure

Conventional toxicological approaches and concepts based on the premise of constant exposure of chemicals are not any longer adequate to deal with time varying exposures of chemicals, such as short term high peaks. Therefore variable exposure has increasingly become an attractive field of research, both for experimental (see e.g. Hodson *et al.*, 1983; Holdway *et al.*, 1994; Barry *et al.*, 1995a & 1995b) and modelling studies (Mancini, 1983; Landrum *et al.*, 1991; McCarty *et al.*, 1992; Bedaux & Kooijman, 1994; Hickie *et al.*, 1995; Meyer *et al.*, 1995).

The model presented in this thesis incorporates the dynamics of exposure concentrations of toxic substances. Derivation of the toxicokinetics-based survival model ultimately leads to a relatively simple mathematical expression (Chapter 6). This makes it possible to estimate, from survival data, the degradation or disappearance rate of a chemical (k_0), the elimination rate (k_2) and the ultimate ILC_{50} (μ); the latter was defined as the initial concentration in the medium that is just high enough to kill ultimately 50% of the animals before the concentration decreases to a level that does not have an effect.

As the model contains only three parameters, the assumptions leading to its erection are necessarily a simplified representation of reality. One point in particular is the assumption that toxicokinetics are invariant across doses and with time. This assumption will not hold if rate constants change with exposure levels, *e.g.* due to behavioral changes during accumulation, affecting uptake, or induced metabolism, affecting depuration. The data tested to this model indicated that concentration-dependent uptake may be a significant factor contributing to differences between observations and model expectations (Chapters 6 & 7).

In addition to the bioindication potentials of *P. reticulata* as demonstrated in field studies, the research presented in this thesis shows the usefulness of this fish species as a test animal in aquatic toxicity experiments with metals. In terms of toxicokinetics and mortality patterns, the field guppy responded to Zn exposure in a predictable manner, so it is feasible to use it for further ecotoxicological analysis and interpretations (Chapter 7).

The inclusion of a basal physiological Zn concentration parameter in fish (A) as a parameter in the toxicokinetic model was necessary because Zn is a regulated metal. The high accumulation rate (k_1) implies that guppies can rapidly take up Zn from water, since no dietary Zn was offered during the experiments. It indicates that Zn uptake from an aqueous source by guppies can contribute significantly to the body burden. Upto now, no consensus exists in the literature on the relative importance of Zn uptake from dietary or aqueous sources in fish (see e.g. Giesy *et al.*, 1980; Dallinger *et al.*, 1987; Timmermans *et al.*, 1992; Köck & Bucher, 1997).

Due to the fact that the elimination rate (k_2) was also high, and accordingly the biological half-life (t_{05}) was shorter, the Biological Concentration Factor (BCF) of Zn in guppies was relatively low. Elimination rates found in the present study were also higher compared to those of freshwater isopods, *Asellus aquaticus* (Van Hattum *et al.*, 1993); however values of toxicokinetics parameters from the present study, except for the elimination rate, were not extremely high (compared to e.g. Timmermans *et al.*, 1992). It seems that under acute exposure concentrations (10 mg/L), the guppies tend to increase their elimination rates (k_2), so the biological half-life of Zn was shortened.

The simulated decreasing concentrations of Zn (Chapter 7) seemed to be in agreement with the expectation since it resulted in baseline survival of guppies, which was similar to the response of the terrestrial isopod *Porcellio scaber* in a study using the non-persistent pesticide diazinon (Chapter 6). High mortality in the initial period of the experiments, especially in experiment I where mortality halted at 32 h, is a common phenomenon in pulsed-exposure toxicity tests in which test animals are exposed briefly to a high concentration of a toxicant followed by a decreasing or absence of exposure concentrations (see e.g. Holdway *et al.*. 1994).

Estimated values of initial exposure concentrations (C_0s), decreasing rates of exposure concentrations (k_0) and elimination rates (k_2) of Zn found from the curve fitting of survival data from toxicity experiments using the proposed model were convincingly close to the corresponding values from the independent toxicodynamics and toxicokinetics curve fittings. This shows that the model is capable of being used to explain the variation of survival data in terms of toxicokinetics and toxicodynamics parameters of Zn in *P. reticulata*. The estimated value of elimination rates which practically show no difference among three independent experiments are of particular interest.

High standard errors for the estimates of ultimate initial LC_{50} (μ) and elimination rate (k_2) may be related to the nature of data. These high standard errors may be responsible for the unsatisfying fit between the model and survival data at the higher exposure concentrations. More chronic survival data may be needed to test the model and to investigate whether high standard errors for these parameters will also appear.

Finally, this modelling exercise suggests that: (1) When coping with urban industrial discharges of toxicants the use of conventional toxicity experiments, assuming a constant exposure concentration, may be misleading, because the toxicity values derived thereafter will overestimate the real toxicity values when the exposure concentrations vary, and (2) a slightly complicated toxicity experiment allowing for a time varying exposure, with an endpoint as simple as mortality, can provide useful insight into the toxicokinetics and toxicodynamics of toxicant.

References

Anonymous (1994). Environmental Quality Objectives in the Netherlands. Risk Assessment and Environmental Quality Division, Directorate for Chemicals, External Safety and Radiation Protection, Ministry of Housing, Spatial Planning and the Environment. The Hague.

Barry, M.J.; D.C. Logan; J.T. Ahokas & D.A. Holdway (1995a). Effects of esfenvalerate pulse-exposure on the survival and growth of larval Australian crimson-spotted rainbow fish *(Melanotaenia fluviatilis)*. Environ. Toxicol. Wat. Qual. 10: 267-274.

Barry, M.J.; D.C. Logan, R.A. Van Dam; J.T. Ahokas & D.A. Holdway (1995b). Effects of age and weight-specific respiration rate on toxicity of esfenvalerate pulse-exposure to the Australian crimson-spotted rainbow fish (*Melanotaenia fluviatilis*). Aquat. Toxicol. 32: 115-126.

Bedaux, J.J.M. & S.A.L.M. Kooijman (1994). Statistical analysis of bioassay based on hazard modelling. Environ. Ecol. Statistics 1: 303-314.

Berger, B. & R. Dallinger (1993). Terrestrial snails as quantitative indicators of environmental metal pollution. Environ. Monit. Assess. 25: 65-84.

Cairns, J. Jr. & B.R. Niederlehner (1996). Developing a field of landscape ecotoxicology. Ecol. Appl. 6: 790-796.

Dallinger, R.; F. Prosi; H. Segner & H. Back (1987). Contaminated food uptake of heavy metals by fish: a review and a proposal for further research. Oecologia 73: 91-98.

Dallinger, R.; B. Berger & S. Birkel (1992). Terrestrial isopods: Useful biological indicators of urban metal pollution. Oecologia 89: 32-41.

Donker, M.H.; C. Zonneveld & N.M. Van Straalen (1993). Early reproduction and increased reproductive allocation in metal-adapted populations of the terrestrial isopod *Porcellio scaber*. Oecologia 96: 316-323.

Frazer, D.F. & J.F. Gilliam (1992). Nonlethal impacts of predator invasion:

facultative suppression on growth and reproduction. Ecology 73: 959-970.
Giesy, J.P.; J.W. Bowling & Henry J. Kania (1980). Cadmium and zinc accumulation and elimination by freshwater crayfish. Arch. Environ. Contam. Toxicol. 9: 683-697.
Hickie, B.E.; L.S. McCarty & D.G. Dixon (1995). A residue-based toxicokinetic models for pulse-exposure toxicity in aquatic systems. Environ. Toxicol. Chem. 14: 2187-2197.
Hodson, P.V.; B.R. Blunt; U. Borgmann; C.K. Minns & S. McGraw (1983). Effect of fluctuating lead exposures on lead accumulation by rainbow trout *Salmo gairdneri*. Environ. Toxicol. Chem. 2: 225-238.
Holdway, D.A.; M.J. Barry; D.C. Logan; D. Robertson; V. Young & J.T. Ahokas (1994). Toxicity of pulse-exposed fenvalerate and esfenvalerate to larval Australian crimson-spotted fish (*Melanotaenia fluviatilis*). Aquat. Toxicol. 28: 169-187.
Hopkin, S.P. (1989). Ecophysiology of Metals in Terrestrial Invertebrates. Elsevier Applied Sciences, London.
Hopkin, S.P.; C.A.C. Hames & S. Bragg (1989). Terrestrial isopods as biological indicators of zinc pollution in the Reading area, South England. Monitore zool. ital. (N.S.) Monogr. 4: 477-488.
Köck, G. & F. Bucher (1997). Accumulation of Zinc in rainbow trout (*Oncorhynchus mykiss*) after waterborne and dietary exposure. Bull. Environ. Contam. Toxicol. 58: 305-310.
Landrum, P.F., B.J. Eadie & W.R. Faust (1991). Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphiphod *Diporeia sp*. Environ. Toxicol. Chem. 10: 35-46.
Low, K.S. & G. Balamurugan (1991). Urbanization and urban water problems in South East Asia: A case of unsustainable development. J. Environ. Man. 32: 195-209.
Mancini, J.L. (1983). A method for calculating the effects on aquatic organisms, of time varying concentrations. Water Res. 17: 1335-1362.
Mat, I. & M.J. Maah (1994). Sediment trace metal concentrations from the mudflats of Kuala Juru and Kuala Muda of Malaysia. Bull. Environ. Contam. Toxicol. 53: 740-746.
Mat, I.; M.J. Maah & A. Johari (1994). Trace metals in sediments and potential availability to *Anadara granosa*. Arch. Environ. Contam. Toxicol. 27: 54-59.
McCarty, L.S. & D. Mackay (1993). Enhancing ecotoxicological modelling and assessment. Environ. Sci. Technol. 27: 1719-1727.
McCarty, L.S.; D. Mackay; A.D. Smith; G.W. Ozburn & D.G. Dixon (1992). Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: neutral narcotic organics. Environ. Toxicol. Chem. 11: 917-930.
McDowall, R.M. (1994). On size and growth in freshwater fish. Ecology of Freshwater Fish 3: 67-79.
Meyer, J.S.; D.D. Gulley; M.S. Goodrich; D.C. Szmania & A.S. Brooks. (1995). Modeling toxicity due to intermittent exposure of rainbow trout and common shiners to monochloramine. Environ. Toxicol. Chem. 14: 165-175.
Pizl, V. & G. Josens (1995). Earthworm communities along a gradient of urbanization. Environ. Pollut. 90: 7-14.
Prudente, M.S.; H. Ichishashi & R. Tatsukawa (1994). Heavy metal concentration in sediments from Manila Bay, Philippines and inflowing rivers. Environ. Pollut. 86: 83-88.
Reznick, D.A.; F.H. Shaw; F.H. Rodd & R.G. Shaw (1996). Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science 275: 1934-1937.
Smock, L.A. (1983). Relationships between metal concentrations and organism size in aquatic insects. Freshwater Biol. 13: 313-321.

Timmermans, K.R.; E. Spijkerman; M. Tonkes & H. Govers (1992). Cadmium and zinc uptake by two species of aquatic invertebrate predators from dietary and aqueous sources. Can. J. Fish. Aquat. Sci. 49: 655-662.

Van Capelleveen, H.E. (1987). Ecotoxicity of heavy metals for terrestrial isopods. PhD Thesis, Vrije Universiteit, Amsterdam.

Van Hattum, B.; K. Timmermans & H.A.J. Govers (1991). Abiotic and biotic factors influencing in situ trace metal levels in macroinvertebrates in freshwater ecosystems. Environ. Toxicol. Chem. 10: 275-292.

Van Hattum, B.; G. Korthals; N.M. Van Straalen; H.A.J. Govers & EN.G. Joosse (1993). Accumulation patterns of trace metals in freshwater isopods in sediment bioassays - influence of substrate characteristics, temperature and pH. Wat. Res. 27: 669-684.

Von Steiger, B,; R. Webster, R. Schulin & R. Lehmann (1996). Mapping heavy metals in polluted soil by disjunctive kriging. Environ. Pollut. 94: 205-215.

Widianarko, B.; K. Vink & N.M. Van Straalen (Eds) (1994). Environmental Toxicology in South East Asia. VU University Press, Amsterdam.

SAMENVATTING

Ecotoxicologie van het stedelijk milieu: ruimtelijke en temporele variatie in verontreiniging

The potentiële bedreigingen die uitgaan van verontreiniging van het stedelijk milieu zullen naar verwachting in de toekomst sterk toenemen. Tegen het jaar 2000 zullen in de regio Zuid-Oost Azië tenminste vijftien steden een inwoneraantal van meer dan een miljoen mensen hebben, met drie megasteden van meer dan tien miljoen inwoners. Deze groei van de stedelijke bevolking zal een grote druk veroorzaken op het milieu in de stad en in de omgeving daarvan.

Aangezien ecotoxicologie de wetenschap is die de toxische effecten van chemische en fysische agentia op levende organismen bestudeert, is het noodzakelijk dat zij een belangrijke rol gaat spelen bij de ontwikkeling van stedelijke planvorming. Er zijn echter slechts enkele ecotoxicologische studies gedaan, zeker in Zuid-Oost Azië, die zich gericht hebben op de problemen van verontreiniging in de stad.

Uit een bibliografisch onderzoek naar wetenschappelijke artikelen verschenen in zeven ecotoxicologische tijdschriften over de jaren 1993 tot en met 1995 (hoofdstuk 2) blijkt dat milieuwetenschappers in Zuid-Oost Azië een keur aan verschillende onderwerpen bestuderen. Daarbij valt op dat men zich nogal baseert op algemeen aanvaarde methoden, zoals toxiciteits-experimenten met één soort en studies naar de ruimtelijke verdeling van verontreinigende stoffen. De meeste studies worden gedaan aan zware metalen (41.7%), gevolgd door organische contaminanten exclusief bestrijdingsmiddelen (22.2%), bestrijdingsmiddelen (19.4%) en luchtverontreiniging (16.7%). Van de bestudeerde systemen wordt het stedelijk-industrieel milieu het minst bestudeerd.

Het uitgangspunt van dit proefschrift is dat voor de studie van verontreiniging in het stedelijk milieu conventionele benaderingen tekort schieten. De verontreiniging in de stad wordt gekenmerkt door drie aspecten: (1) de ruimtelijke verdeling van verontreinigende stoffen is sterk heterogeen, (2) in de meeste gebieden worden organismen blootgesteld aan relatief lage concentraties over lange periodes, (3) in sommige gebieden kunnen gedurende korte periodes hoge pieken in de blootstelling voorkomen. De ecotoxicologie van het stedelijk milieu moet zich daarom met name richten op de ruimtelijke en temporele variatie van verontreinigende stoffen.

Ruimtelijke verdeling van metalen

Urbanisatie gaat gewoonlijk gepaard met verhoogde concentraties van zware metalen zoals lood, cadmium, koper en zink. In hoofdstuk 3 van dit proefschrift wordt de ruimtelijke verdeling van metalen gegeven in sedimenten van waterlopen in de stad Semarang (Midden Java), bemonsterd op 101 monsterlokaties in een raster van 2 x 2 km. Het grootste deel (75%) van de onderzochte sedimenten bleek een lage metaalconcentratie te hebben, echter op sommige monsterplaatsen waren zeer hoge concentraties aanwezig: zink tot 1257 µg/g, lood tot 2666 µg/g en koper tot 448 µg/g. Uit het onderzoek kunnen gemiddelde achtergrondconcentraties afgeleid worden; de voorgestelde waarden zijn 25.6 µg/g voor Pb, 132 µg/g voor Zn en 40.7 µg/g voor Cu. Voor Zn en Cu verschillen deze waarden niet veel van de referentiewaarden die in het Nederlandse bodembeschermingsbeleid aangehouden worden als indicatoren voor een goede bodemkwaliteit; de voorgestelde waarde voor Pb is aanmerkelijk lager.

Voor de beoordeling van sedimenten die met meerdere metalen verontreinigd zijn wordt in hoofdstuk 3 een nieuwe methode voorgesteld. De concentraties van elk van de metalen worden gedeeld door hun bijbehorende achtergrondwaarde en de quotiënten worden vervolgens vermenigvuldigd; de logaritme van het produkt wordt gedefinieerd als de index W. Gebaseerd op een indeling van W in vier categorieën kan de ruimtelijke verdeling van de sedimentkwaliteit in de stad Semarang overzichtelijk weergegeven worden.

Relaties tussen metalen in sediment, water en vis

In waterlopen van de stad Semarang is de gup (*Poecilia reticulata*) zeer algemeen. Het voorkomen van guppen bleek niet gerelateerd te zijn aan de mate van sedimentverontreiniging, hetgeen deze vissoort geschikt maakt als potentieel monitoring-organisme (hoofdstuk 4). Voor het metaal lood werd een significante invloed van lichaamsgrootte gevonden: de loodconcentratie nam af met toenemend gewicht van de vis. Voor koper en zink was een dergelijke invloed van lichaamsgewicht niet aanwijsbaar.

De relaties tussen metaalconcentraties (water, sediment en vis) en diverse waterkwaliteitsparameters en sedimenteigenschappen werden onderzocht door middel van correlatie-analyse. Het bleek dat de multipele correlaties tussen de 14 variabelen gedomineerd werden door de associatie tussen metaalgehaltes in sediment en vis. Er was echter geen eenduidige relatie tussen het gehalte in de vis en het gehalte in het water. De andere factoren hadden relatief weinig invloed.

Gebruik van *Poecilia reticulata* voor bioindicatie

In een detailleerde vergelijking van guppenpopulaties op vier monsterlokaties (twee verontreinigd en twee schoon) bleken mannetjes op de vervuilde lokaties een lager gewicht te hebben dan de mannetjes op de schone lokaties (hoofdstuk 5). Voor vrouwtjes werd een dergelijk effect niet gevonden. De mannetjes van de verontreinigde plekken hadden echter ook een hoger totaalgehalte aan reservestoffen (glycogeen, eiwit en vet). De betekenis van de verschillen in gewicht en lichaamssamenstelling zijn nog niet duidelijk en vereisen een nadere studie.

Ook in de reproduktieve parameters van de guppenpopulaties blijken verschillen tussen de lokaties te bestaan. De populaties van de vervuilde lokaties hadden minder vrouwtjes, waarvan echter een groter percentage zwanger was, terwijl er een lichte trend was voor een lagere vruchtbaarheid op de verontreinigde lokaties. De resultaten zijn vergelijkbaar met die verkregen in een guppenstudie gedaan in Trinidad, waarin de aanwezigheid van roofvissen resulteerde in kleinere, korter levende guppen met meer kleinere nakomelingen, vergeleken met predator-vrije populaties.

Model voor sterfte na blootstelling aan een verdunde puls

De conventionele toxicologische benadering waarbij de blootstellings-concentratie constant verondersteld wordt is niet langer geldig bij blootstelling aan kortdurende pieken. In hoofdstuk 6 van dit proefschrift werd een model ontwikkeld voor een bepaald type veel voorkomende blootstelling, namelijk een plotseling optredende piek, gevolgd door exponentieel verval van de concentratie; dit wordt een "verdunde puls" genoemd.

Uitgaande van het principe dat de kans per tijd om te sterven evenredig is met het interne gehalte van de toxicant kan een relatief eenvoudige wiskundige vergelijking afgeleid worden waarin het aantal overlevende dieren gegeven wordt als functie van de beginconcentratie van de toxicant, de verdwijnsnelheid van de stof uit het blootstellingsmedium, de eliminatiesnelheid van de stof door het dier en de toxiciteit van de stof. Als in een experiment de beginconcentraties bekend zijn kunnen uit het verloop van de sterfte de kinetische parameters geschat worden. De toxiciteit kan uitgedrukt worden als de beginconcentratie die juist hoog genoeg is om na lange blootstellingstijd 50% van de dieren te laten overlijden (ILC_{50}, hoofdstuk 6).

Experimenten met tijdsvariabele blootstelling aan zink

In hoofdstuk 7 worden experimenten beschreven waarin vissen (*Poecilia reticulata*) in het laboratorium blootgesteld werden aan een experimenteel gesimuleerde "verdunde puls" van zink in het water. In overeenstemming met de modelvoorspellingen werd gevonden dat bij intermediaire beginconcentraties niet alle vissen dood gingen, maar dat een bepaald gedeelte bleef leven doordat de zinkconcentratie tijdig een lage waarde bereikt had. Uit het verloop van de sterfte werden de toxicokinetische snelheidsconstanten voor eliminatie en verdunning geschat; deze parameterschattingen vertoonden een opvallend goede overeenkomst met schattingen voor dezelfde parameters uit een ander experiment waarbij de zinkconcentraties in vis en water direct gemeten werden.

De resultaten in hoofdstuk 7 laten zien dat de gekozen modelformulering zeer geschikt is om gebruikt te worden bij de analyse van toxiciteitsexperimenten waarbij de blootstellingsconcentratie exponentieel vervalt. Het zou interessant zijn de benadering uit te breiden door incorporatie van een interne drempelwaarde (geen-effect niveau) en subletale effecten. De relatief grote standaardfouten van de parameterschattingen doen echter vermoeden dat de gegevens zoals die gewoonlijk verzameld worden in toxiciteitsexperimenten nauwelijks een uitbreiding van het aantal te schatten parameters toelaten.

De in dit proefschrift ontwikkelde benaderingen laten zien dat bij de analyse van tijdsvariabele blootstellingssituaties in het stedelijk milieu conventionele toxiciteitsexperimenten, waarin de blootstelling constant gehouden wordt, misleidend kunnen zijn omdat de daaruit afgeleide toxiciteitscriteria de echte toxiciteit kunnen overschatten als de blootstellingsconcentratie feitelijk varieert. Door het uitvoeren van experimenten die slecht weinig ingewikkelder zijn dan de conventionele kan meer inzicht verkregen worden in de toxicokinetiek en toxicodynamiek van de toxicant.

RINGKASAN

Ancaman pencemaran kota (urban pollution), yang diprediksi akan semakin meningkat, merupakan bidang penelitian yang menantang. Pada tahun 2000, di Asia Tenggara saja, paling sedikit terdapat 15 kota yang berpenduduk lebih dari sejuta, dan 3 megacity dengan penduduk lebih dari 10 juta. Sebagai akibatnya, tentu saja, tekanan yang cukup berat akan diterima oleh lingkungan di sekitar kota-kota besar tersebut. Karena ekotoksikologi adalah cabang ilmu yang mempelajari tentang efek beracun dari agen kimia dan fisika terhadap kehidupan organisme, khususnya pada populasi dan komunitas yang hidup dalam ekosistem tertentu, maka sudah selayaknya jika disiplin ilmu ini berperan penting dalam manajemen lingkungan perkotaan. Namun sayangnya, sampai saat ini masih sedikit studi ekotoksikologis, khususnya di Asia Tenggara, yang diarahkan pada masalah pencemaran kota. Berdasarkan survei di tujuh jurnal internasional tentang toksikologi lingkungan, selama periode tahun 1993 - 1995, ekosistem perkotaan merupakan jenis ekosistem yang paling kurang digarap oleh para peneliti Asia Tenggara (Chapter 2).

Meskipun demikian, prospek studi ekotoksikologi perkotaan (urban ecotoxicology) di Asia Tenggara, seperti di kawasan lain di dunia, amatlah menjanjikan. Ekotoksikologi, atau sering pula disebut sebagai toksikologi lingkungan (environmental toxicology), memperoleh perhatian yang terus meningkat di Asia Tenggara. Masih sangat terbuka peluang untuk melakukan studi ekotoksikologi perkotaan, baik untuk pengembangan ilmu maupun untuk aplikasi manajemen lingkungan. Berbagai bentuk dukungan untuk pertumbuhan bidang penelitian yang penting ini sudah selayaknya tersedia, mengingat persoalan lingkungan perkotaan langsung berkatian dengan eksistensi dan kehidupan sebagian besar manusia. Terlebih lagi, kota-kota di Asia Tenggara merupakan model yang tepat bagi kawasan perkotaan dunia yang tumbuh pesat. Kondisi di atas menciptakan peluang bagi penelitian ekotoksikologi di Asia Tenggara untuk segera mengarah pada masalah pencemaran perkotaan. Disertasi ini mendemonstrasikan bahwa dengan sifat-sifatnya yang khusus pencemaran perkotaan tidak dapat distudi menggunakan pendekatan dan metoda ekotoksikologi konvensional. Pencemaran perkotaan dicirikan oleh 3 sifat utama : (1) distribusi spasial senyawa pencemar cenderung membentuk agregat (patchy), (2) pajanan (exposure) substansi pencemar di sebagian besar kawasan kota terdapat dalam dosis rendah dan berlangsung secara kronik dalam jangka waktu panjang, (3) di beberapa kawasan substansi pencemar terdapat dalam kosentrasi yang sangat tinggi namun dalam jangka waktu yang pendek. Sebagai konsekuensinya, studi ekotoksikologi perkotaan harus memperhitungkan heterogenitas spasial dan temporal dari substansi pencemar. Studi tentang distribusi logam berat yang dilaporkan dalam disertasi ini memberikan informasi seutuhnya tentang tingkat pencemaran

logam berat di kota Semarang (Chapter 3). Informasi tersebut juga mencakup status pencemaran logam berat pada sedimen di kawasan pantai yang berimplikasi penting terhadap kesehatan lingkungan, khususnya berkaitan dengan konsumsi seafood dari kawasan tersebut. Kecuali beberapa lokasi dengan konsentrasi logam yang sangat tinggi, yaitu sampai dengan 1257 mg/g, 2666 mg/g, 448 mg/g untuk Zn, Pb dan Cu, sebagian besar lokasi memiliki konsentrasi logam yang rendah. Konsentrasi ketiga jenis logam berat yang diperoleh dalam studi ini tidak jauh berbeda dari hasil studi-studi lain di Asia Tenggara. Tujuh puluh lima persen lokasi memiliki konsentrasi logam yang rendah, yaitu Pb < 30,0 mg/g, Zn < 172 mg/g dan Cu < 49,3 mg/g. Kondisi ini memberikan justifikasi untuk melakukan derivasi konsentrasi latar bagi ketiga logam berat tersebut. Nilai rujukan (reference value) yang diusulkan adalah 25,6 mg/g, 132 mg/g dan 40,7 mg/g, berturut-turut untuk Pb, Zn dan Cu. Nilai rujukan yang diusulkan ini ternyata tidak berbeda jauh dari apa yang disebut sebagai "background concentrations" dalam baku mutu sedimen di Negeri Belanda. Meskipun nilai-nilai rujukan tersebut diperoleh secara empirik, namun mempertimbangkan ketiadaan baku mutu sedimen, nilai-nilai tersebut dapat dipergunakan sebagai dasar penetapan indeks gabungan untuk pencemaran logam (combined metal contamination index), W. Berdasarkan nilai W, diusulkan empat kategori pencemaran, yaitu tidak tercemar (unpolluted), tercemar ringan (slightly polluted), tercemar (polluted) dan tercemar berat (heavily polluted). Pendekatan dalam studi ini cukup prospektif, terutama dalam kaitannya dengan kebutuhan baku mutu sedimen untuk pemantauan pencemaran logam, baik di kota Semarang, maupun kota-kota lain di Asia Tenggara. Data keberadaan ikan seribu (*Poecilia reticulata*) di 63 sungai/selokan di kota Semarang menunjukkan bahwa jenis ikan ini tidak menyingkir dari lokasi-lokasi yang tercemar berat (Chapter 4). Dengan demikian, ikan seribu merupakan spesies bioindikator yang menjanjikan. Keberadaannya di lokasi tercemar akan memungkinkan dilakukannya determinasi perubahan berbagai parameter ekotoksikologis pada populasi ikan seribu akibat pencemaran logam berat. Adanya perbedaan yang nyata antara konsentrasi Pb dan Zn serta muatan (body burden) Pb dalam tubuh ikan dari lokasi dengan tingkat pencemaran logam yang berbeda tidak dapat ditafsirkan secara sederhana. Penjelasan yang mungkin diajukan adalah bahwa ikan dari lokasi tercemar dan tercemar berat telah mengembangkan suatu bentuk adaptasi fisiologis dengan mengakumulasi lebih banyak logam. Dalam penelitian ini, teramati adanya kecenderungan penurunan kadar Pb seiring dengan peningkatan ukuran tubuh ikan. Fenomena akumulasi ini merupakan salah satu dari tiga jenis hubungan antara konsentrasi logam dan ukuran tubuh, yaitu : peningkatan, penurunan dan tiadanya perubahan konsentrasi dengan bertambahnya ukuran tubuh, seperti didemonstrasikan oleh berbagai studi pada invertebrata akuatik. Untuk dua logam esensial, Zn dan Cu, jelas terlihat bahwa konsentrasi keduanya independen terhadap bobot tubuh. Tampaknya, konsentrasi kedua logam tersebut dalam tubuh ikan seribu diatur dan dipertahankan pada tingkat konsentrasi tertentu. Analisis korelasi yang melibatkan konsentrasi logam (dalam sedimen, air dan ikan), parameter fisik-kimia (air dan sedimen), dan ukuran tubuh ikan, menghasilkan 17 koefisien korelasi yang signifikan dari 91 hubungan bivariate yang ada. Secara umum parameter abiotik dan ukuran tubuh tidak berpengaruh terhadap aliran logam dari sedimen ke air dan ke tubuh ikan. Asosiasi yang signifikan hanya terdapat antara konsentrasi logam dalam sedimen dan tubuh ikan (Chapter 4). Hasil analisis korelasi parsial menunjukkan bahwa konsentrasi logam dalam sedimen merupakan faktor terpenting yang menentukan konsentrasi logam dalam tubuh ikan, karena tak satupun parameter abiotik (sedimen dan air) yang berperan sebagai covariate. Penelitian ini menunjukkan bahwa ikan seribu (*P. reticulata*) dari perairan di daerah perkotaan merupakan bioindikator yang potensial untuk pencemaran logam berat, khususnya ditunjang oleh (1) distribusi spasialnya yang mencakup seluruh kawasan tercemar, dan (2) adanya variasi akumulasi logam akibat derajat pencemaran yang berbeda. Hambatan pertumbuhan, tercermin dari ukuran tubuh yang lebih kecil, dijumpai pada ikan jantan yang hidup di lokasi tercemar. Tetapi, uniknya hal yang sama tidak diperagakan oleh ikan betina (Chapter 5). Meskipun secara statistika tidak signifikan, panjang tubuh ikan betina dari lokasi tercemar justru sedikit lebih panjang dari ikan betina dari lokasi tidak tercemar. Perbedaan yang signifikan dalam kandungan energi total pada ikan jantan dari lokasi tidak tercemar dan tercemar, yang bertolak belakang dengan hasil analisis ukuran tubuh, kemungkinan menunjukkan adanya perbedaan komposisi jaringan antar ikan dari lokasi yang berbeda. Penelitian lebih lanjut masih diperlukan untuk mengungkap fenomena ini. Diantara parameter reproduktif yang dikaji dalam penelitian ini, terlihat bahwa rasio kelamin (sex ratio) dan rasio betina hamil/total (ratio of pregnant to total females) merupakan dua parameter yang menjanjikan untuk bioindikasi. Dengan menggabungkan nilai kedua rasio tersebut, dapat diprediksi bahwa populasi ikan seribu di ekosistem tercemar cenderung memiliki lebih sedikit anggota betina, namun dengan aktivitas reproduktif yang lebih tinggi dibandingkan populasi di ekosistem yang tidak tercemar.

Untuk mempelajari pajanan senyawa pencemar yang tidak konstan dalam waktu (time-varying exposure), seperti konsentrasi tinggi dalam jangka waktu singkat, pendekatan dan konsep ekotoksikologi konvensional, yang mengandalkan premis pajanan konstan, tidaklah memadai. Maka, belakangan ini, pajanan yang tidak konstan telah menjadi topik penelitian yang menarik, baik dari aspek ekperimental maupun pemodelan. Dalam disertasi ini, sebuah model yang melibatkan dinamika pajanan substansi pencemar telah diusulkan (Chapter 6). Derivasi model survival berbasis toksikokinetika ini akhirnya menghasilkan ekspresi matematika yang relatif sederhana. Model ini memberi peluang untuk pendugaan laju penurunan konsentrasi substansi pencemar di lingkungan (k0), laju eliminasi (k2) dan nilai ultimate ILC50 (μ). Parameter terakhir ini didefinisikan sebagai konsentrasi awal yang mengakibatkan kematian 50%

hewan uji sebelum konsentrasi tersebut menurun sampai ke tingkat tak berpengaruh lagi. Selanjutnya model baru ini diaplikasikan pada percobaan toksisitas Zn menggunakan *P. reticulata* (Chapter 7). Selain potensinya sebagai bioindikator, ternyata jenis ikan ini juga menunjukkan kegunaan sebagai hewan uji dalam percobaan toksisitas akuatik. Dalam uji toksiko kinetika dan mortalitas, ikan seribu ternyata memberikan tanggapan sesuai dengan prediksi, sehingga memungkinkan analisis dan interpretasi ekotoksikologis lebih lanjut. Akhirnya studi pemodelan ini merekomendasikan bahwa (1) dalam evaluasi risiko pencemaran untuk pajanan yang tidak konstan akibat pelepasan substansi pencemar oleh kegiatan industri-perkotaan, penggunaan percobaan toksisitas konvensional, yang mengandalkan asumsi konsentrasi pajanan yang konstan, akan mengakibatkan kekeliruan, karena nilai toksisitas yang diperoleh akan terlalu tinggi (overestimate) dibanding nilai sesungguhnya, (2) percobaan toksisitas yang sedikit lebih rumit, yang memungkinkan perlakuan pajanan yang bervariasi dalam waktu (time-varying exposure), dengan tolok ukur sederhana seperti mortalitas, akan memberikan deskripsi yang lebih mendalam tentang toksikokinetika dan dinamika substansi pencemar di ekosistem.

ACKNOWLEDGEMENTS

One never notices what has been done; one can only see what remains to be done (Marie Curie, 1894)

I hope that the completion of this thesis is by no means the endpoint of my scientific "journey", but, at least my lengthy days in Pawiyatan Luhur empat-satu and De Boelelaan duizendzevenentachtig, and my extended evenings in Tegal Rejo, Pudak Payung and Uilenstede, have paid off. As to its collective nature, this thesis would never have been completed without support from others.

First and foremost, I would like to express my gratitude to my promotor, Prof. N.M. van Straalen (Nico), for his sustainable support to my academic advancement. His genuine empathy and willingness to listen to my dreams and difficulties deserves my heartfelt admiration. I am also grateful to my copromotor Dr. C.A.M. van Gestel (Kees). I count myself particularly fortunate to have had the chance to be supervised by an excellent combination of the two of them. Nico is a scientist with a rigorous authority and far-reaching vision, whereas Kees adds conciseness and accuracy.

The continuation of this PhD study would not have been possible without support from the former leadership of the Unika Soegijapranata (Romo Dr. Ir. P. Wiryono, S.J.; Ibu Lucia H. Patworo and Pak A.M.S. Darmawan), and the chairman of Yayasan Sanjojo (Prof. R.J. Djokomoeljanto). I am particularly grateful to Pak P.J. Supratignya who introduced me to Romo Wiryono. I highly appreciate the present Rector, Romo Dr. Al. Purwa Hadiwardoyo, MSF, for sustaining Unika's support to this programme. I would also like to thank the Department of Ecology and Ecotoxicology and the Faculty of Biology of the VU, for providing partial support to my PhD programme.

I am thankful to Rudo Verweij for his assistance in metal analyses. Rudo, I am sorry to have kept you busy with a huge number of samples everytime I came to Amsterdam. I am also thankful to Axel, Maaike, Novi Susetyo Adi and Felix Sholeh for performing experiments and field surveys. For their secretarial support (word processing, filing, mail, fax, e-mail, bills etc.), I would like to thank Al. Dwiyoga, Hermin, Edi, Kahono, Vivin and Teguh at Unika, and Désirée Hoonhout and Chris Dubbeldeman at the VU. A special word of thank goes to Miranda Aldham-Breary for correcting the English of my thesis, and to Luigi Sanna for his excellent drawings.

I am grateful to the referees Prof. Dr. Wim Admiraal, Prof. Dr. S.A.L.M. Kooijman, Dr. Joop L.M. Hermens, Dr. A.G.M. (Bert) van Hattum and Dr. Ir. Jan E. Kammenga for their willingness to sacrifice their valuable time to evaluate this thesis.

The special "climate" in the Department of Ecology and Ecotoxicology, VU, has influenced my perception (and also misperception) of the Dutch people and culture. What impressed me most is their modesty in lifestyle. I enjoyed my interaction with three generations of PhD students: Leo Posthuma - Marianne Donker - Martien Janssen - Matty Berg, Trudie

Crommentuijn - Timco van Brummelen, Daphna Lavy - Els Smit - René Hogervorst - Ed Stam - Paul Hensbergen. I would like to thank in particular Leo Posthuma, who introduced me, for the first time, to a toxicity experiment, in which I made a series of Cd concentrations 1000 times higher than the plan. I also took pleasure from my acquaintance with staff members in the department: Ab Kessler (thanks for the room and the "Macintosh"), Rik Zoomer (thanks for the "Budel" beer that made my "alcohol-free" face turning red), Ger Ernsting (thanks for the guppy literature and discussion), Piet Kakes and Tineke (thanks for the dinner and Wieteke van Dort's CD), Jan Simons (thanks for the dinner), Kees Verhoef and Anneke.

After some years of searching, I finally found the Dechene family in Lunteren. I am most grateful to Oom Eddy, Tante Melanie, Harry and David, who always welcomed me in a real "Kayumanis" atmosphere. In Lunteren, I always felt home. When you go back to Indonesia, please never forget to visit Semarang, you are most welcome. My understanding of other cultures has increased substantially due to friendship with Hayo van Schreeven, Eric and Alice van Gorp, Mohammed and Hala El-Bakary. Hayo, I am really happy that you were prepared to design the cover of this thesis. I am also thankful for your "lecture" on classical music and painting, our trips to Friesland and Rotterdam, and the dinners. Next time it will be my turn to take you to the best "warung sate kambing" in Semarang. Eric and Alice, the "concert-gebouw" experience was incredible, thank you for bringing me there. It will be your "mistake" if I start to love classical concerts. I relished several visits to your nice home. Mohammed and Hala, I enjoyed our lengthy chats on many evening occasions. I think I will miss your Egyptian tea, goat-milk rice and "halal" fried chicken. I hope we can meet again in the future.

During my last visit to finalize this thesis, I took pleasure from my stay in the "South-East Asian Corridor" of Uilenstede 102, where I met Tony B. Mayo, Hoa and Be, Rully, Ihot, Ferdy Rondonuwu, Marten Ndoen, Hendrawan and family. Our evening gatherings were very refreshing for someone working under time-pressure like myself. I wish you all the best with your programmes.

When I was at UKSW, I have benefited from the inspiring discussions with Agna S. Krave, Rully A. Nugroho and Sucahyo. I am also indebted to my senior fellows, Dr. Parakitri Simbolon (Gramedia, Jakarta) and Prof. Dr. Bambang Hidayat (Observatorium Boscha, Lembang), who have enriched my perspective on the role and responsibility of intellectuals and scientists in Indonesia. I also enjoyed my involvement in the FOSKO-21 discussion group; I appreciate Budi Pranowo, Anto Prabowo and Herudjati Purwoko for being my critical sparring partners. Back to Unika, I was inspired by the activities of my colleagues: Pudjo Koeswhoro, Djoko Suwarno, Bambang Sudarsono, Paulus Haryono, Andreas Lako, Tyas B. Susanti, Tjahjono Rahardjo, Rustina Untari, and Rudiyanto Susilo. Last but not least, I would like to express my sincere thanks to the staff members of the Faculty of Agricultural Technology: Lindayani, Retnaningsih, Rika Pratiwi, Sri Lestari, Sudarini and Sumardi for their support and cooperation. In particular, I am grateful to Lindayani and Sumardi, who took care of the "empty" faculty during my final visit to Amsterdam. I hope after finishing this job I will be able to devote more time to our faculty.

Finally, I would like to express my most sincere gratitude to Ime, for her everlasting support, understanding, patience and love. I don't know how to "reimburse" her very hard times to stay alone with our two kids, when I was away from home. I owe to my sons, Arya and Angga, for often neglecting my paternal duty to play with them during their spare times and school holidays. I hope I can spend more time to play badminton, uno card and computer games with them after this.

CURRICULUM VITAE

Budi Widianarko was born on November 23, 1962 at Semarang, Central Java, Indonesia. He completed his elementary school (SD Bernardus, Semarang) in 1974, junior high school (SMP Pangudi Luhur, Salatiga) in 1977, and high school (SMAN Salatiga) in 1981. From August 1981 to February 1986 he did his tertiary education and earned a Sarjana degree (the best graduate, period I - 1986) from the Faculty of Animal Husbandry, Diponegoro University, Semarang.

After a 9 months work in a multinational poultry breeding farm company at Tangerang, West Java, in December 1986 he started his academic carrier at the Faculty of Biology, Universitas Kristen Satya Wacana (UKSW), Salatiga. At this faculty, he had been involved in various tasks, including teaching (animal physiology, biometrics, ecotoxicology etc), research (pesticide impacts on the terrestrial isopod *Porcellio scaber*) and administration (head of department, vice dean etc).

Upon receiving a NUFFIC scholarship, in October 1989 he started his MSc programme at the Department of Theoretical Biology and Department of Ecology and Ecotoxicology, Vrije Universiteit Amsterdam (VUA), under supervision of Prof. Dr. S.A.L.M. Kooijman and Dr. N.M. Van Straalen, respectively. He received his MSc certificate in May 1991. He was the coeditor (with K. Vink & N.M. Van Straalen, 1994) of "Environmental Toxicology in South East Asia" - VU University Press, Amsterdam. This book is an outgrowth of the first Conference on Environmental Toxicology in South East Asia, Salatiga - 1992, which was also under his chairmanship.

In November 1993, he initiated a sandwich PhD programme at the Department of Ecology and Ecotoxicology, VUA and the Faculty of Biology, UKSW, dealing with the ecotoxicological impacts of non-persistent pesticides. Due to his move to the Universitas Katolik Soegijapranata, Semarang in August 1994, the PhD programme was interrupted. And it was restarted in January 1995, with a new emphasis on urban metal pollution. In this programme, Prof. Dr. N.M. Van Straalen and Dr. C.A.M. Van Gestel acted as the promotor and copromotor.

Currently he works as a lecturer and the Dean of the Faculty of Agricultural Technology, Universitas Katolik Soegijapranata. Besides his teaching and administrative tasks, he is now initiating a research programme on "pollution and food safety".

SELECTED LIST OF PUBLICATIONS

Book

Widianarko, B. ; K. Vink & N.M.van Straalen (eds.). 1994. Environmental Toxicology in South East Asia. The Free University Press. Amsterdam.

Article

Widianarko, B. & N.M. Van Straalen (199x). Scientific Research on Environmental Toxicology in South East Asia: A View of Its Position in the International Arena. (Submitted to Australasian Journal of Ecotoxicology)

Widianarko, B.; R.A. Verweij; C.A.M. Van Gestel & N.M. Van Straalen (199x). Spatial Distribution of Trace Metals in Sediments from Urban streams of Semarang, Central Java, Indonesia. (Submitted)

Widianarko, B.; R.A. Verweij; C.A.M. Van Gestel & N.M. Van Straalen (199x). Association Between Trace Metals in Sediment, Water and Guppy, *Poecilia reticulata* (Peters) from Urban Streams of Semarang. (Submitted)

Widianarko, B. (199x). Heavy Metals in Urban Streams of Semarang: Bioindication Potential of Body Size and Reproductive Parameters of The Guppy (*Poecilia reticulata*) (Submitted)

Widianarko, B.; F.X.S. Kuntoro; C.A.M. Van Gestel & N.M. Van Straalen (199x). Toxicokinetics and Toxicity of Zinc Decreasing-Exposure in Field Guppy, *Poecilia reticulata* (Peters). (Submitted)

Widianarko, B. (1997). Urban Pollution: A Challenge for Eco(toxico)logy. A keynote presentation at the Conference on The Challenge of Urbanization: Socio-Cultural and Spatial Transformation, Semarang, Indonesia January 7-8, 1997

Widianarko, B. & N.M. van Straalen (1996). Toxico-kinetics based survival analysis in bioassays using non-persistent chemicals. Environmental Toxicology & Chemistry 15(3): 402-406

Widianarko, B. (1996). Heavy Metals in Semarang's Urban Streams: Spatial Distribution and Bioindication Using the Guppy (*Poecilia reticulata*). A paper presented at the 1996 Pacific Basin Conference on Hazardous Waste, Kuala Lumpur, Malaysia, November 4-8,1996

Abdullah, A.R.; A.H. Sulaiman & **B. Widianarko** (1996). Pesticides in The Tropical Environment: The Contribution of Ecotoxicological Research to Environmental Protection. The 1st Pan Pacific Symposium on Pesticide Science. Kobe, Japan, October 28-31, 1996.

Selected List of publication (continued)

Widianarko, B. (1996). Demographical Characteristics of The Guppy from Nonpolluted and Polluted Urban Streams in Semarang. A paper presented at the The 13rd National Conference of Environmental Study Center, Denpasar, Bali, Indonesia, October 22-24, 1996 (in Bahasa Indonesia).

Widianarko, B. (1996). Demographical Structure of the Guppy (*Poecilia reticulata*) from Clean and Metal Contaminated Streams of Semarang, Central Java. A paper presented at the International Symposium on Environmental Chemistry and Toxicology 1996 (InterSect'96), Sydney, Australia, July 14-18, 1996

Widianarko, B. & N.M. van Straalen (1995). Environmental Toxicology in South East Asia. The 2nd Society of Environmental Toxicology and Chemistry (SETAC) World Congress, Vancouver, Canada, 5-9 November 1995

Widianarko, B. (1995). Bioassay and modeling for ecotoxicological risk evaluation of non-persistent chemicals. A paper presented at the XIIth National Conference of Environmental Study Center, Semarang, Indonesia, Jan. 11-13 (in Bahasa Indonesia).

Widianarko, B.; K.H. Timotius & K. Vink. (1994). Ecotoxicological approach for environmental standards with special reference to soil quality. In Widianarko, B.; Vink, K. & N.M. van Straalen (Eds.). Environmental Toxicology in South East Asia. The Free University Press. Amsterdam. pp. 7-18.

Widianarko, B. ; M.H. Donker & N.M. van Straalen. (1994). Growth and Arrhenius activation energy of three *Porcellio scaber* populations - An evaluation of cadmium effects. In Widianarko, B.; Vink, K. & N.M. van Straalen (Eds.). Environmental Toxicology in South East Asia. The Free University Press. Amsterdam. pp. 199-208.

Widianarko, B. (1993). In search of relevant parameters for pesticide risk evaluation in Indonesia. Proceedings of the 1993 Pacific Basin Conference on Hazardous Wastes (16: 93109, 14p), Honolulu, Hawaii, USA. November 8-12, 1993.

Posthuma, L.; R.A. Verweij; **B. Widianarko** & C. Zonneveld. (1993). Life-history patterns in metal-adapted Collembola. OIKOS 67: 235-249.

Widianarko, B. (1991). A simple toxicokinetics model with a stochastic input: A modelling and simulation study. A paper presented at the National Biology Congress IX, Bogor, September 1991 (in Bahasa Indonesia).